Lecture 1

Analysis of single-cell RNA-Seq data: Experimental Design

Physalia course 2025

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Single-cell RNA-seq with R/Bioconductor

Instructors: Orr Ashenberg, Jacques Serizay, Fabrício Almeida-Silva

Overall goals

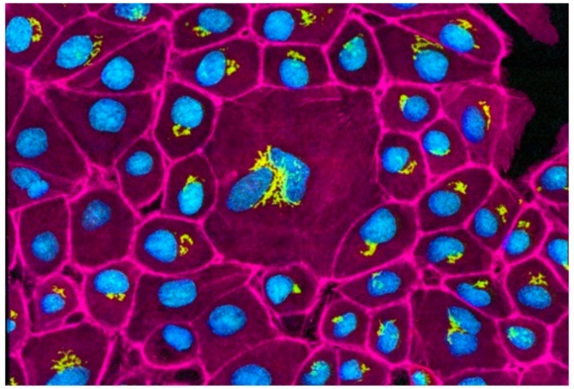
- Introduction to the rapidly expanding world of single-cell transcriptomics
- Focus less on specific software tools but more on underlying concepts - so down the line, you can make informed choices
- Hands on lab exercises analyzing single-cell heterogeneity
- Create a fun, learning, collaborative, and interactive environment over the next week

A few organizational notes

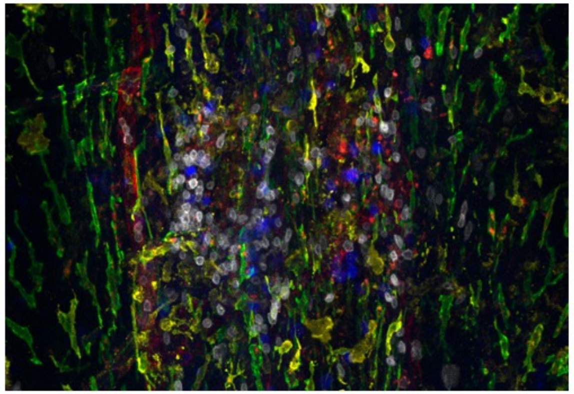
- Workshop link: https://jserizay.com/scRNAseq_Physalia_2025/
- Ask questions or use chat! Raise hand in Zoom (Participants). Please use video, and mute microphone when not in use.
- Write course notes and questions in a shared Google document. We will send you a link to store your flash talk.
- Please be patient with technical issues (network, Zoom, etc...)

Incredible diversity in cell types, states, and interactions across human tissues

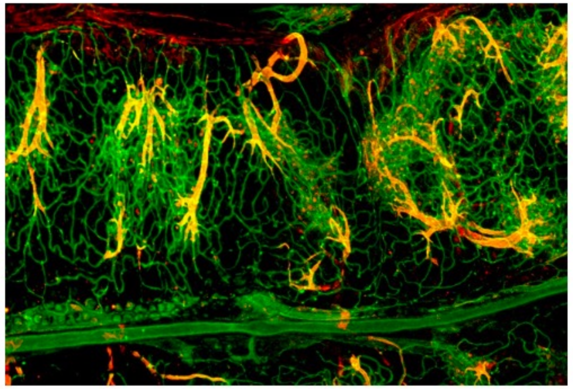
Skin epithelium



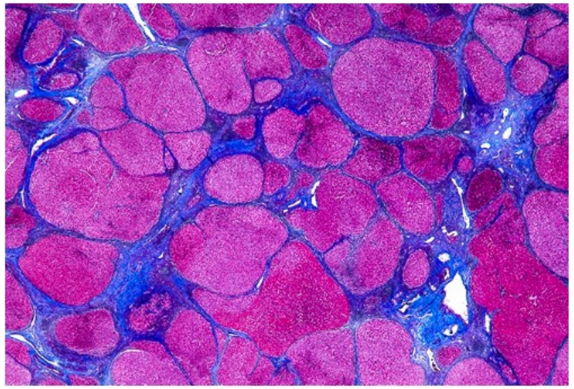
Brain meninges



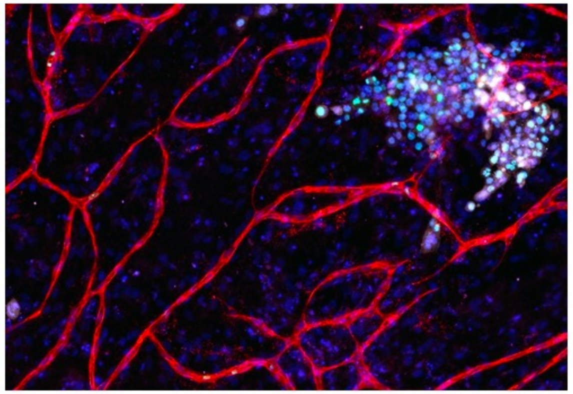
Blood vessels



Small intestine

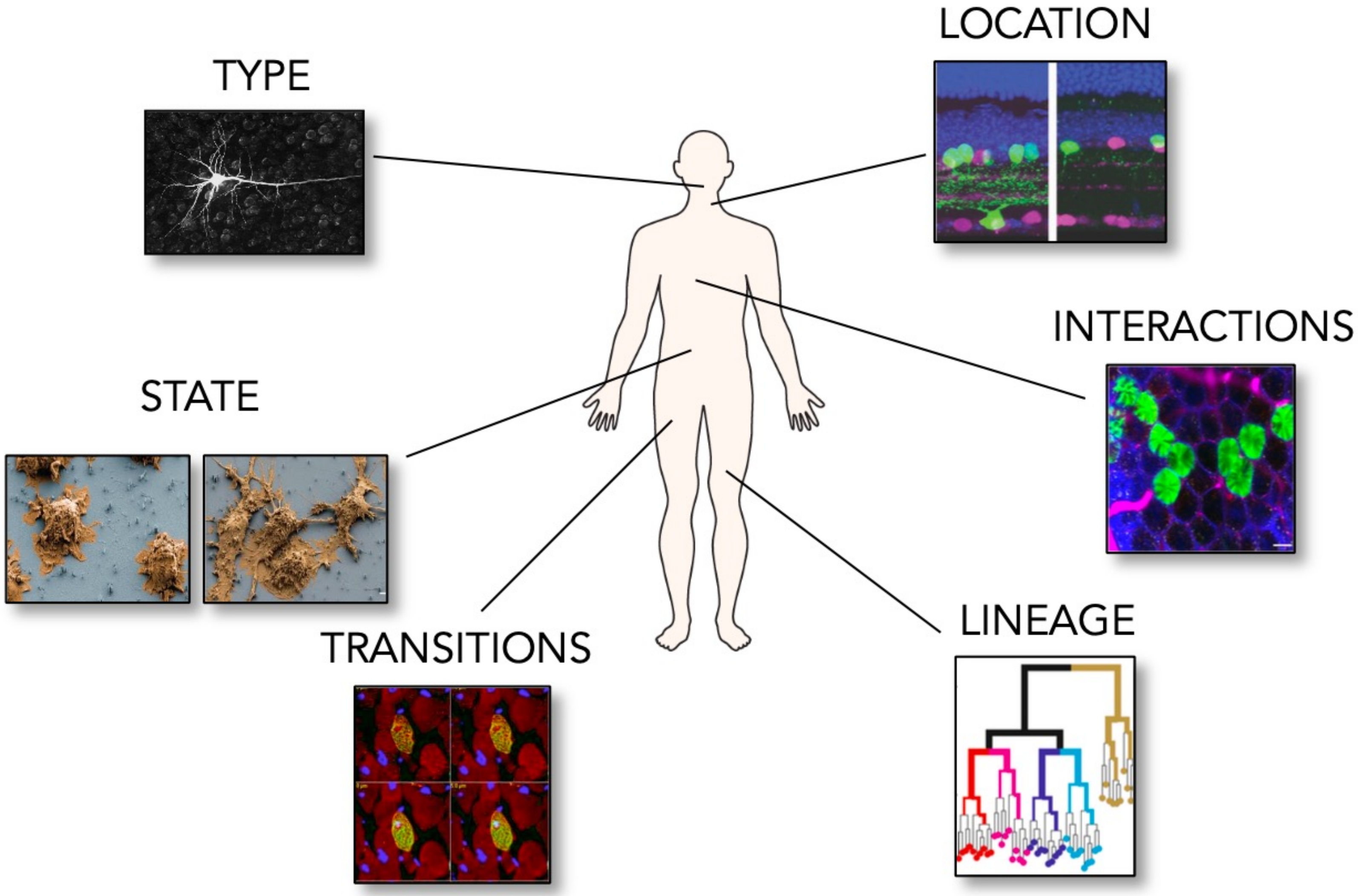


Liver cirrhosis



Breast cancer

A cell’s identity and fate are shaped by many features



TYPE

LOCATION

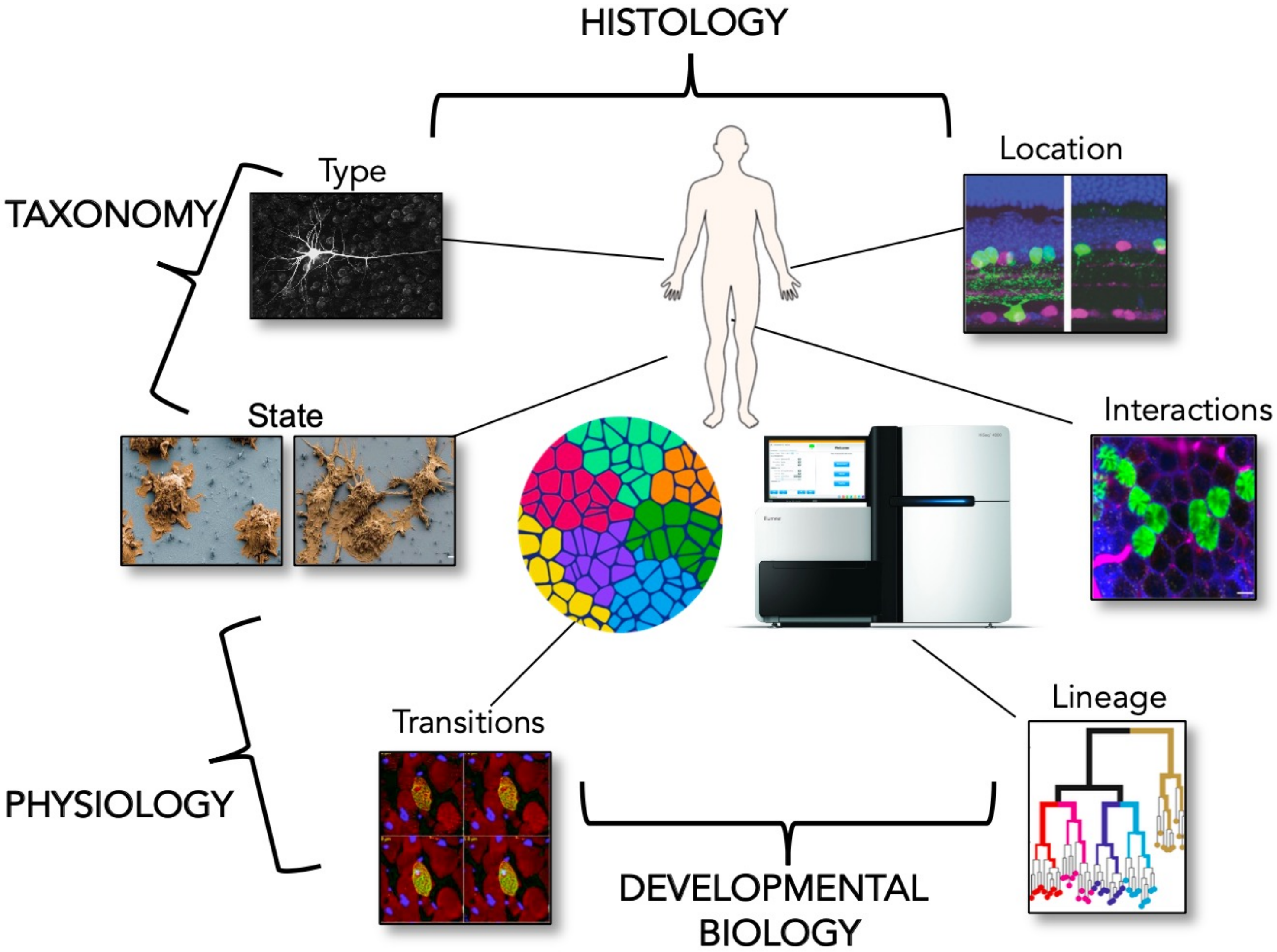
INTERACTIONS

STATE

TRANSITIONS

LINEAGE

A cell's identity and fate are shaped by many features



HISTOLOGY

TAXONOMY

Type

Location

State

Interactions

PHYSIOLOGY

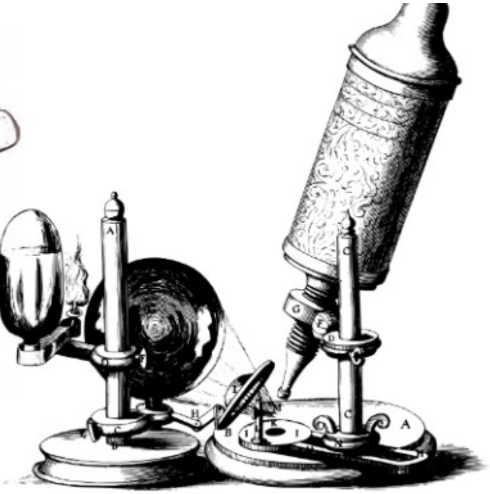
Transitions

Lineage

DEVELOPMENTAL BIOLOGY

Technological innovations allow observation at increasing resolution

"by the help of Microscopes, there is nothing so small as to escape our inquiry"
Robert Hooke



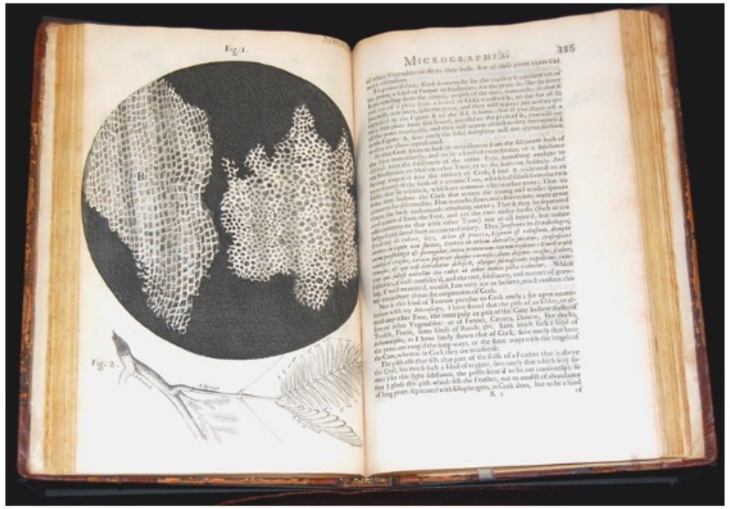
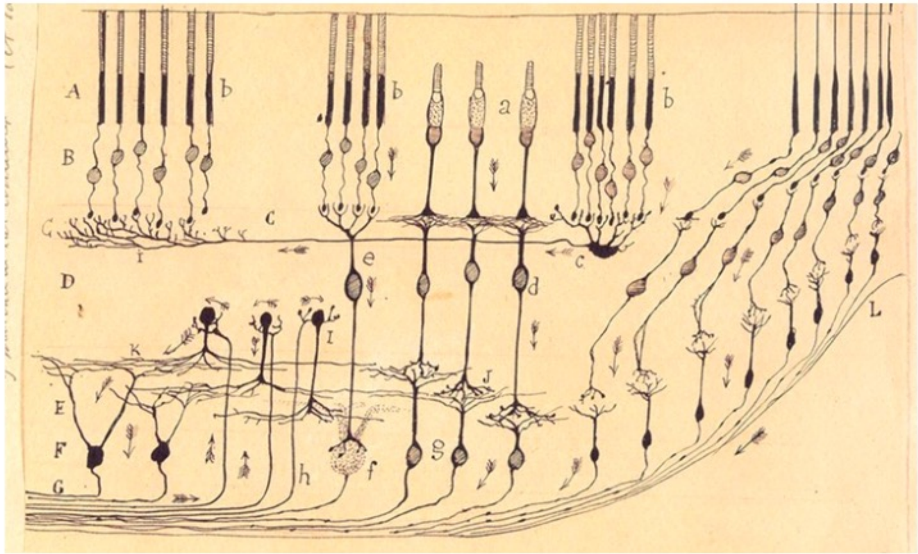


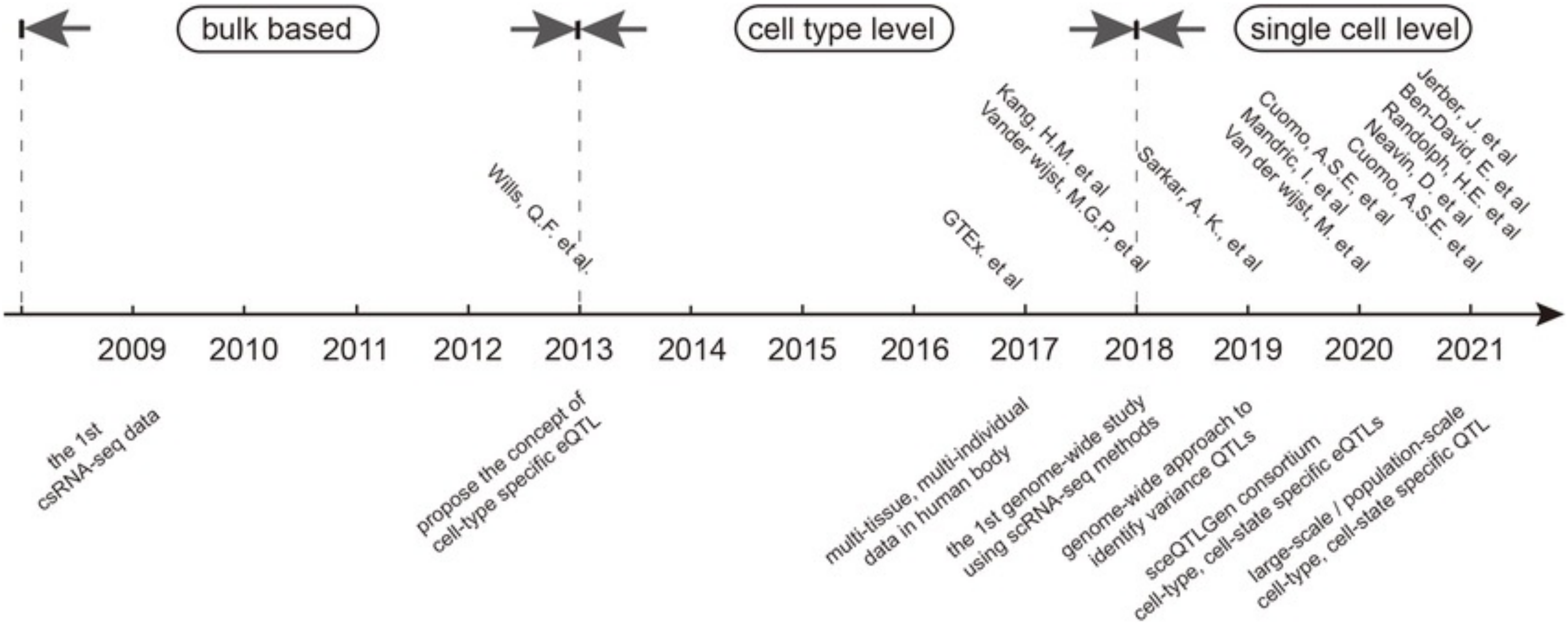
Fig. 1.

MICROGRAPHIA. 115

Fig. 2.

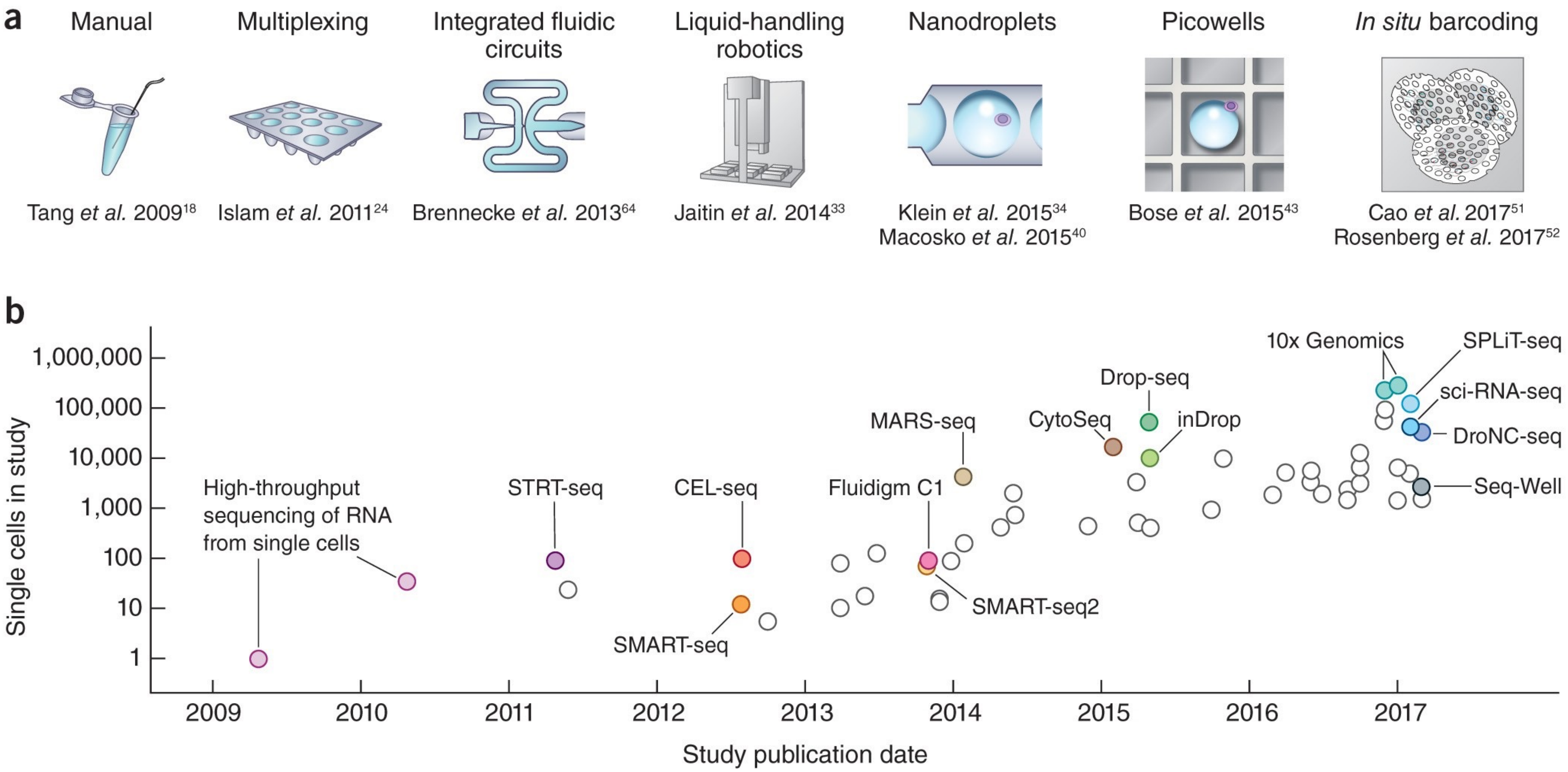


Last decade: emergence of single-cell transcriptomics



Luo et al. Front in Genet 2023

Single-cell RNA sequencing has grown exponentially

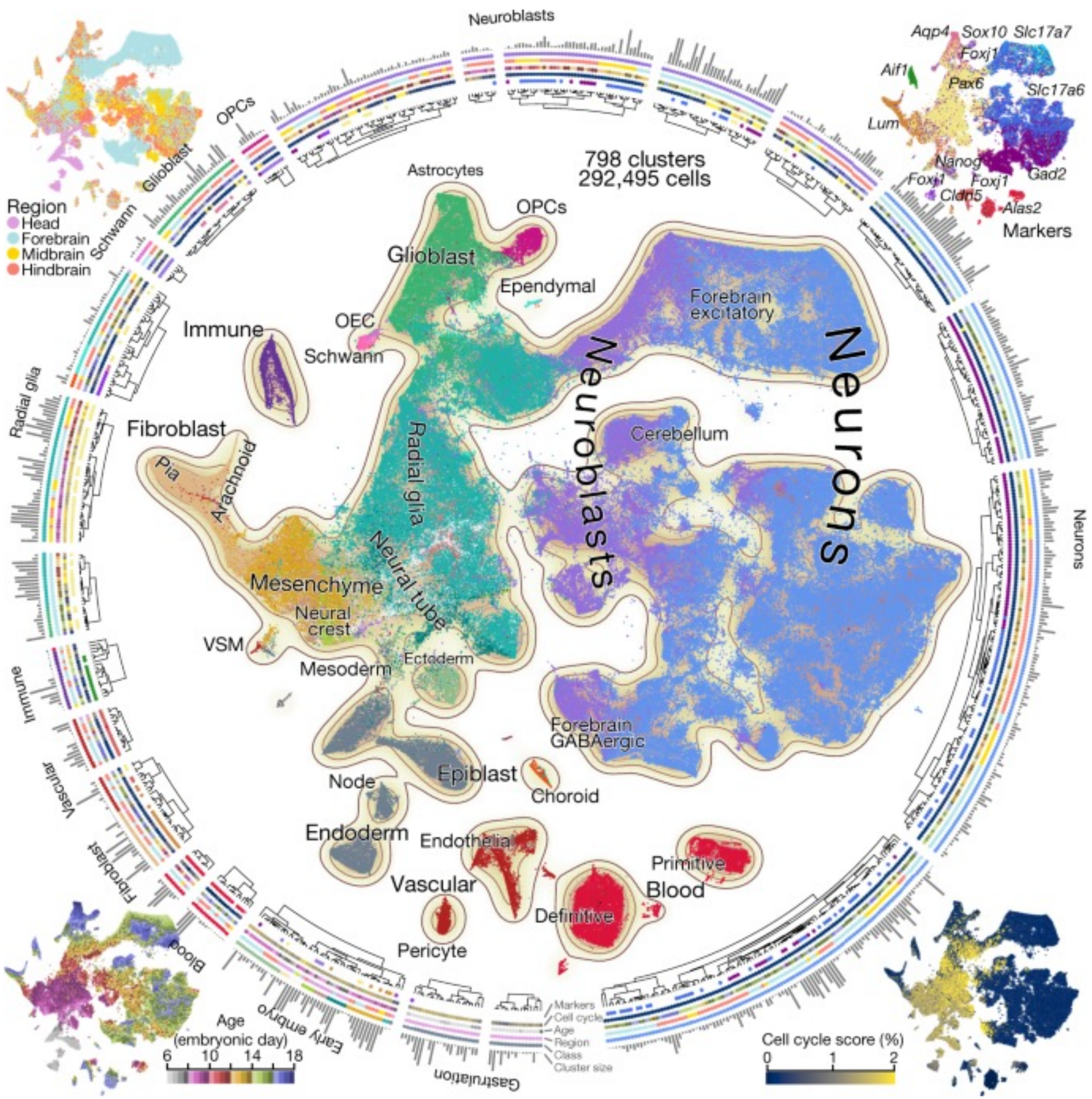


Svensson et al. Nat Protocols 2018

Molecular architecture of the developing mouse brain

Droplet-based single-cell RNA sequencing was used to profile the embryonic mouse brain each day between E7 and E18:

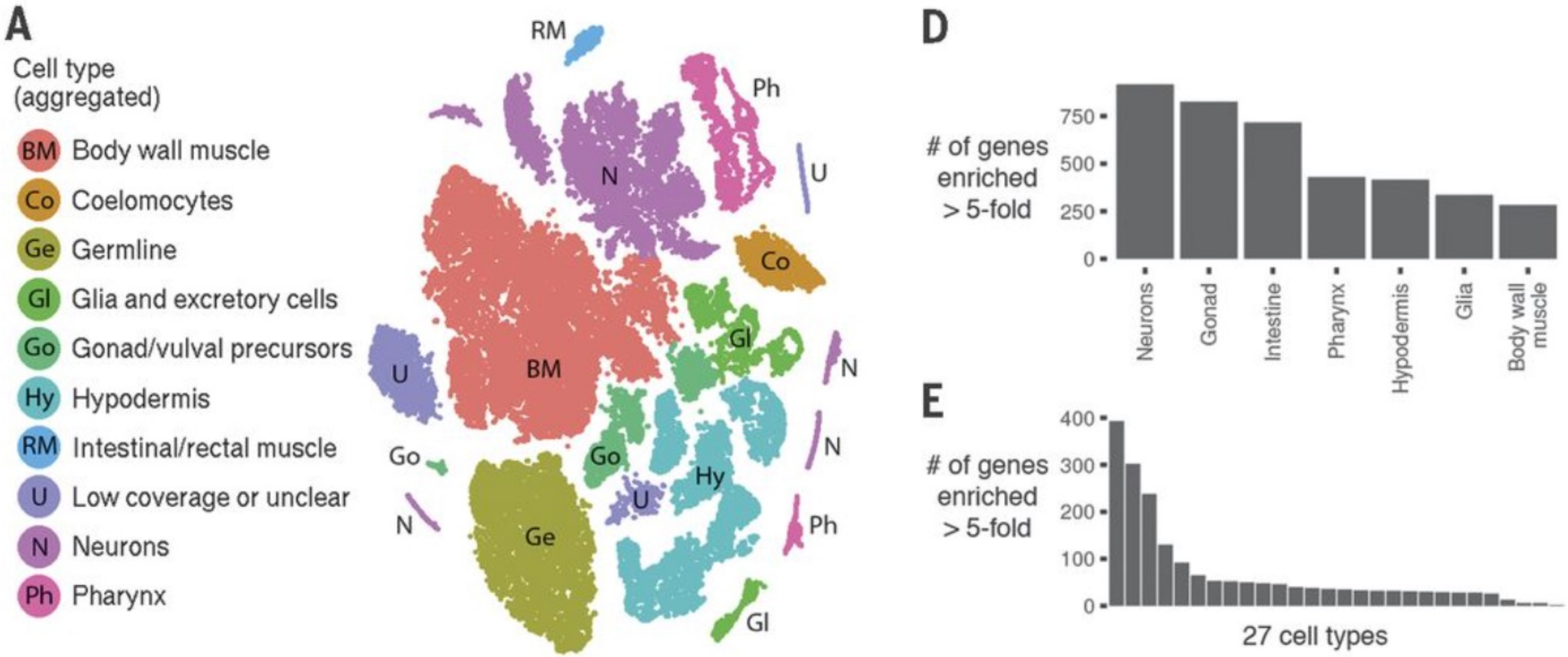
93 samples and 292,495 cells



La Manno et al. Nature 2021

Comprehensive single-cell transcriptional profiling of a multicellular organism

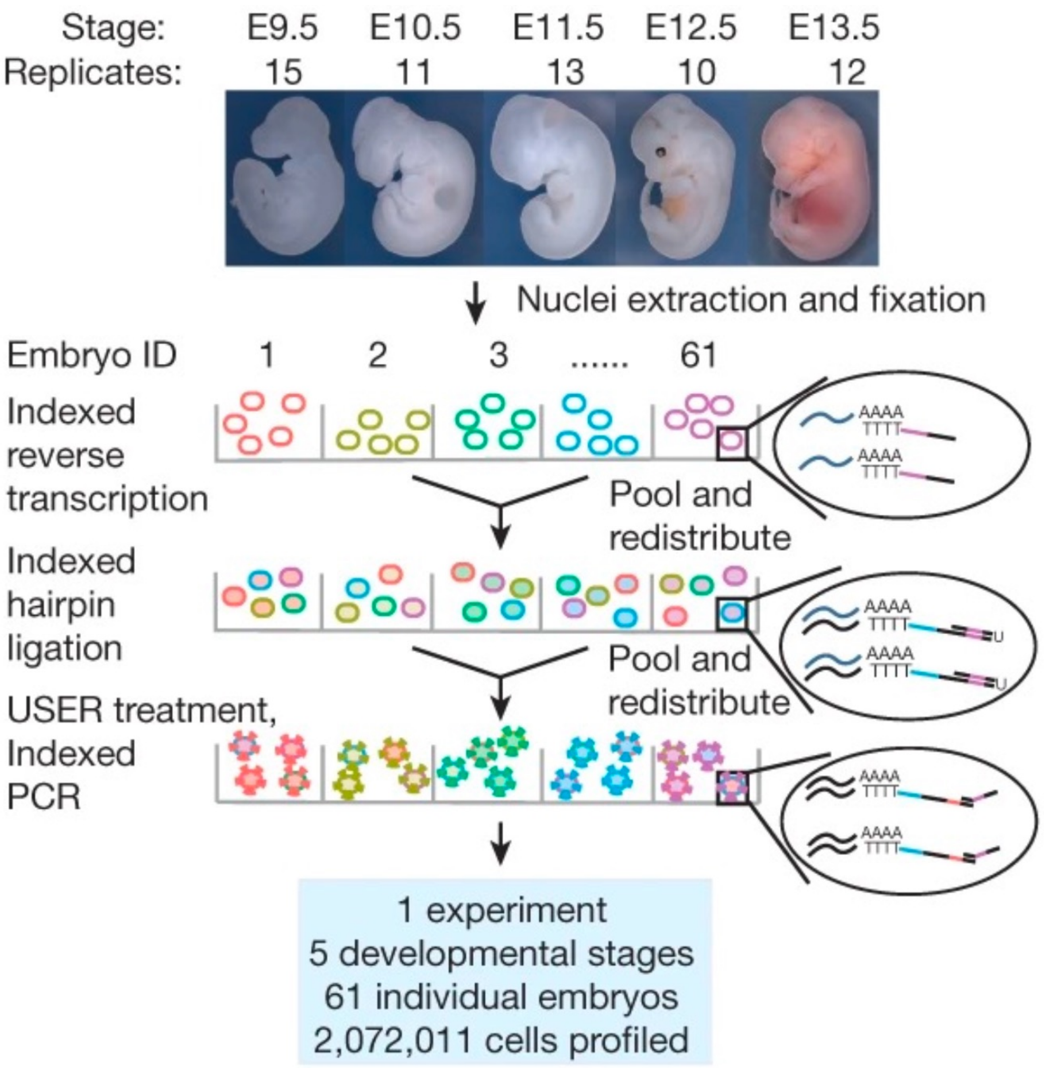
sci-RNA-seq enables profiling of all tissues from a whole organism.



Cao, J., at al. Science 2017

Mouse organogenesis studied by single-cell RNA sequencing

sci-RNA-seq3 enables profiling of 2,072,011 cells from 61 mouse embryos across 5 developmental stages in a single experiment.



Cao, J., at al. Nature 2019

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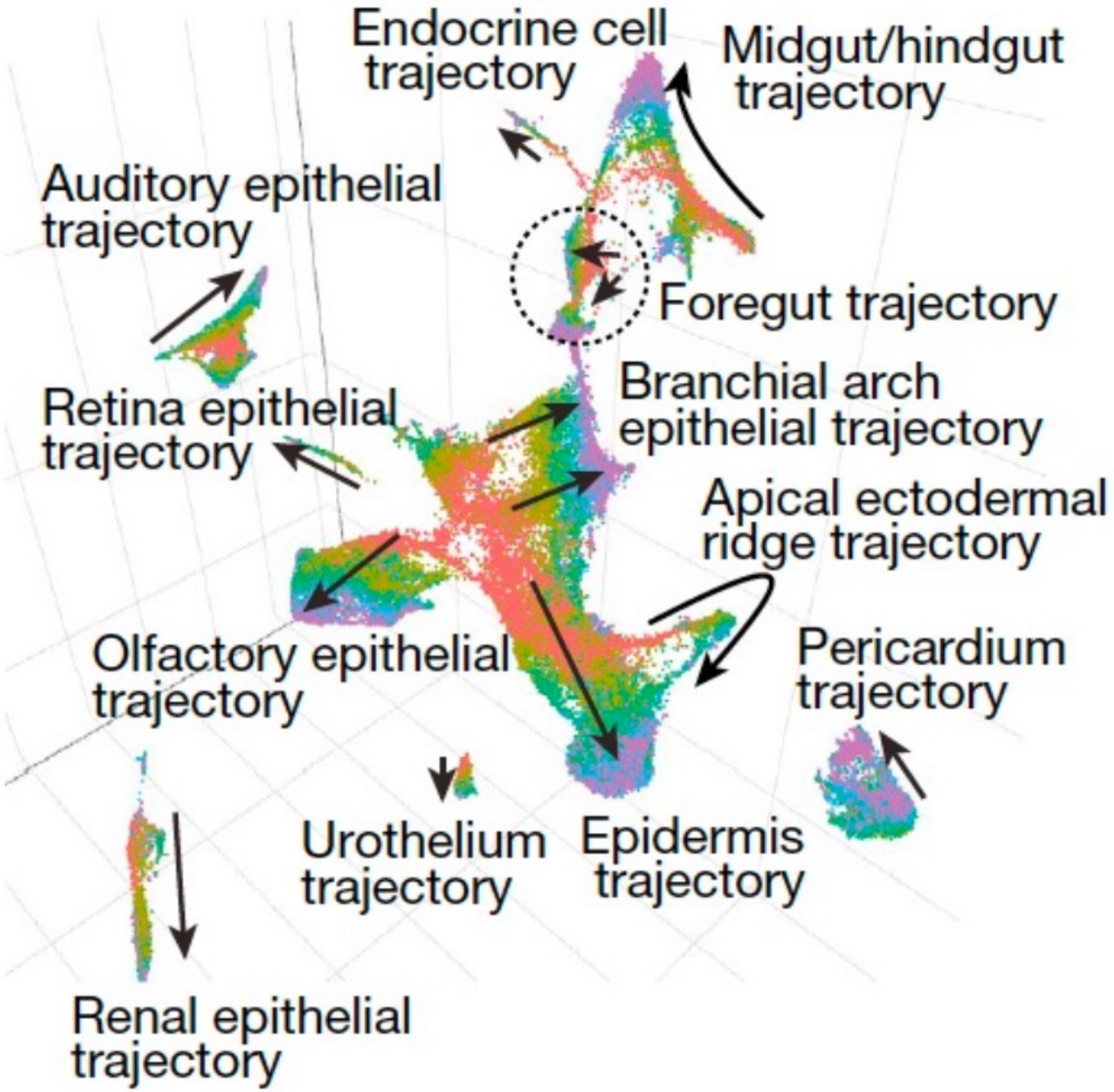


Cao, J., at al. Nature 2019

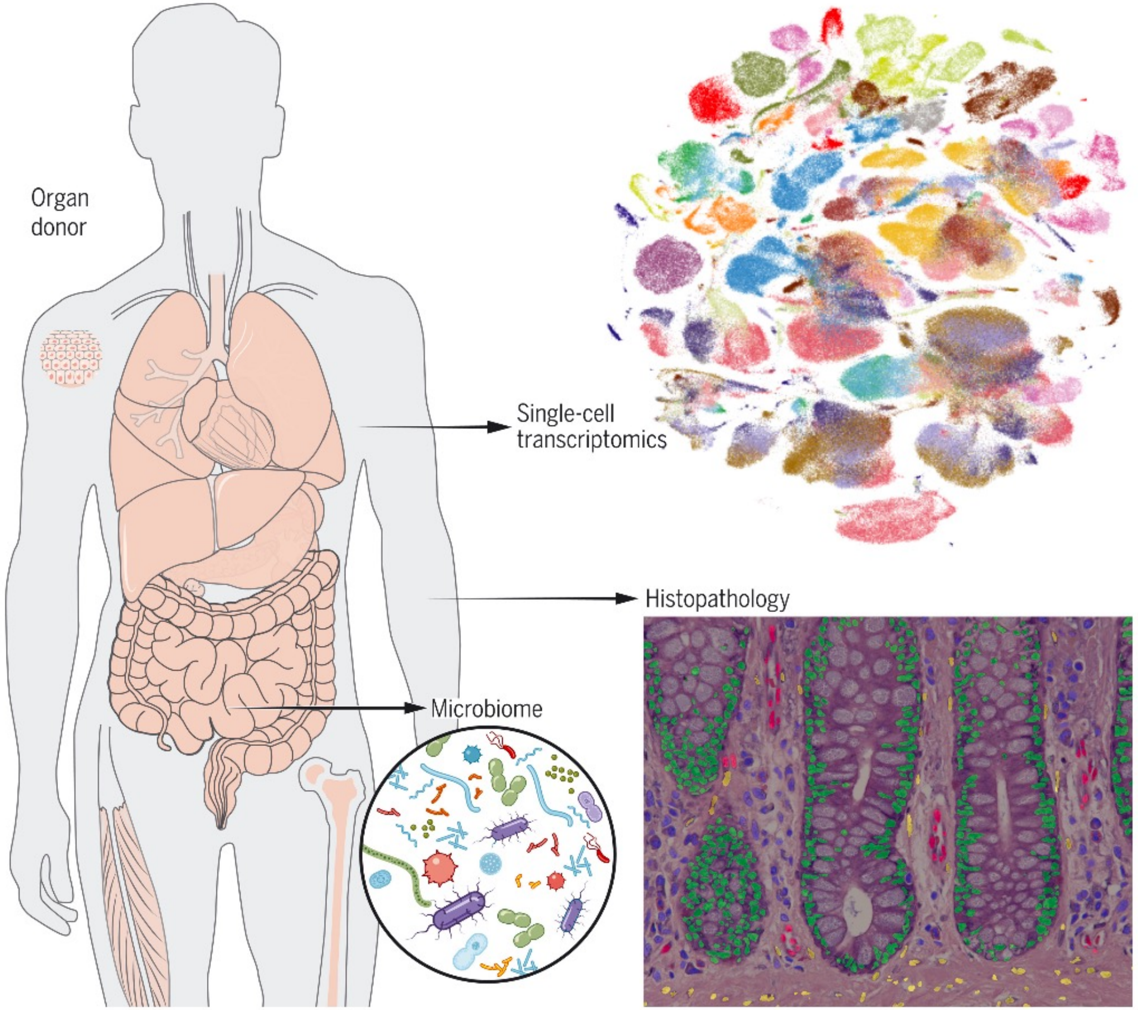
Why so much profiling?

To create a comprehensive reference map of the types and properties of all human cells, the fundamental unit of life, as a basis for understanding, diagnosing, monitoring, and treating health and disease.

"The vestiges of the rupture reveal themselves, if someone brings forward a map of the world and considers carefully the coasts of the three [continents]."
Dutch map maker Abraham Ortelius (1596)

A series of human cell atlases published in May 2022

500,000 cells from 24 different tissues and organs

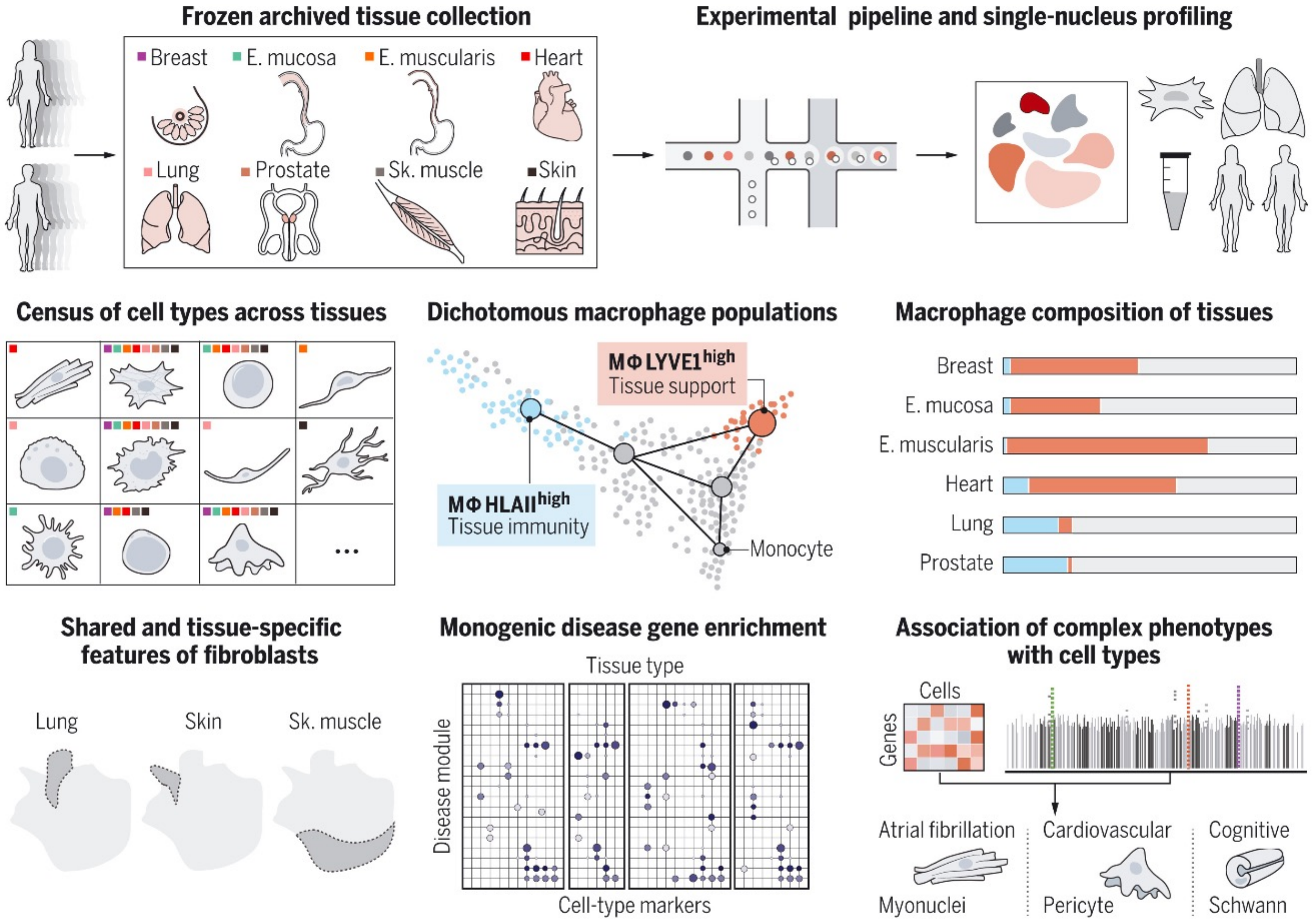


The Tabula Sapiens Consortium. Science 2022

A series of human cell atlases published in May 2022

500,000 cells from 24 different tissues and organs

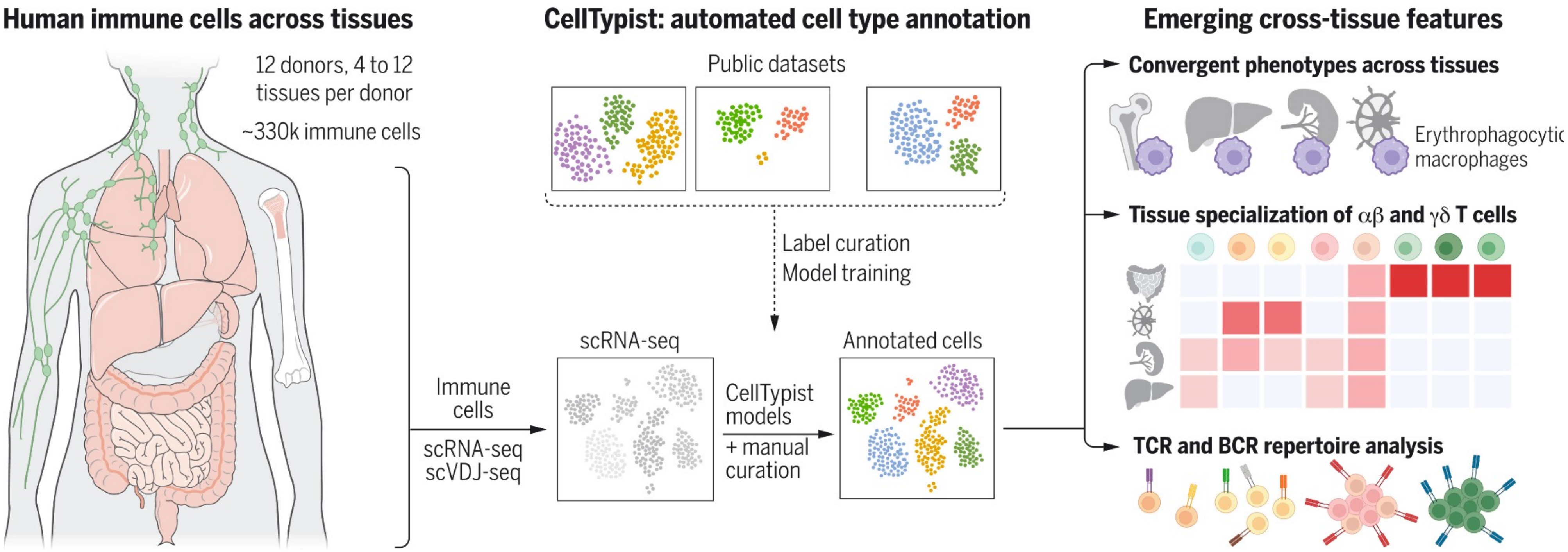
Association of cell types and states with human disease



Eraslan et al. Science 2022

A series of human cell atlases published in May 2022

500,000 cells from 24 different tissues and organs
Association of cell types and states with human disease
300,000 immune cells from 16 different tissues



Domínguez Conde et al. Science 2022

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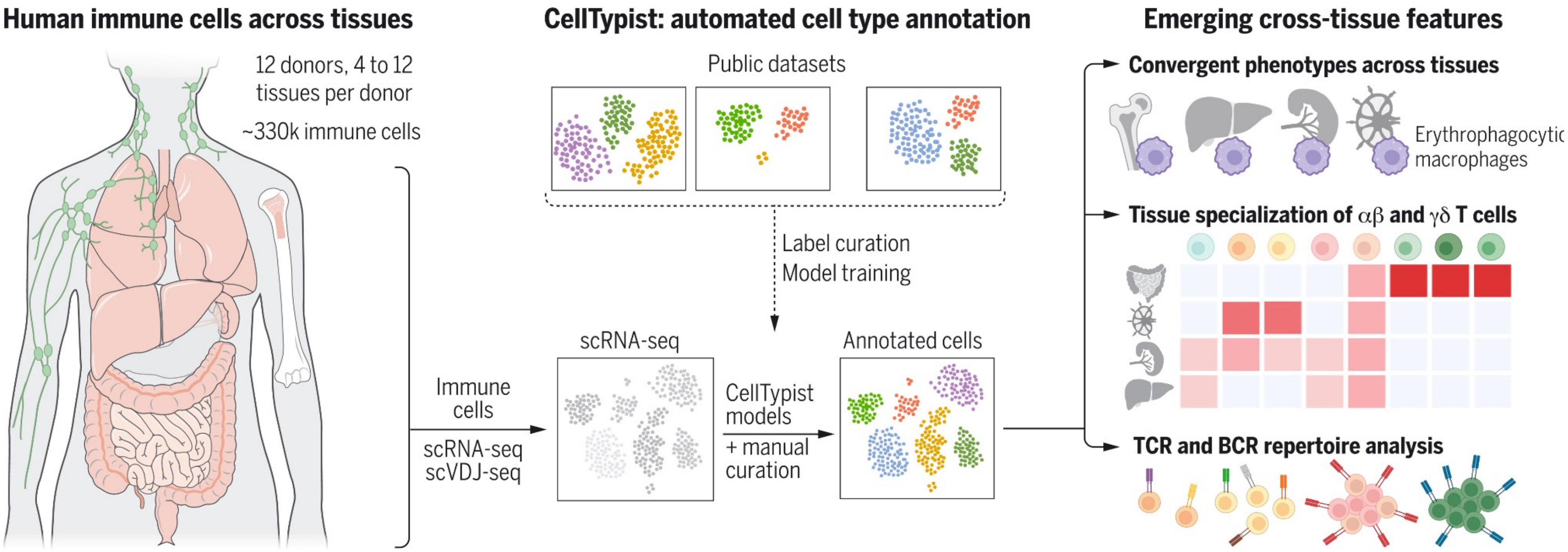

Domínguez Conde et al. Science 2022

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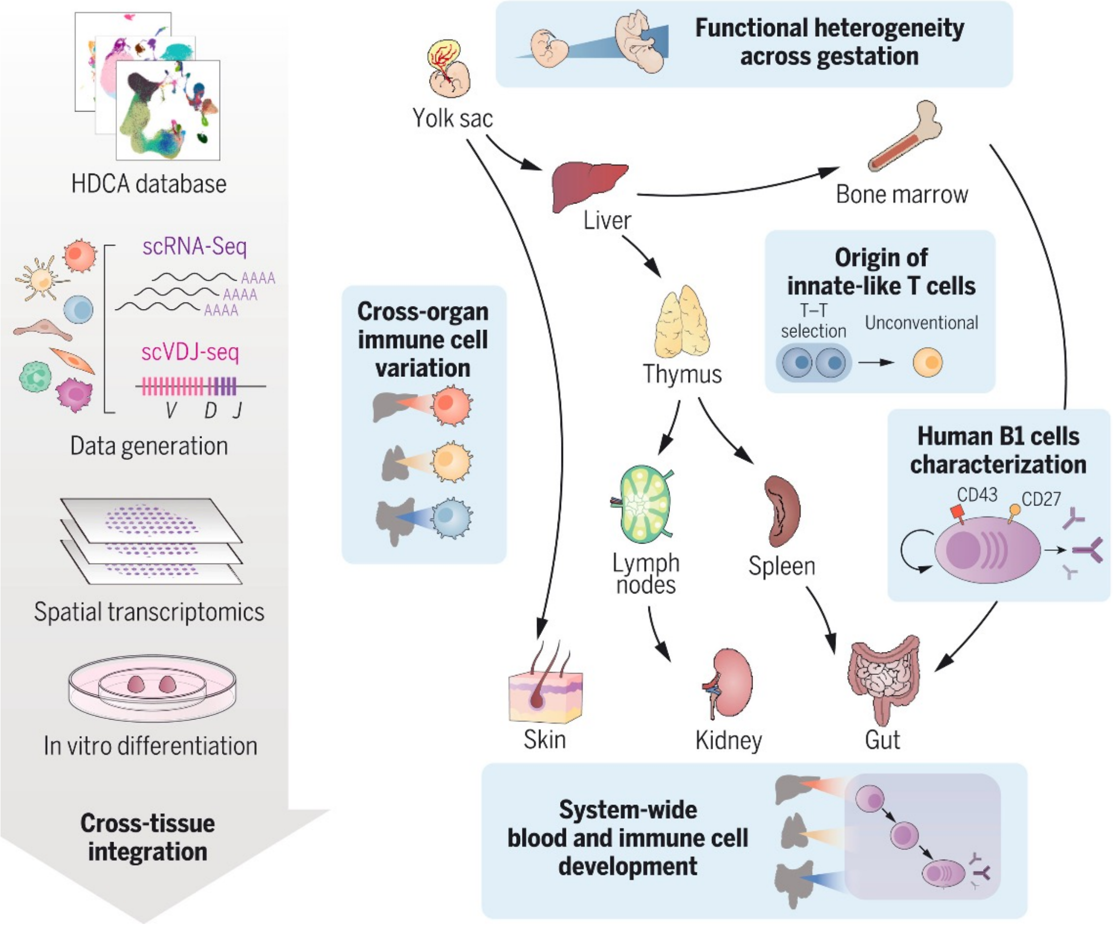
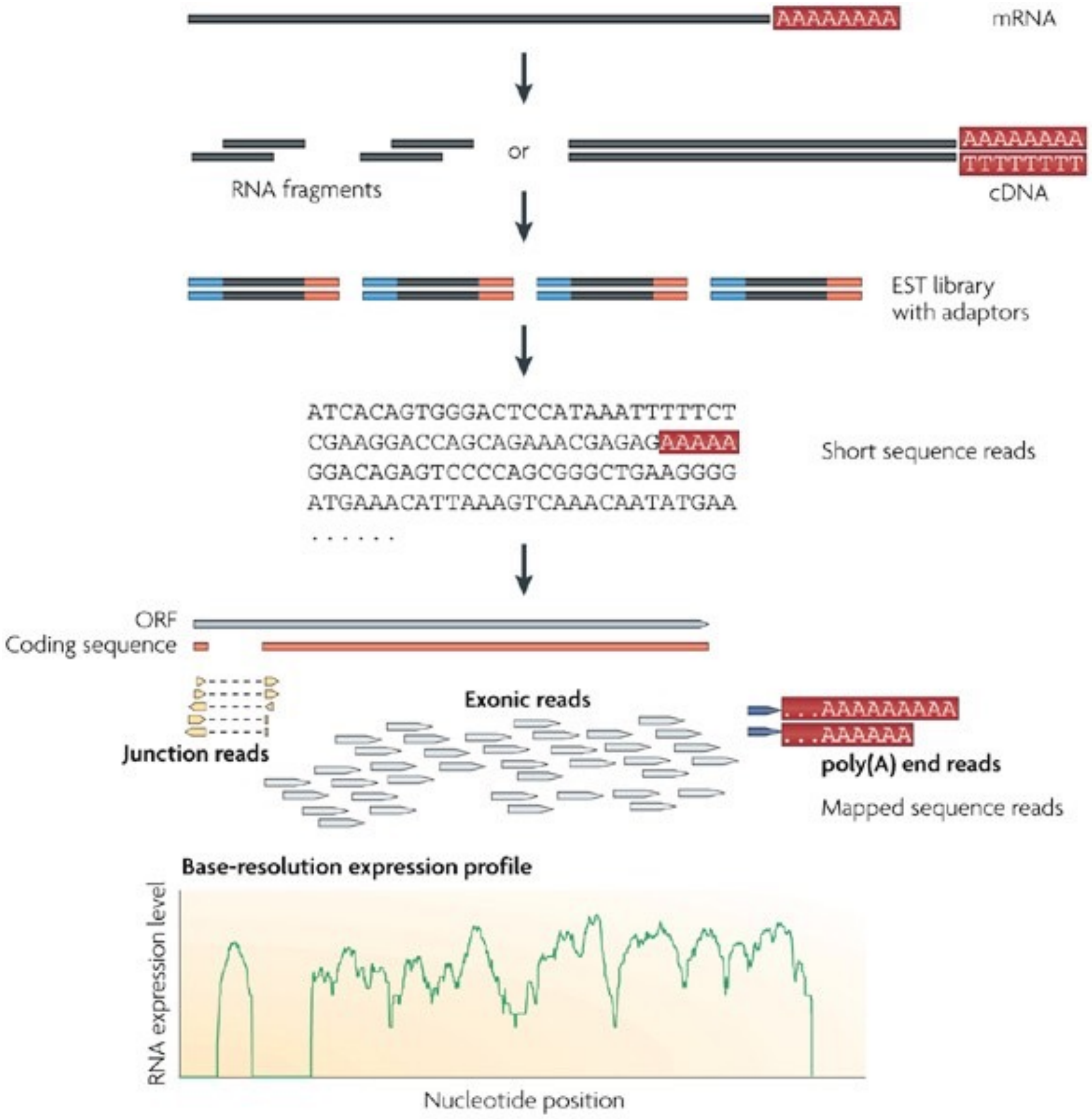
Developing human immune system



Suo et al. Science 2022

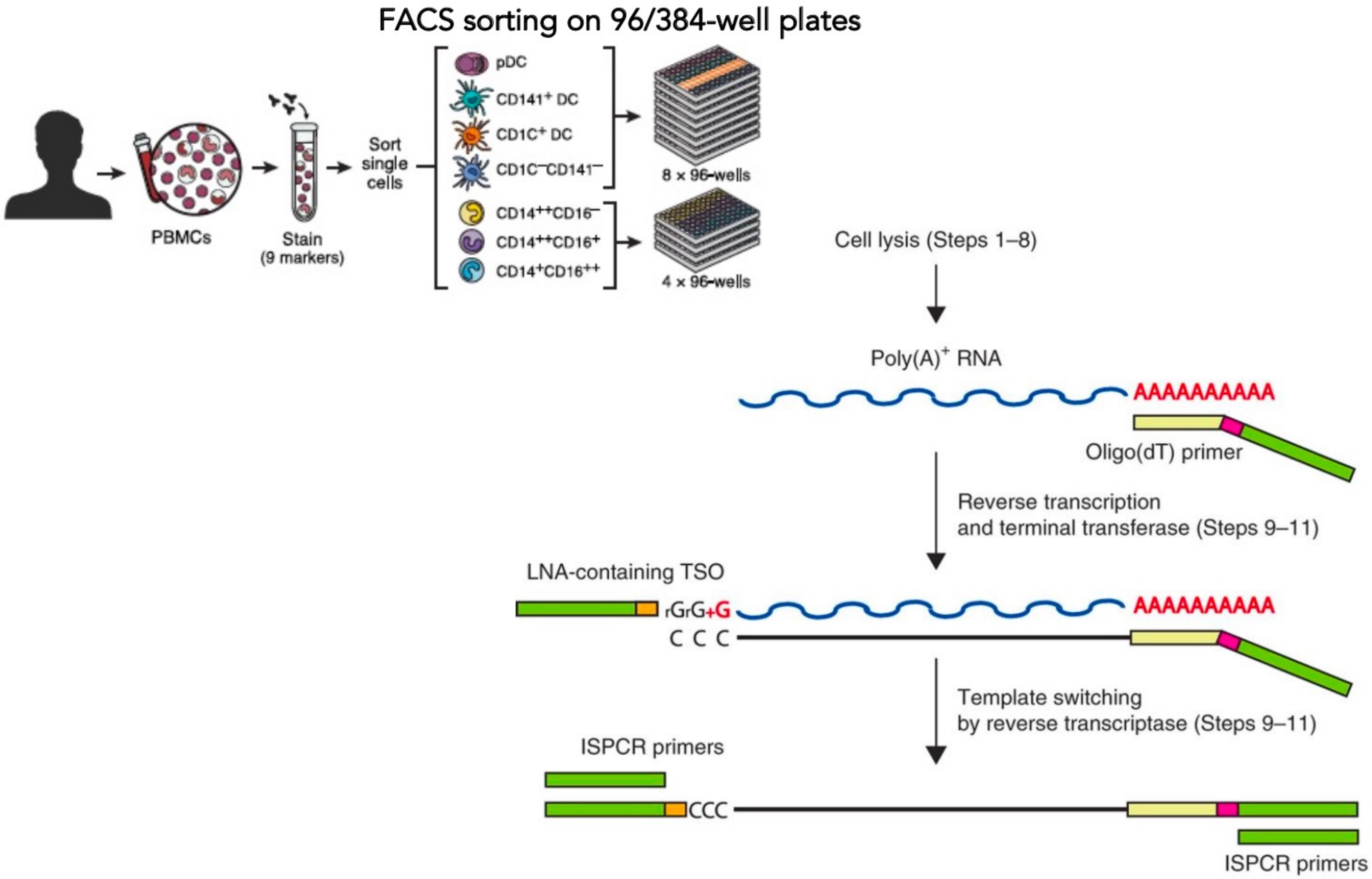
Typical RNA-seq workflow, bulk



Nature Reviews | Genetics

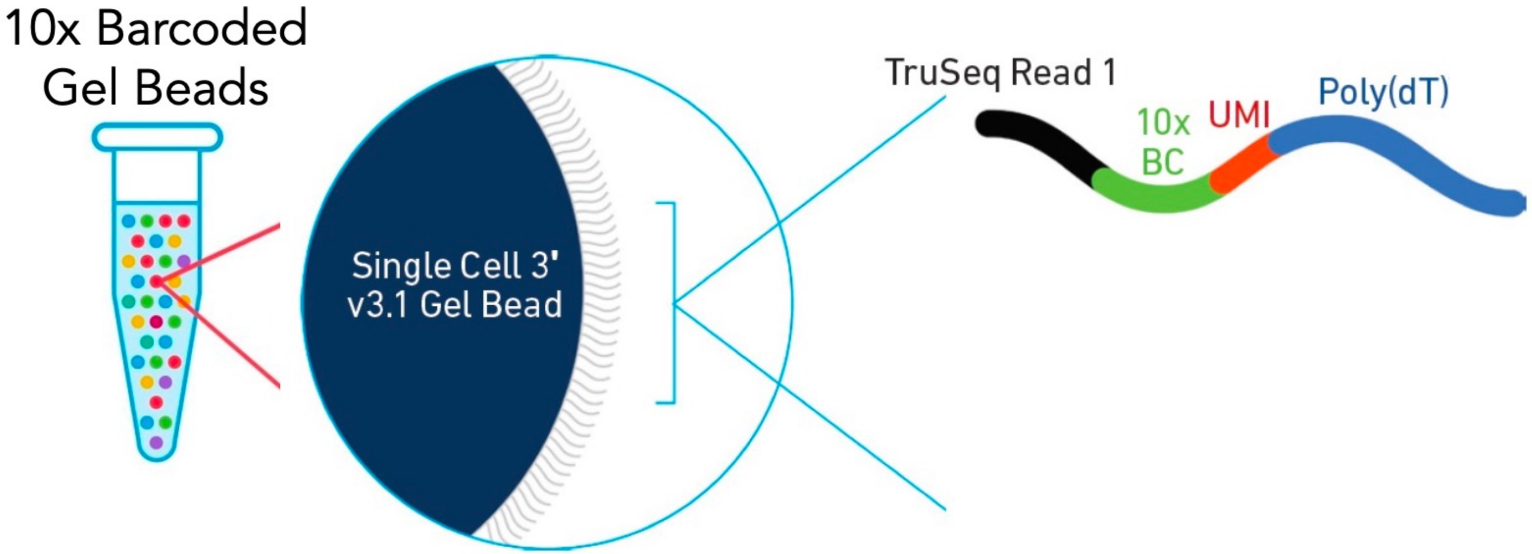
Wang et al., Nat. Rev. Genet. 2009

Typical RNA-seq workflow, single-cell



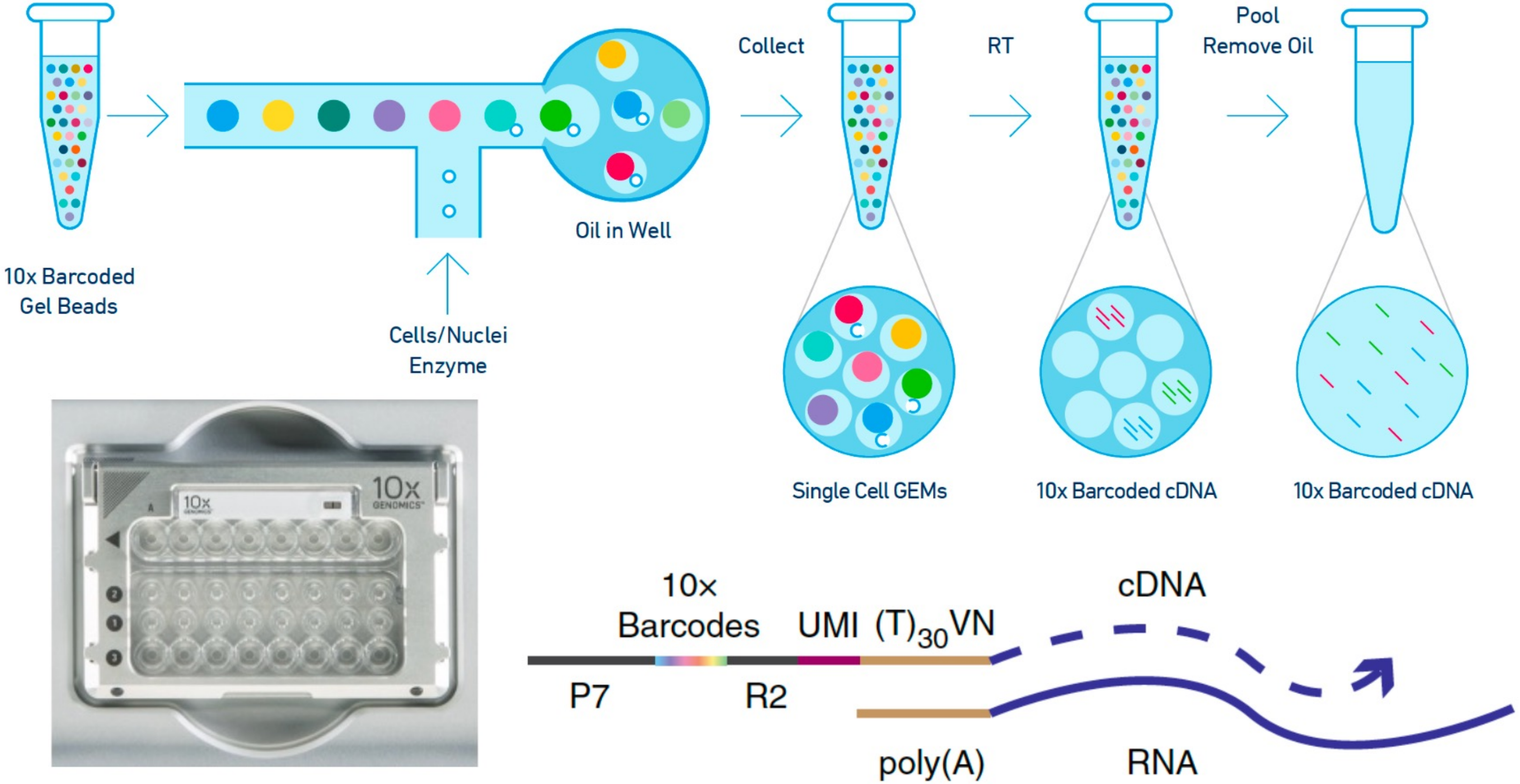
Picelli et al. Nat Protocols 2014; Villani et al. Science 2017

Typical RNA-seq workflow, single-cell (2)



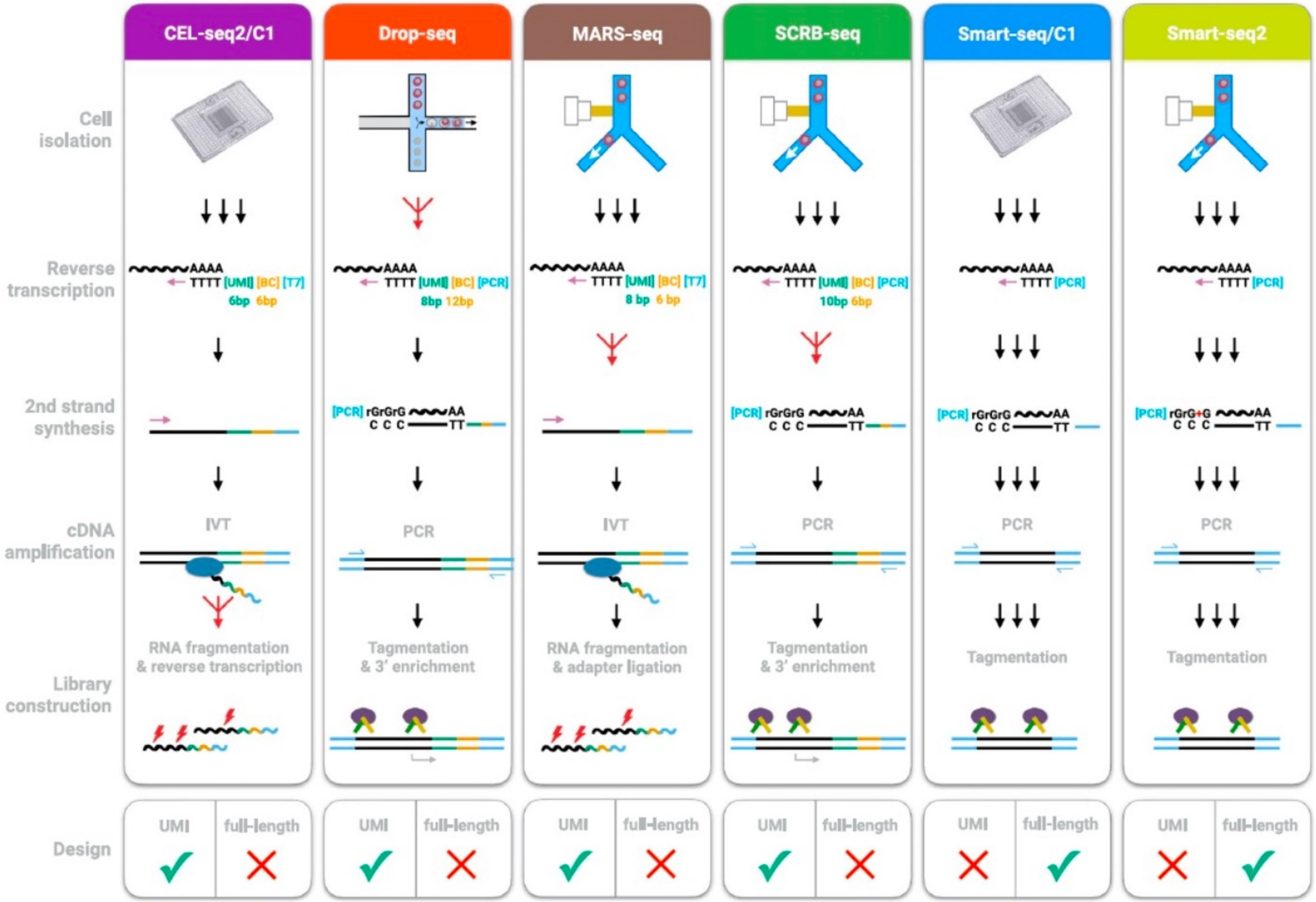
10X genomics

Typical RNA-seq workflow, single-cell (2)



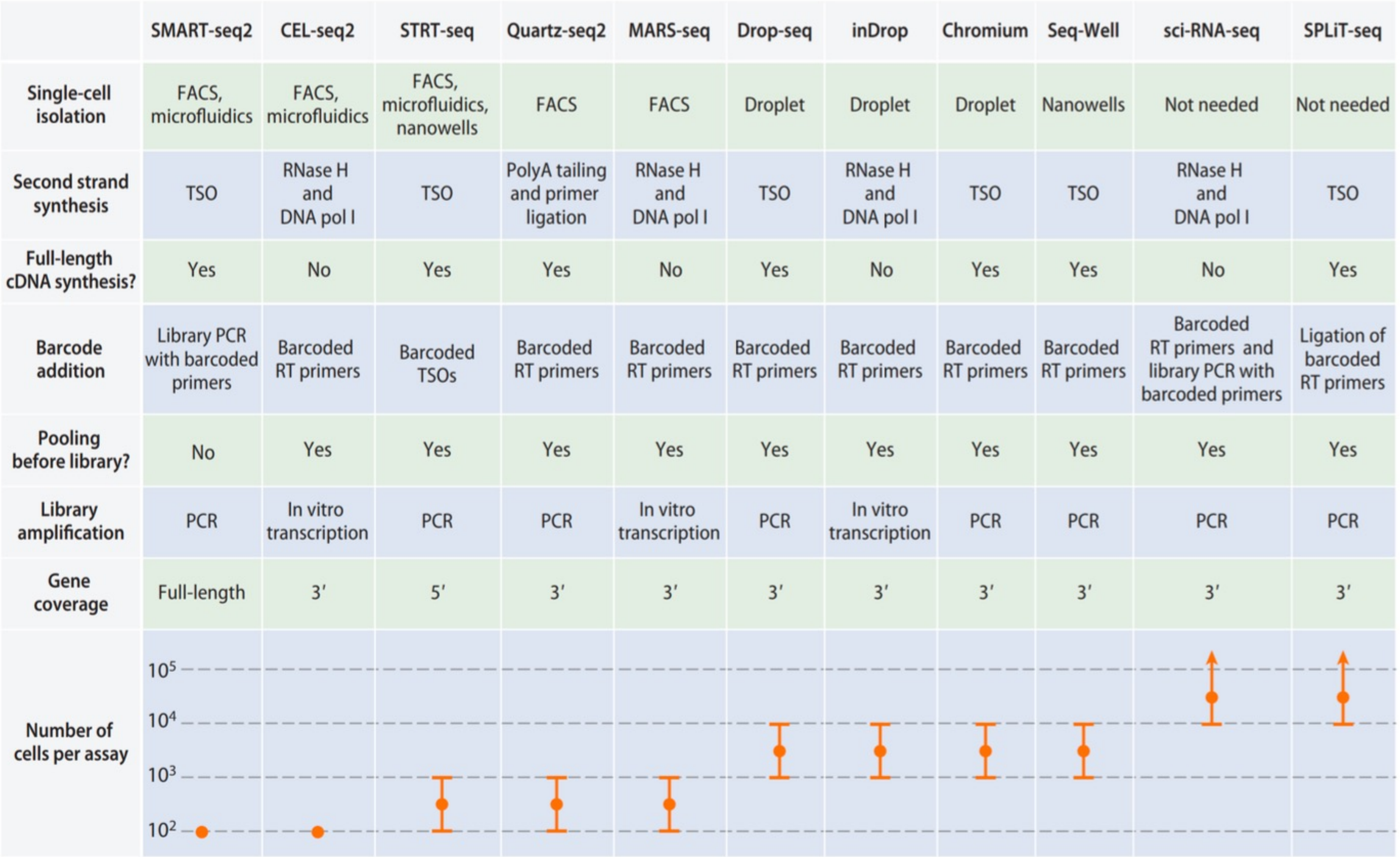
10X genomics

There are many single-cell RNA sequencing methods



Ziegenhain et al. Mol Cell 2017

There are many single-cell RNA sequencing methods



	SMART-seq2	CEL-seq2	STRT-seq	Quartz-seq2	MARS-seq	Drop-seq	inDrop	Chromium	Seq-Well	sci-RNA-seq	SPLiT-seq
Single-cell isolation	FACS, microfluidics	FACS, microfluidics	FACS, microfluidics, nanowells	FACS	FACS	Droplet	Droplet	Droplet	Nanowells	Not needed	Not needed
Second strand synthesis	TSO	RNase H and DNA pol I	TSO	PolyA tailing and primer ligation	RNase H and DNA pol I	TSO	RNase H and DNA pol I	TSO	TSO	RNase H and DNA pol I	TSO
Full-length cDNA synthesis?	Yes	No	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes
Barcode addition	Library PCR with barcoded primers	Barcoded RT primers	Barcoded TSOs	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers and library PCR with barcoded primers	Ligation of barcoded RT primers
Pooling before library?	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Library amplification	PCR	In vitro transcription	PCR	PCR	In vitro transcription	PCR	In vitro transcription	PCR	PCR	PCR	PCR
Gene coverage	Full-length	3′	5′	3′	3′	3′	3′	3′	3′	3′	3′
Number of cells per assay											

Chen et al. Ann Rev of Biomed Data Sci 2018

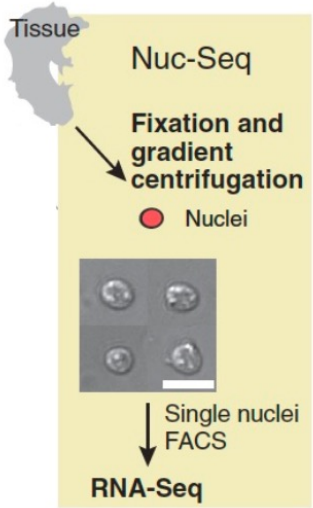
Considerations for single cell RNA-Seq

Choose protocol based on :

- Throughput (# of cells / reaction)
- Sample of origin
- Cost / Labor / Time limitations
- Gene body coverage - 5′, 3′ biased, or full-length?
- UMI vs no-UMI
- Sequencing depth / cell

For example :

- If I want to classify all cell types in a diverse tissue (e.g. brain), I need high throughput
- If I want to re-annotate the transcriptome and discover new isoforms, I need full-length coverage
- If I only have access to archival human samples, I will need to use a method that permits fixed cells (or nuclei)



Habib et al. Science 2016

Some unique features and challenges of single cell RNA-Seq

Features

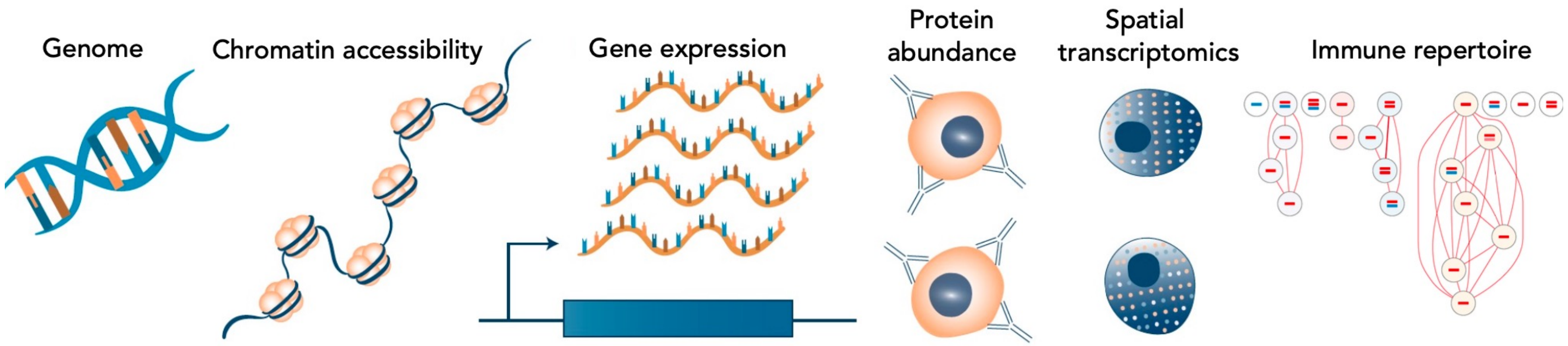
- measures the distribution of expression levels for each gene across a population of single cells
- can study 1e2-1e6 cells in an experiment

Challenges

- amplification during library preparation
- gene dropout
- experimental design and computational analysis

Habib et al. Science 2016

Ongoing developments in single-cell genomics: Many other molecules from single cells may be profiled



DNA and epigenome
- Single cell genomes (WES, WGS)
- Single cell epigenomics (HiC, ChIP, ATAC, mC)

RNA
- Full length (mRNA, total RNA)
- 5′ and 3′ end counting

Proteins
- Multi-parameter flow
- Mass cytometry
- Single cell proteomics

‘multi-omics’
- DNA+RNA (G+T)
- RNA+protein (T+P)
- Epigenome + RNA

Efremova et al. Nat Methods 2020

scRNAseq requires combination of wet and dry lab expertise

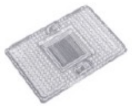
Cell capture and Barcoding

Library Prep

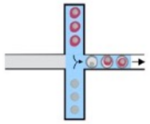
Sequencing

FACS

Micro-capture

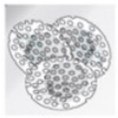


Droplet



Nanowell

Split/pool barcoding



PolyA vs random priming

3′/5′ end tagging vs full-length

PCR vs *in vitro* transcription

…

Miseq

~ 20M reads total

Nextseq

~ 500M reads total

HiSeq 4000

4 billion reads

A few singlecell resources

Comprehensive list of single-cell resources
https://github.com/seandavi/awesome-single-cell https://www.scrna-tools.org/

Computational packages for single-cell analysis
https://satijalab.org/seurat/
https://scanpy.readthedocs.io/
https://bioconductor.org/books/release/OSCA/

eLife Commentary on the Human Cell Atlas (HCA) https://elifesciences.org/articles/27041
Nature Commentary on the HCA https://www.nature.com/news/the-human-cell-atlas-from-vision-to-reality-1.22854

Online courses
https://bioconductor.org/books/release/OSCA/
https://lmweber.org/OSTA-book/
https://uppsala.instructure.com/courses/52011

Single cell data repositories
http://jinglebells.bgu.ac.il/
https://tabula-sapiens-portal.ds.czbiohub.org/
https://www.nxn.se/single-cell-studies/gui
https://tabula-muris.ds.czbiohub.org/
https://data.humancellatlas.org/

Flash talk instructions

Each one of us will give a 1 minute, 1 slide presentation on a question that they are interested in, which could benefit from single-cell RNA-Seq. It can be an area you are currently investigating, something you are broadly interested in and wish to explore in the near future, or an idea for future projects.

The goal of this is for all of us to get to know each other a little more, and for you to see the diversity of research possibilities in this area.

Please prepare a 1 slide pdf for Tuesday, and place the pdf in the shared Google drive.

Include your name in the filename, and please ask for any clarifications.

Any question?