

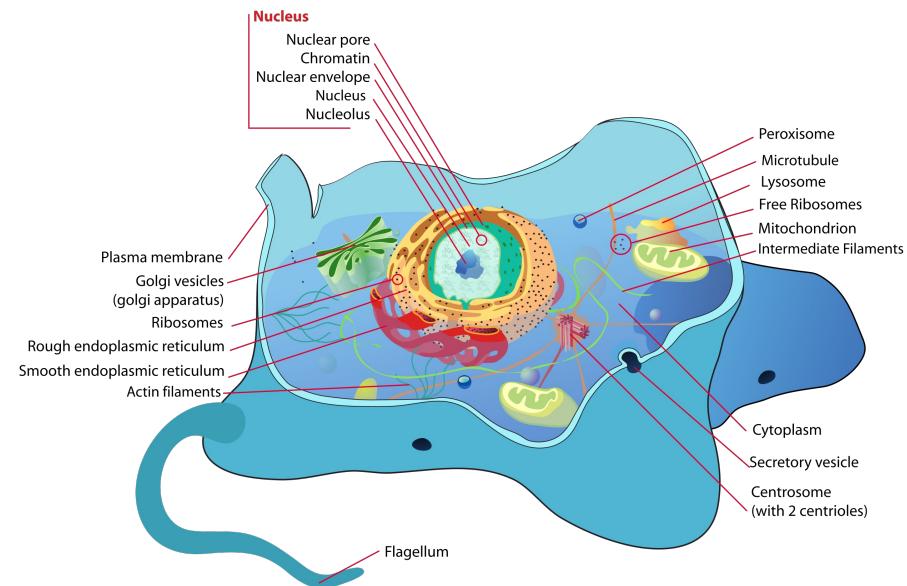
Single cell transcriptomics

Introduction to single cell RNA-seq



Why single cell RNAseq?

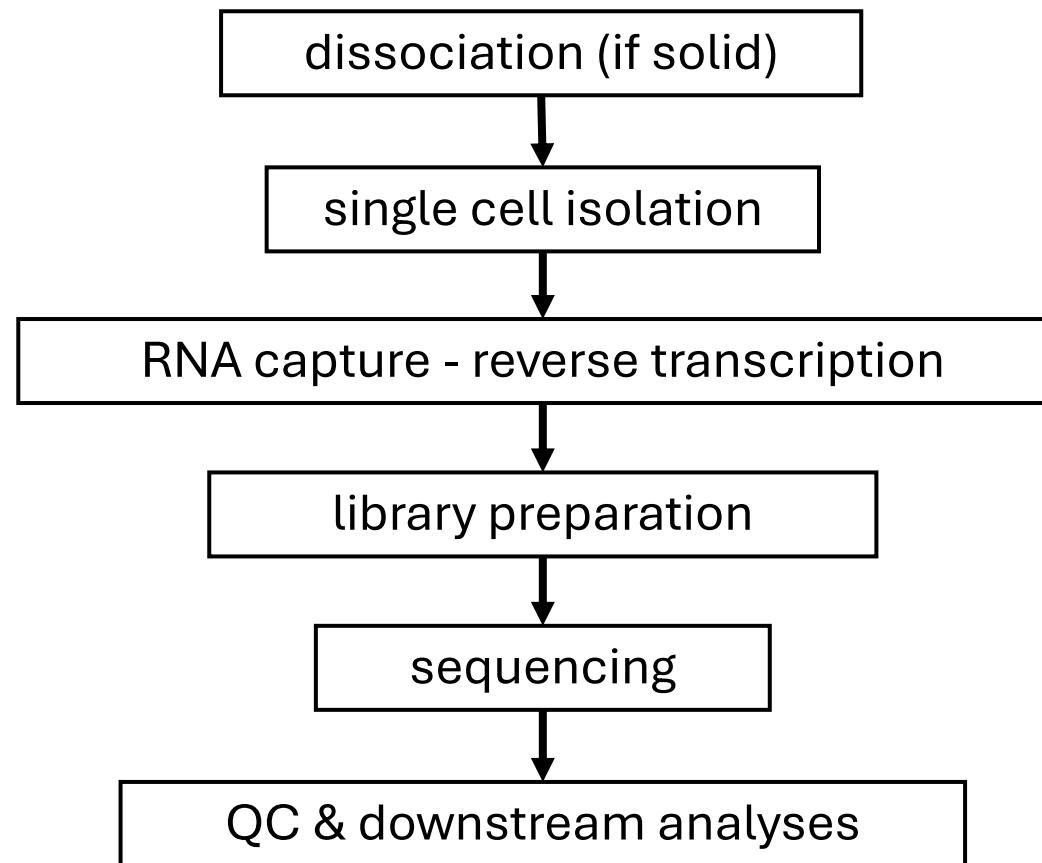
- **Cell:** basic structural and functional unit of life
- Annotation of **cell type** and/or **state**
- Differential gene expression **between** and **within** cell types



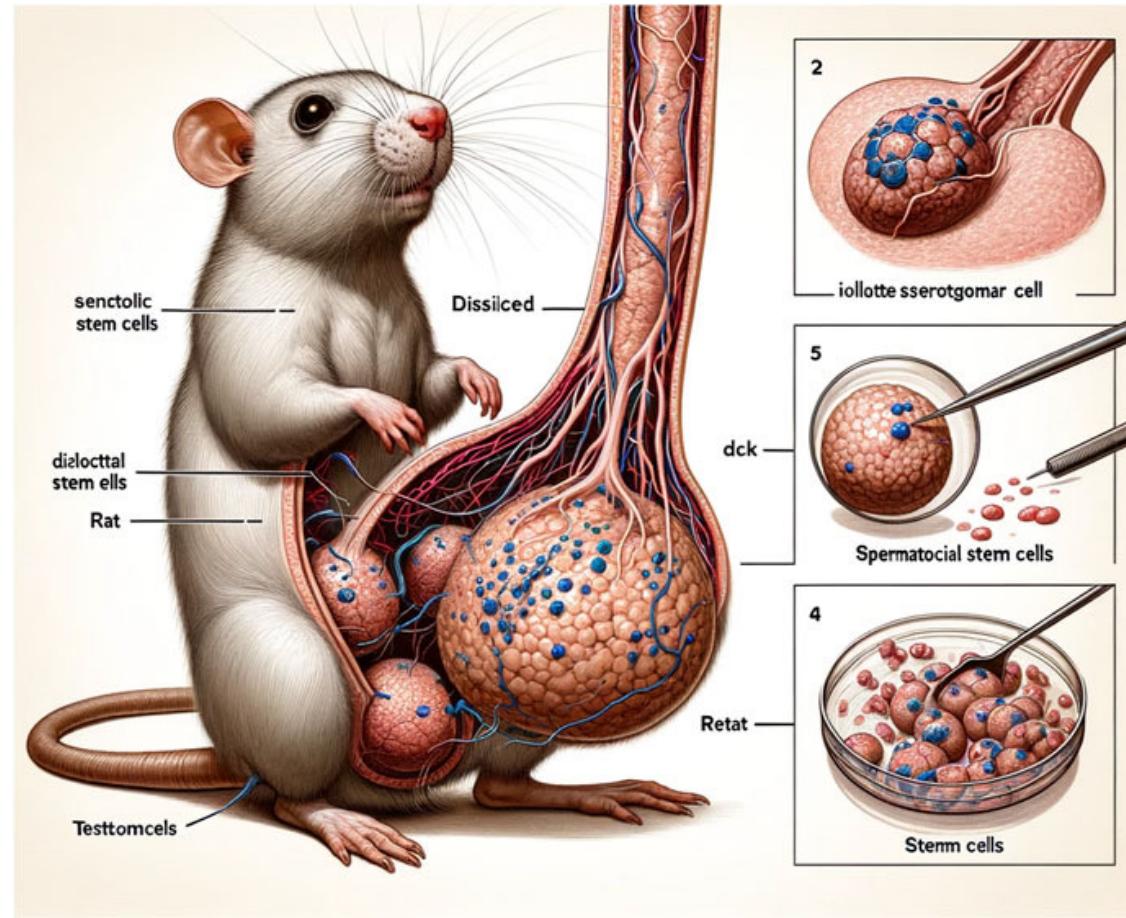
Why single cell RNAseq?



scRNA-seq workflow

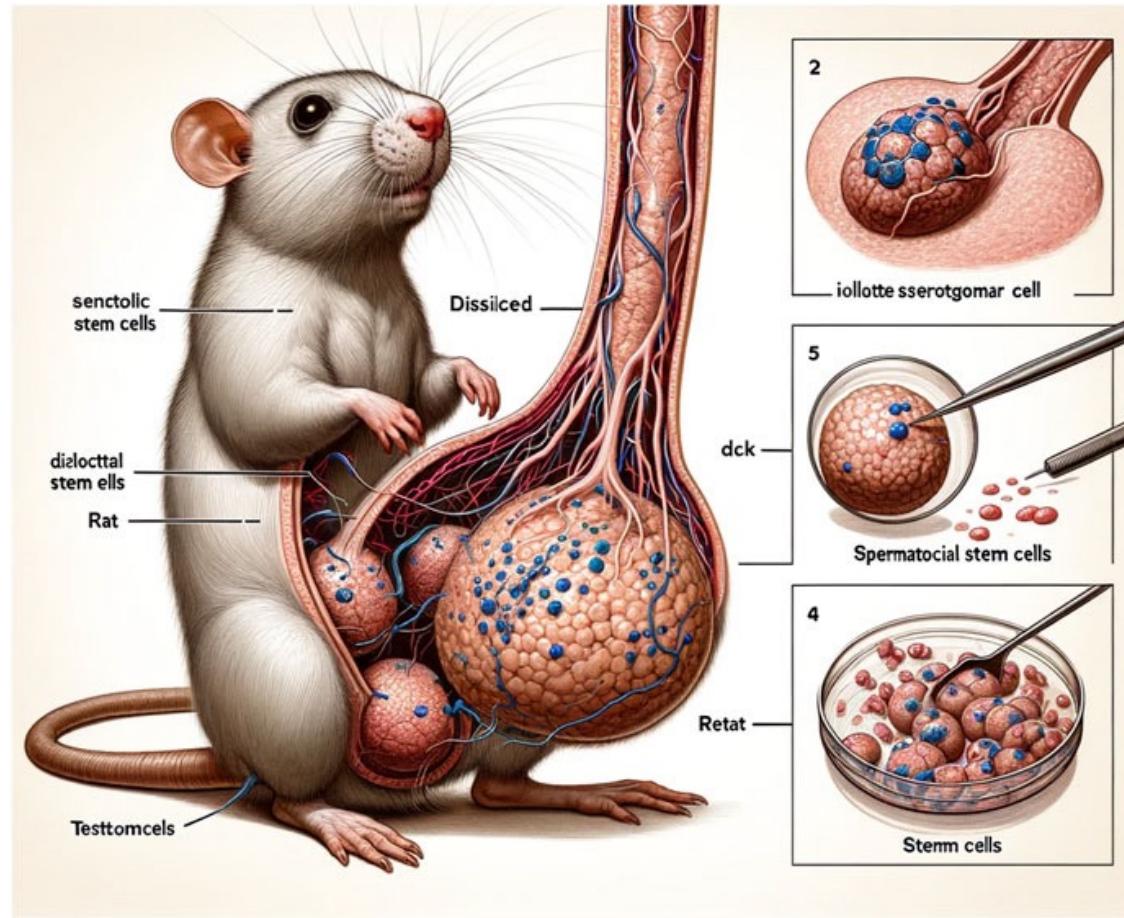


Obtaining cells



Obtaining cells

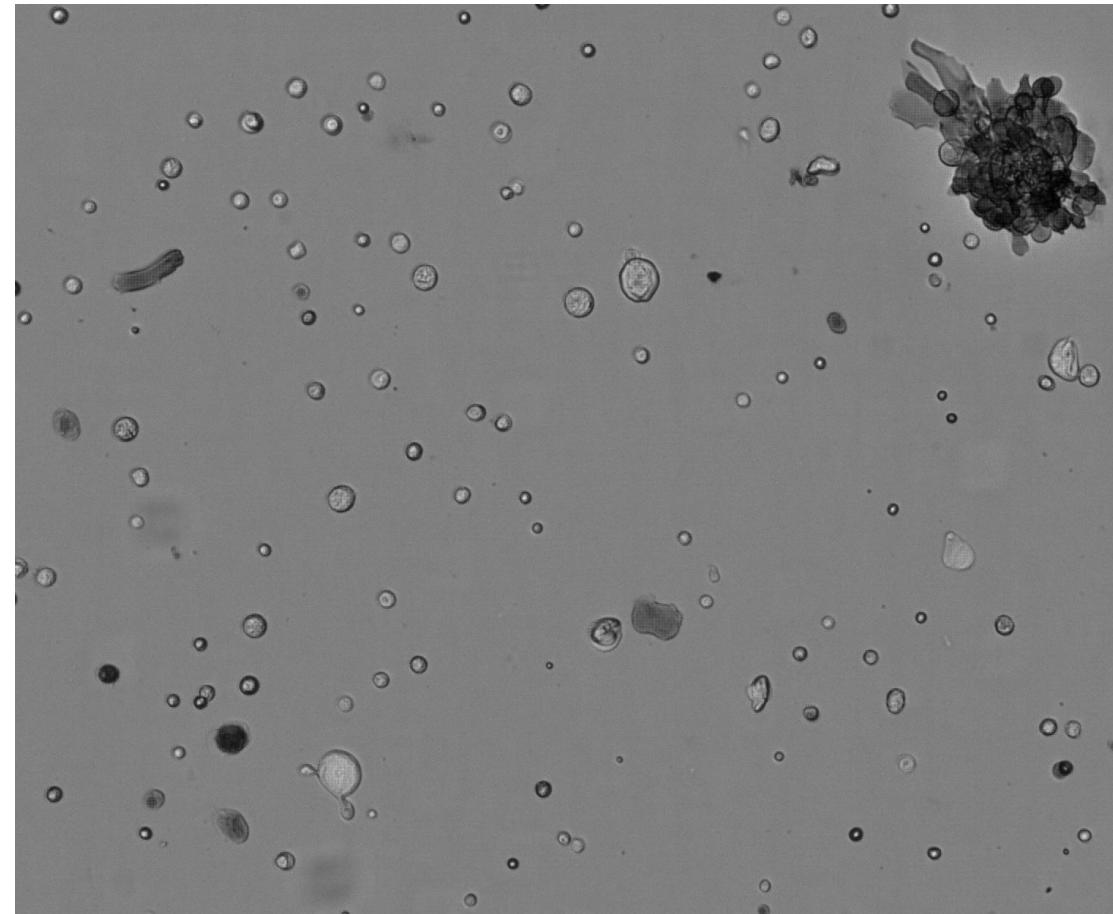
- Yield and quality are dependent on
 - **Tissue:** dissociation ranges from ‘easy’ to ‘impossible’
 - **Lab:** facilities and their proximities
 - **Handling:** gentle handling
- Enrichment of cell types by FACS is possible
- Cell suspensions should be:
 - **Clean:** no clumps
 - **Healthy:** viable
 - **Intact:** no decomposition



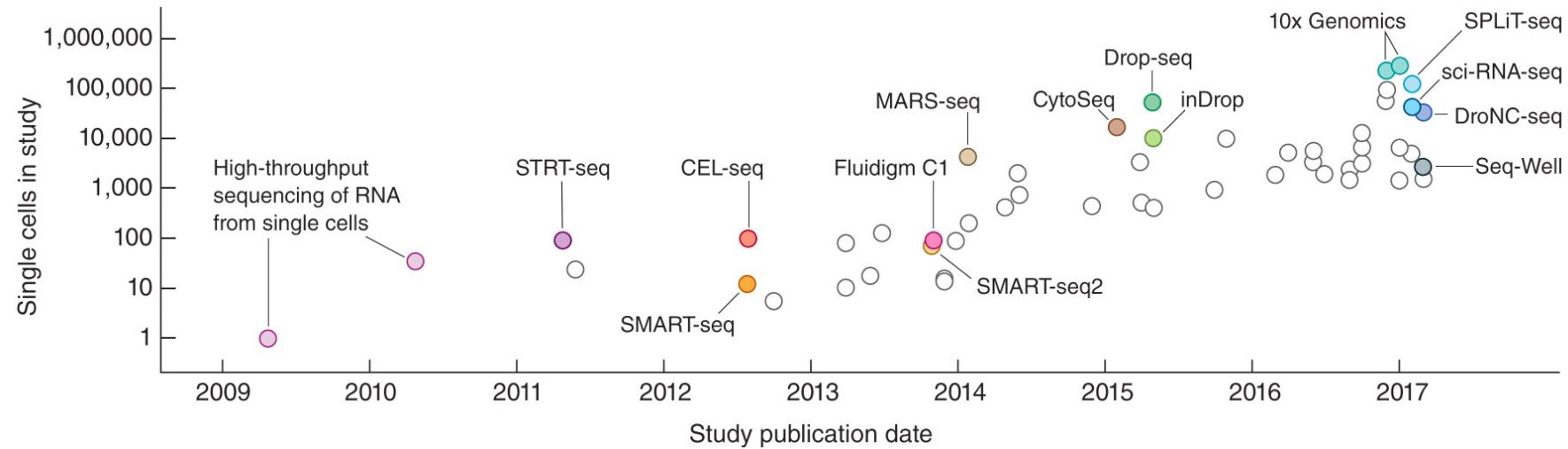
Not like this 😊: <https://doi.org/10.3389/fcell.2023.1339390>

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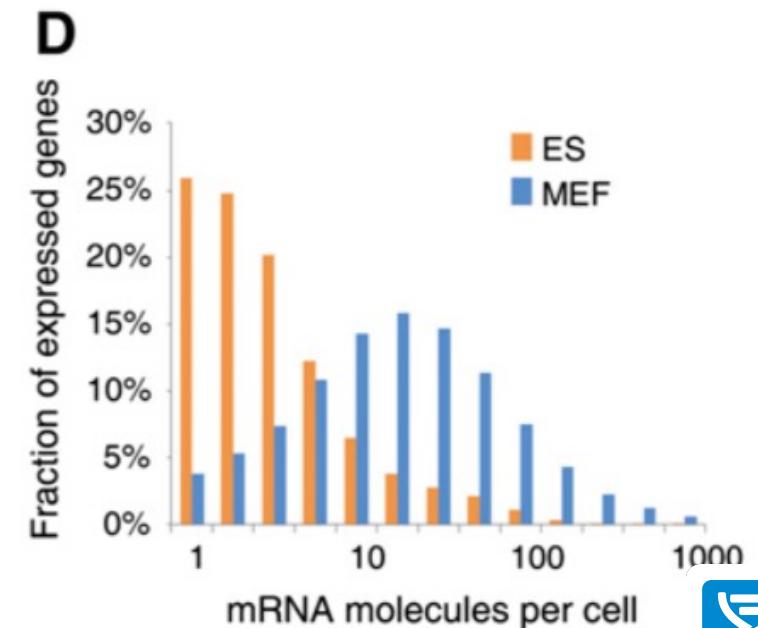
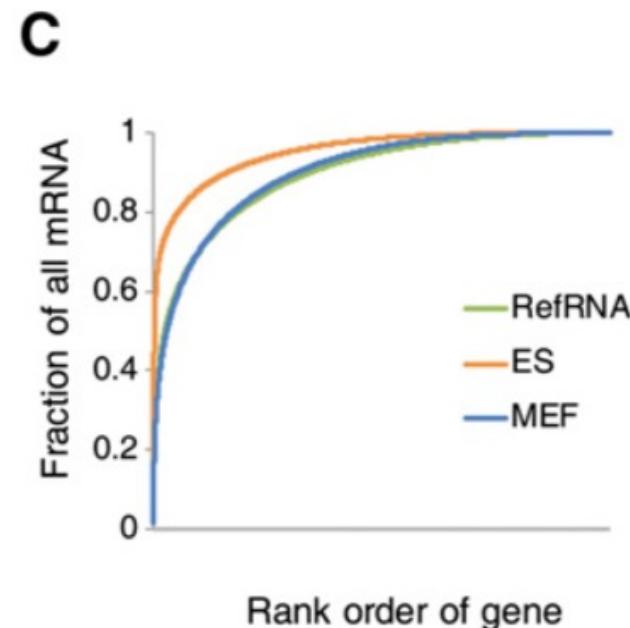
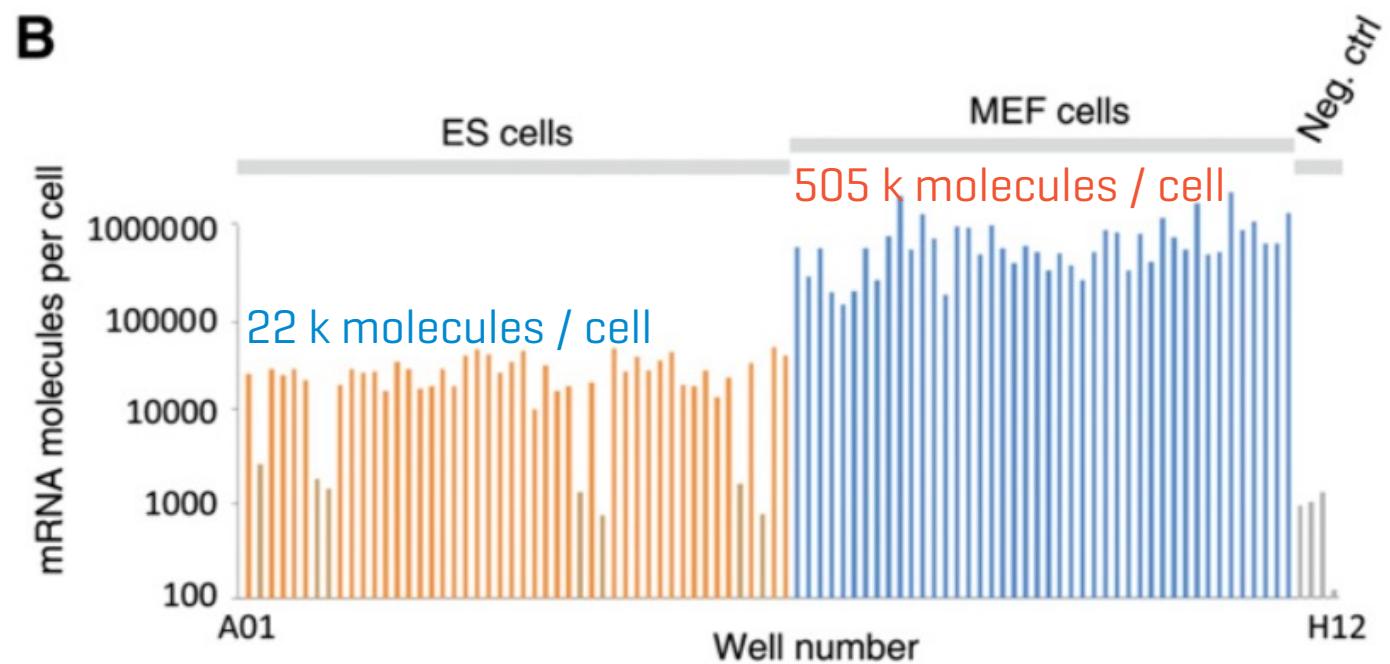


Technologies

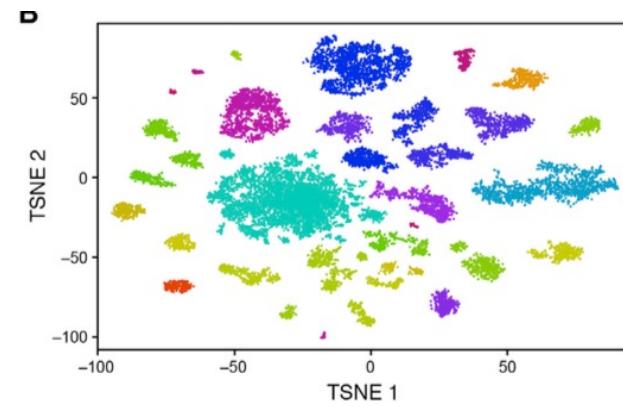
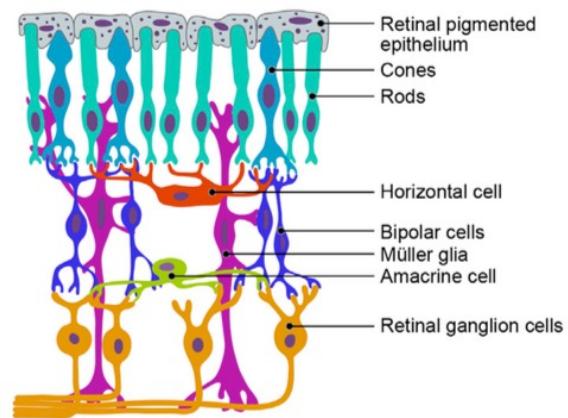


- Plate separation by FACS: e.g. SMART-seq
- Droplet-based: e.g. 10x genomics (3' kit)
- Combinatorial indexing: e.g. SPLiT-seq
- Microwell-based: e.g. BD Rhapsody (CytoSeq)

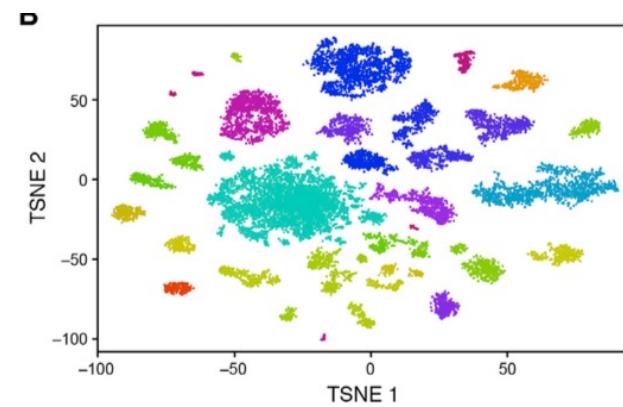
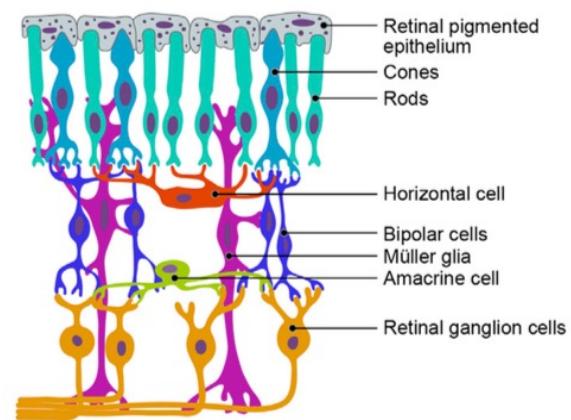
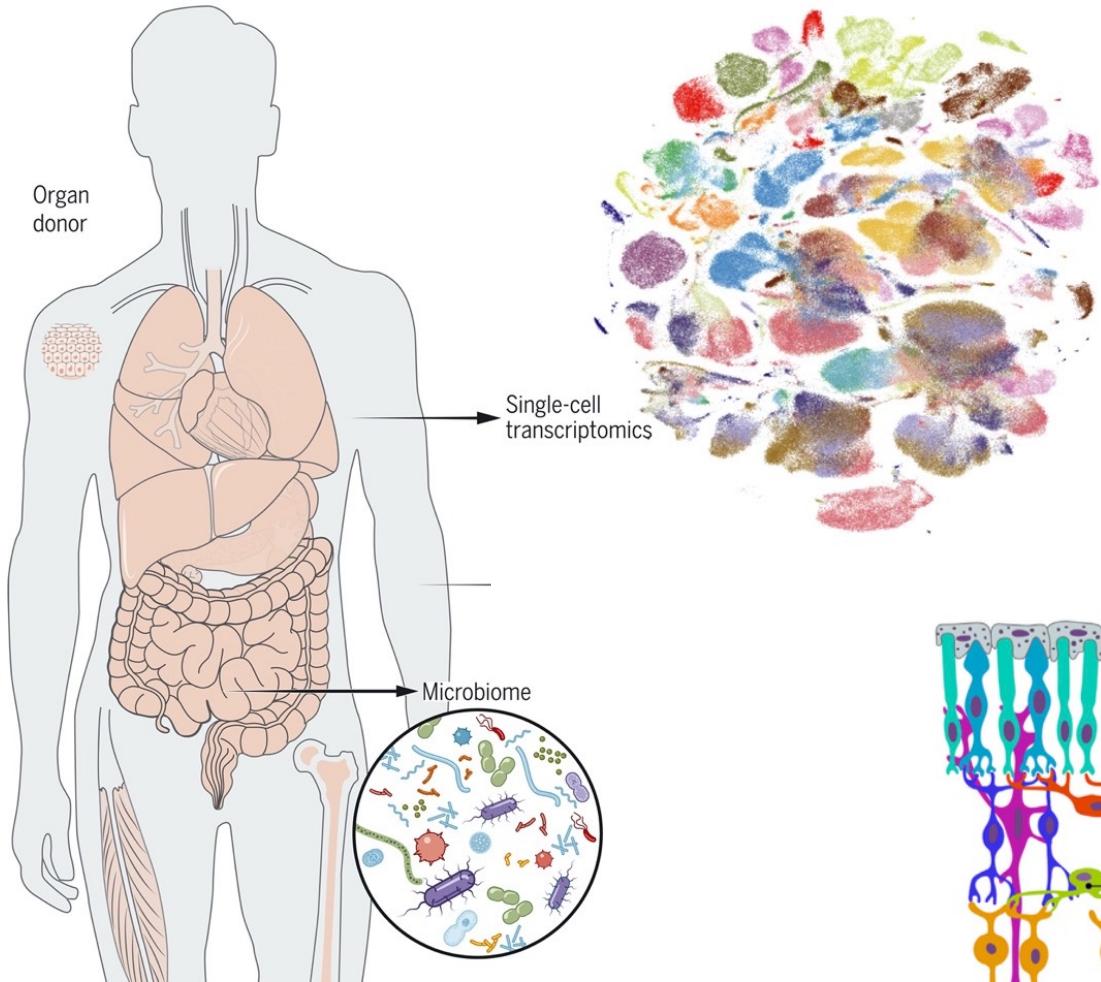
mRNA in cells



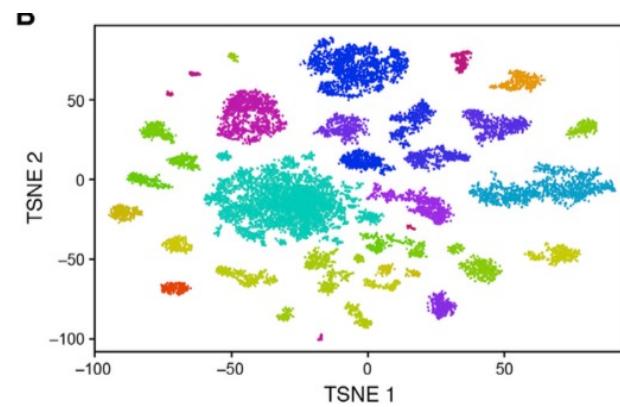
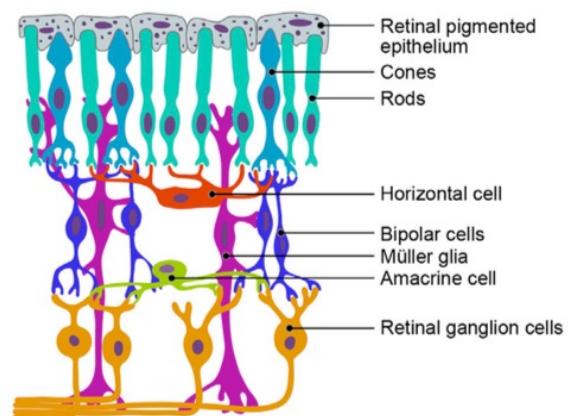
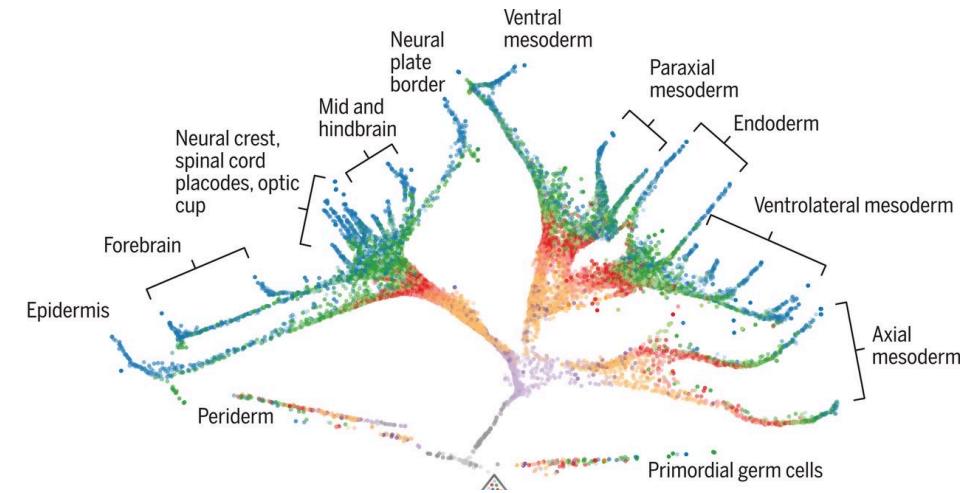
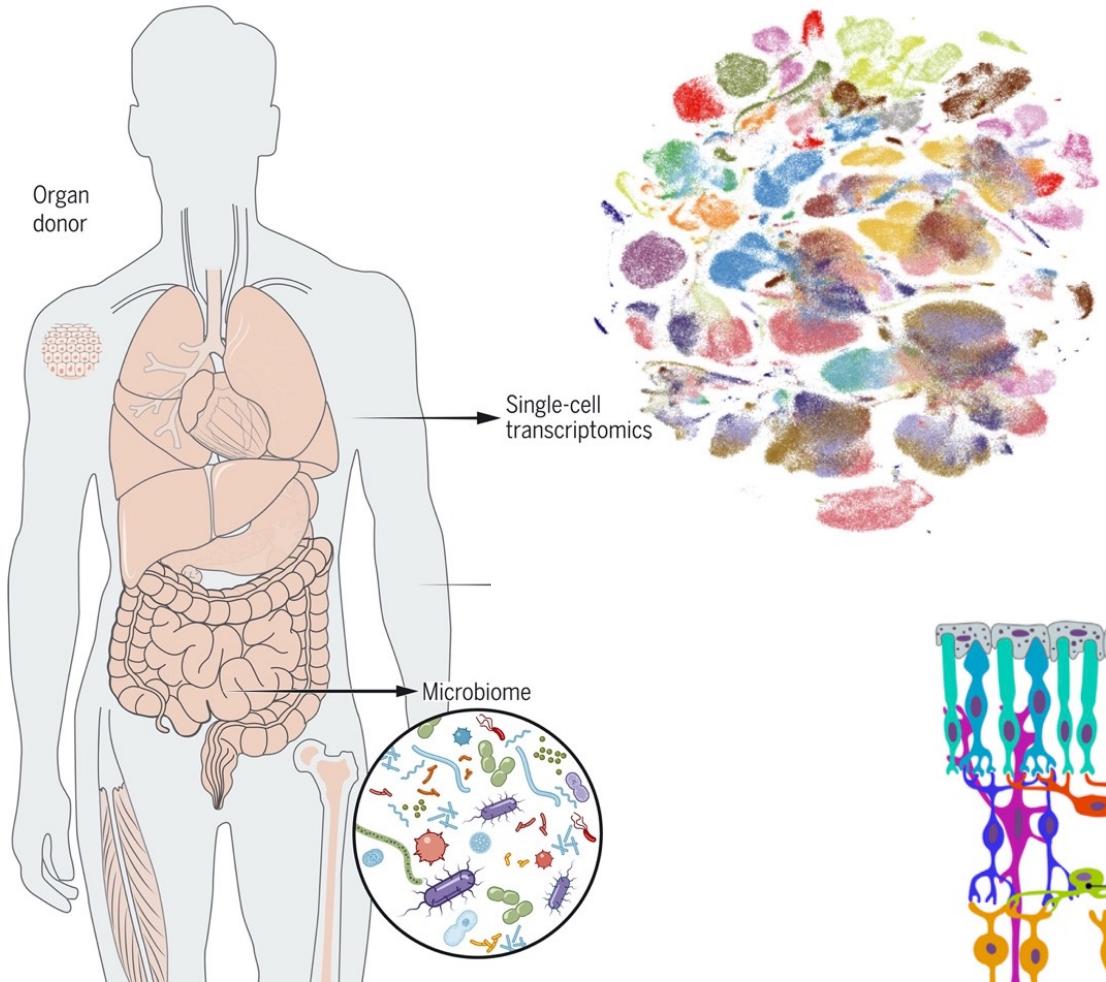
Motivation



Motivation

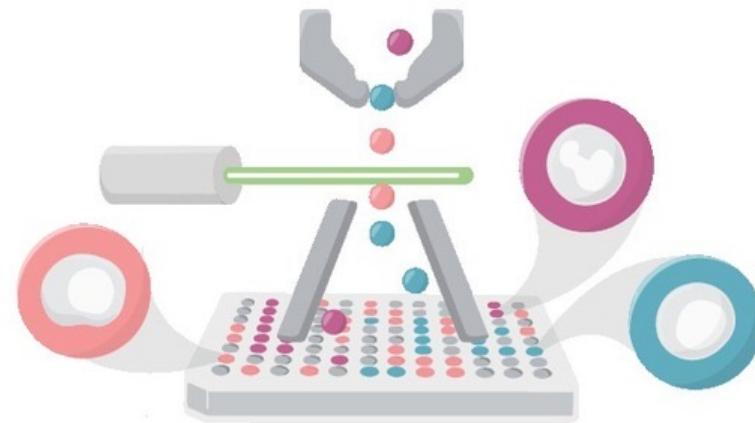


Motivation



SMART-seq

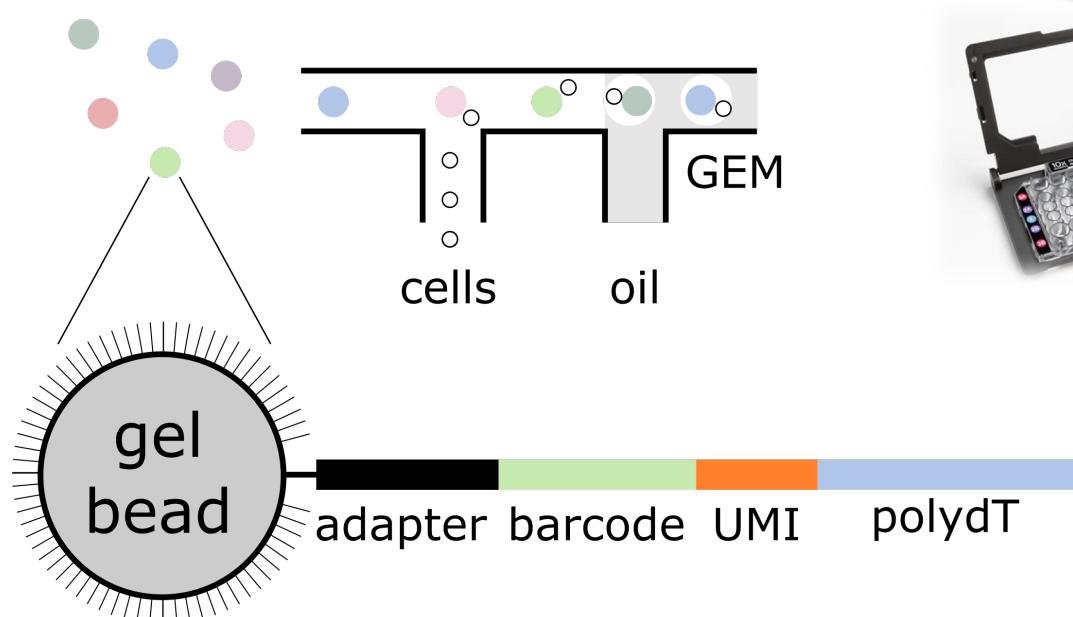
- Use FACS for: 1 well = 1 cell
- Library preparation per cell
- Whole transcript can be sequenced



Hagemann-Jensen M et al. Single-cell RNA counting at allele and isoform resolution using Smart-seq3. *Nat Biotechnol.* 2020;38:708–14.
Image: *Trends in Genetics* DOI: (10.1016/j.tig.2023.10.003)

Droplet based scRNA-seq

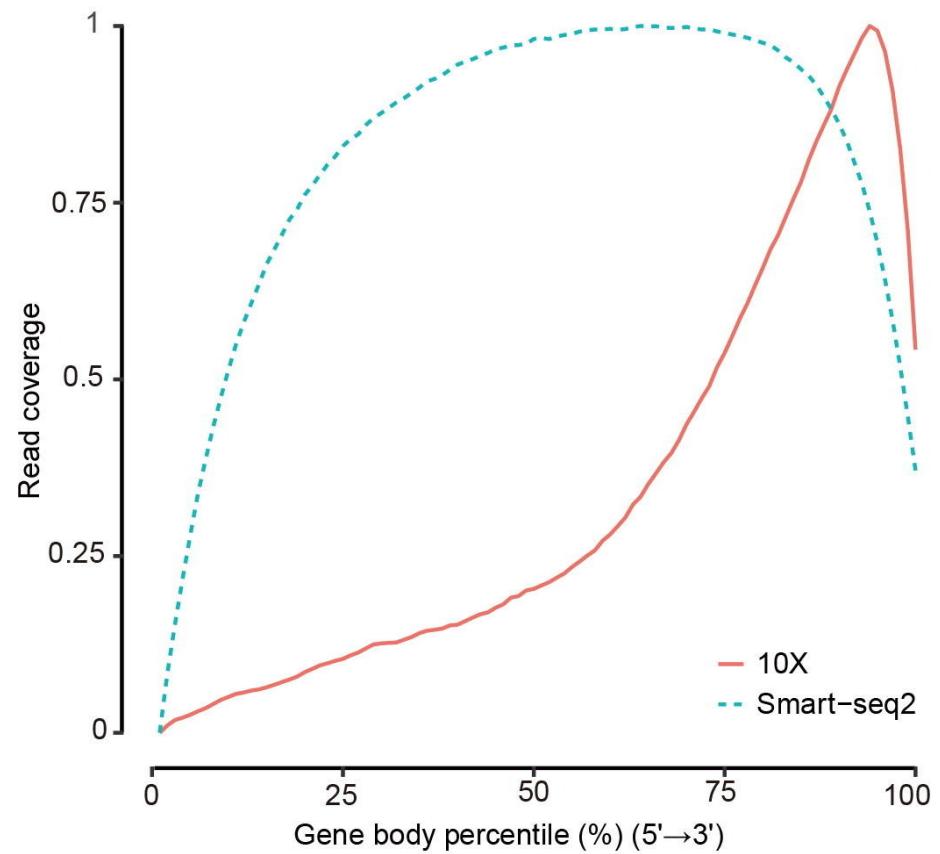
- Cell isolation within GEM (Gel Bead-In EMulsions)
- RNA capture with 1 bead/cell in a droplet
- Sequencing from 3' end
- Only expression



Zheng GXY et al. Massively parallel digital transcriptional profiling of single cells. Nat Commun; 2017;8.

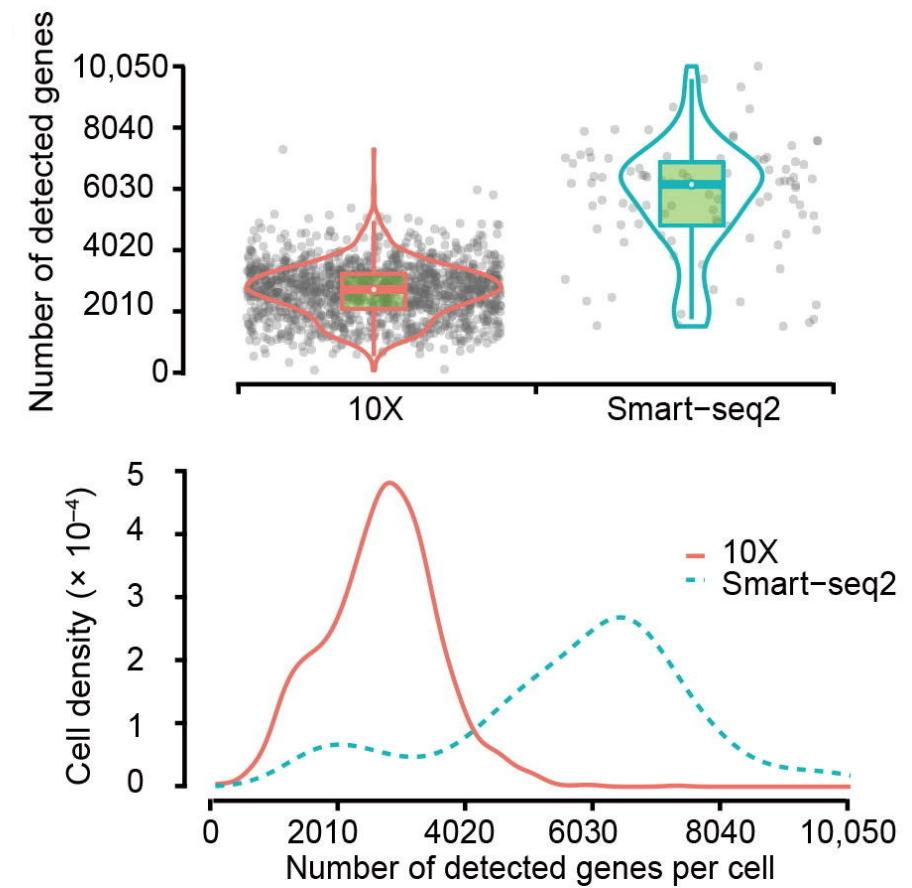
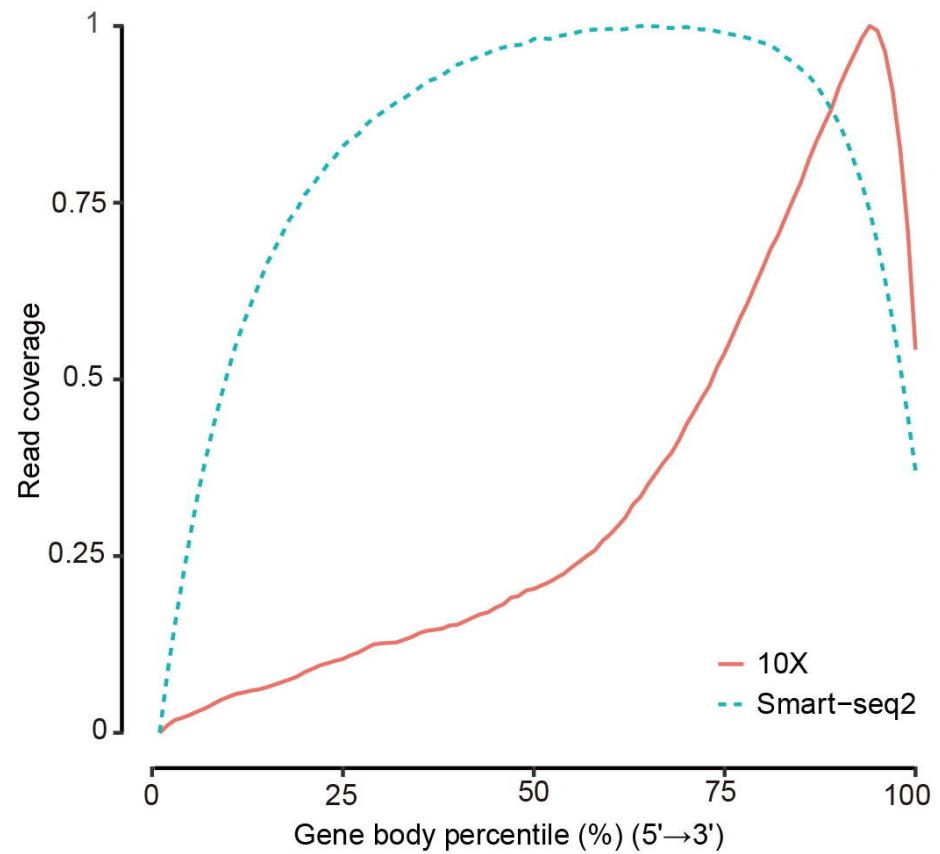
10X
GENOMICS®

IMG



Wang X, et al. Direct Comparative Analyses of 10X Genomics Chromium and Smart-seq2. Genomics Proteomics Bioinformatics; 2021

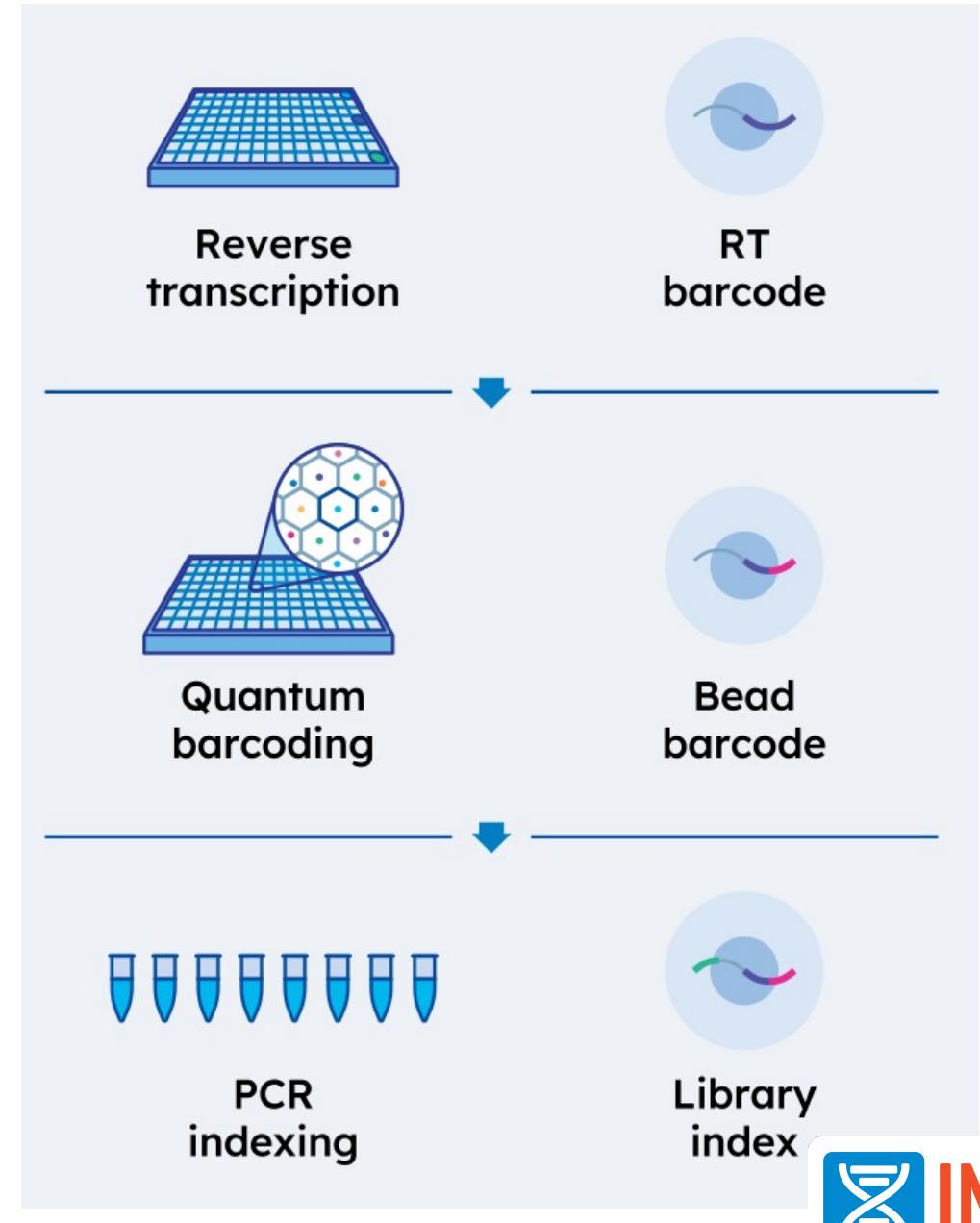




Wang X, et al. Direct Comparative Analyses of 10X Genomics Chromium and Smart-seq2. *Genomics Proteomics Bioinformatics*; 2021

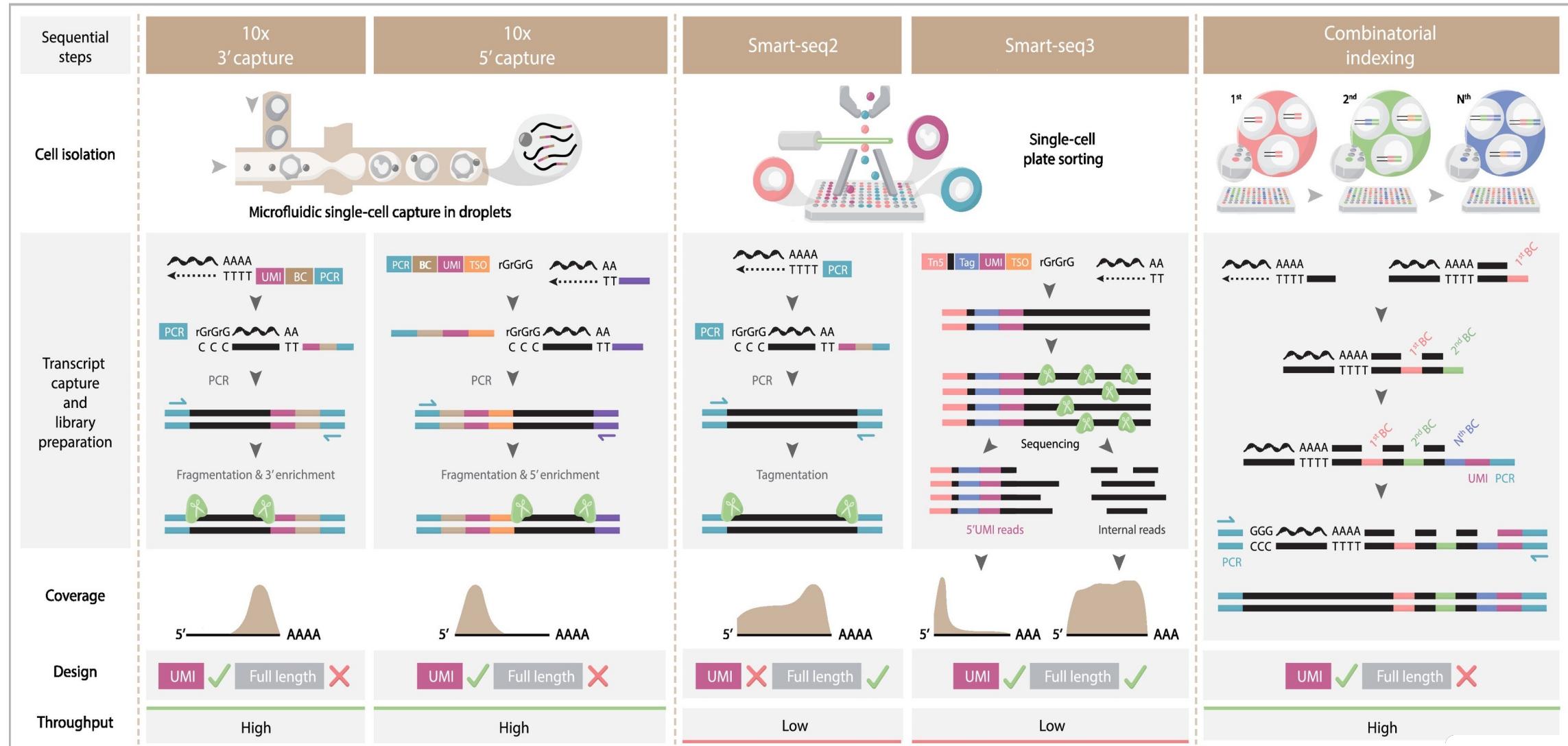
SPLiT-seq

- Based on:
 - formaldehyde fixation
 - in-cell RT + ligation
- several rounds of pooling-splitting and barcoding
- Characteristics:
 - Flexible (fixation + no devices needed)
 - Laborious



Rosenberg AB, et al. Single-cell profiling of the developing mouse brain and spinal cord with split-pool barcoding. *Science*. 2018;360:176–82.

Overview



Quiz Question

Experimental design

- Replication, randomization and blocking

Experimental design

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- Be aware of confounding factors, e.g.:
 - Person performing handling
 - Reagents
 - Sequencing lane/library



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- Different cells of one replicate ≠ replicates!



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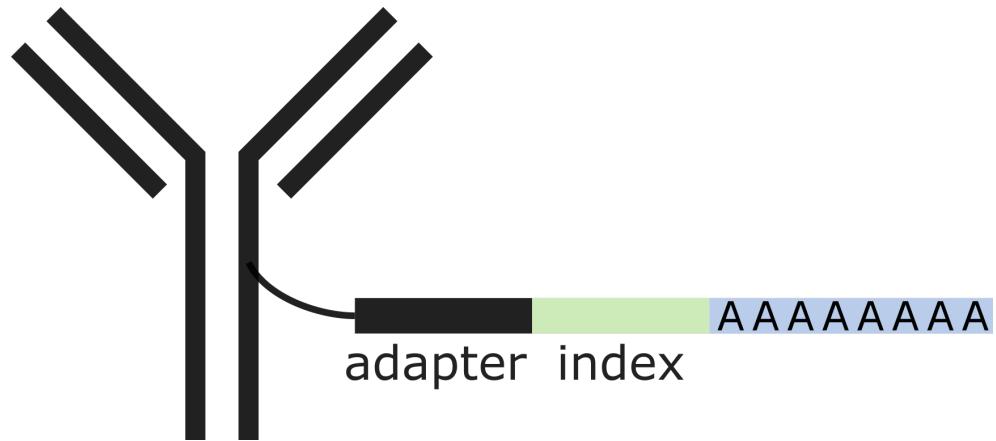


Further reading:

- <https://doi.org/10.3389/fcell.2018.00108>
- <https://doi.org/10.1093/bib/bby007>
- <https://doi.org/10.1093/bfgp/elx035>

Quiz Question

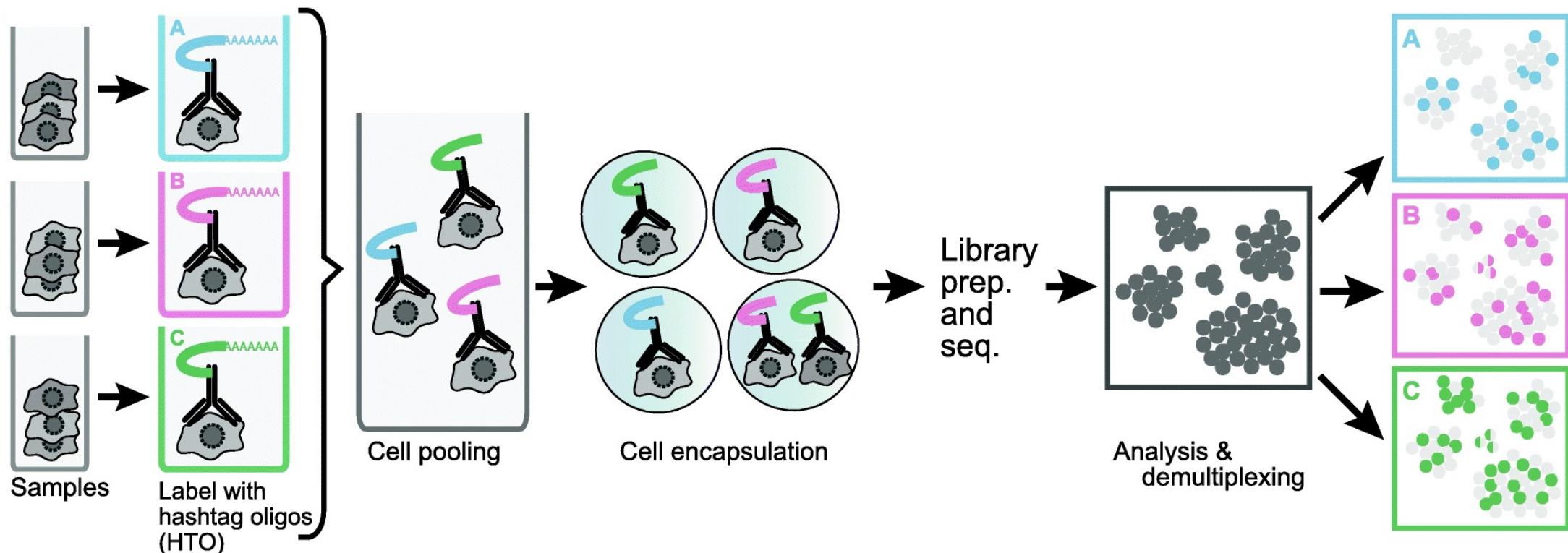
Quantify proteins



- Quantification of (cell-surface) proteins
- Together with transcriptome

Cell hashing

Solution to 1 sample = 1 library



Stoeckius M et al. Cell Hashing with barcoded antibodies enables multiplexing and doublet detection for single cell genomics. *Genome Biol. Genome Biology*; 2018;19:1–12.

Quiz Question

single-nucleus RNA-seq (snRNA-seq)

- Alternative to scRNA-seq
- Required in combination with scATAC-seq
- Also for tissues difficult to dissociate
- No ribosomes -> no translation of transcription factors during processing, less changes of the cell state
- Lower representation of immune cells + surface proteins

Denisenko E, et al. Systematic assessment of tissue dissociation and storage biases in single-cell and single-nucleus RNA-seq workflows. *Genome Biol.*; 2020;21:1–25.



Question time 😊