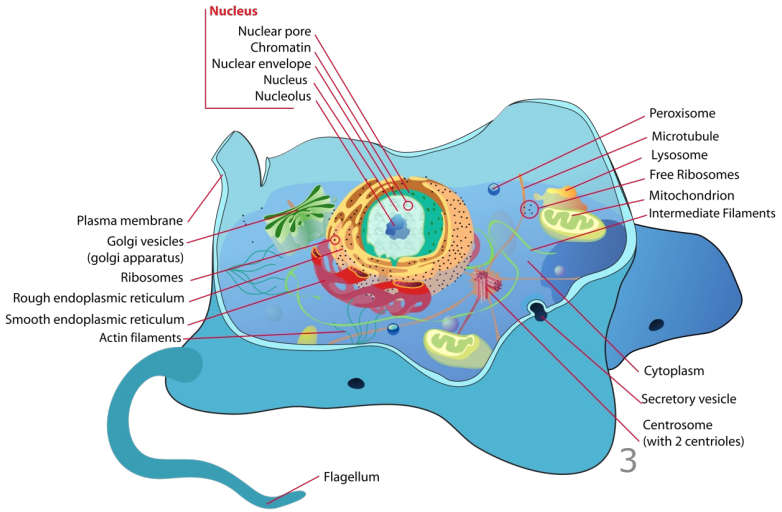
Single cell transcriptomics

Introduction to single cell RNA-seq

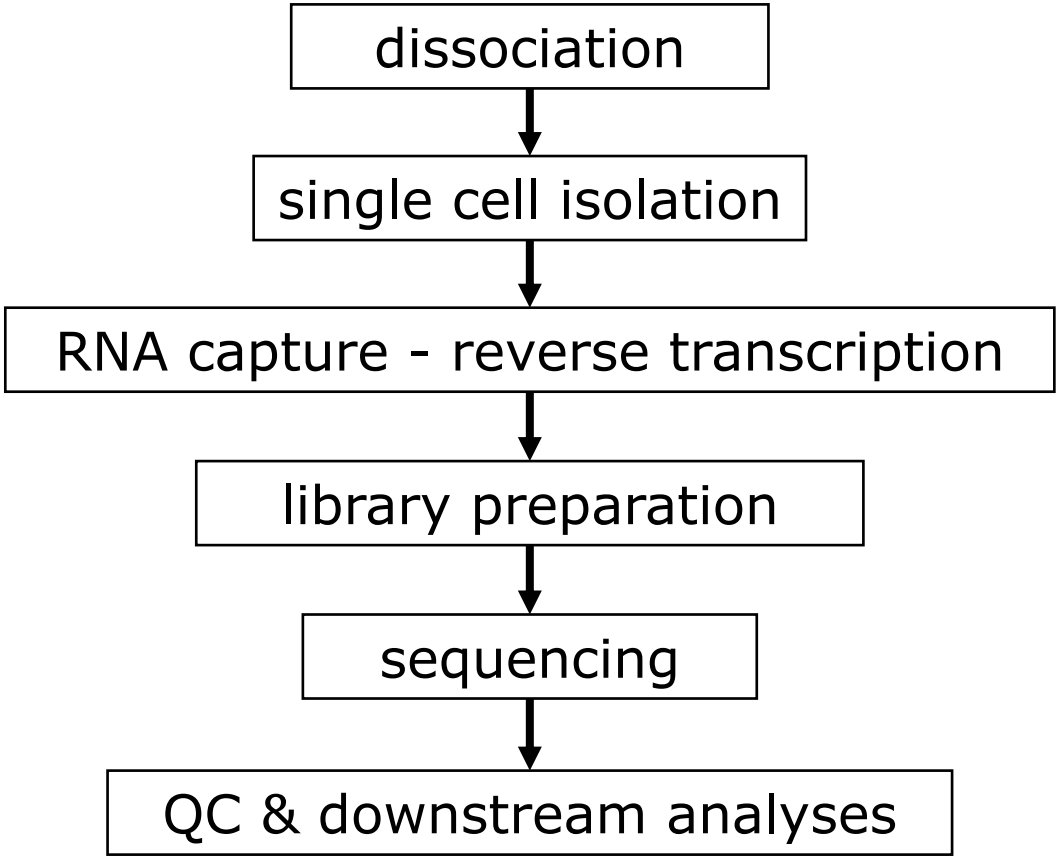
Why single cell RNAseq?

- **Cell**: basic structural and functional unit of life

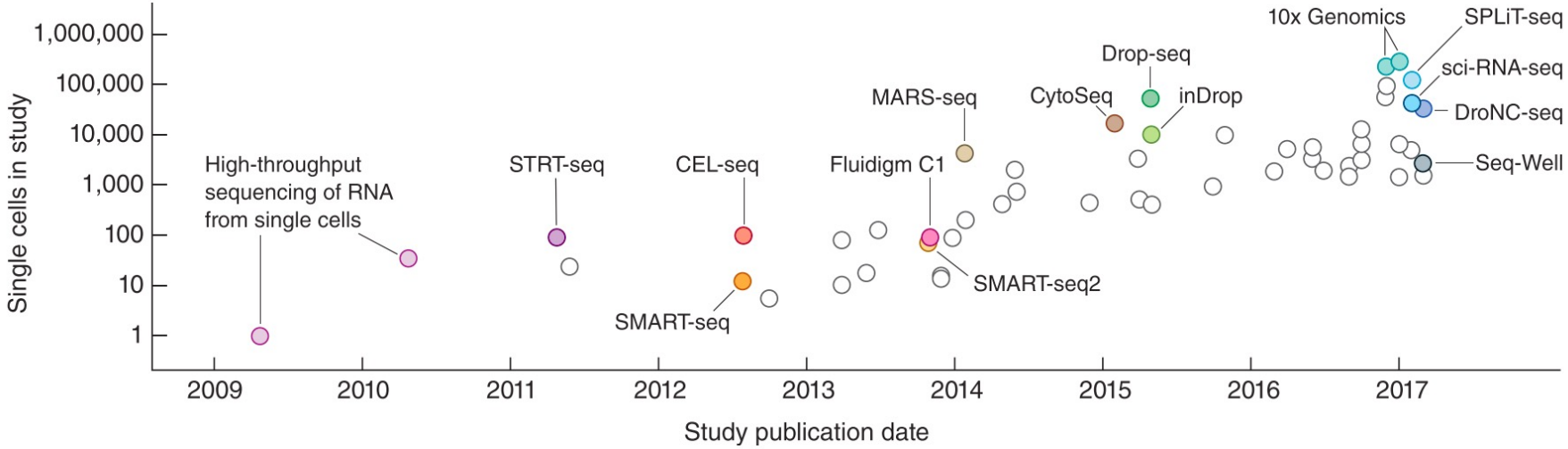
- Annotation of cell type and/or state
- Differential gene expression between and within cell types



scRNA-seq workflow



Technologies

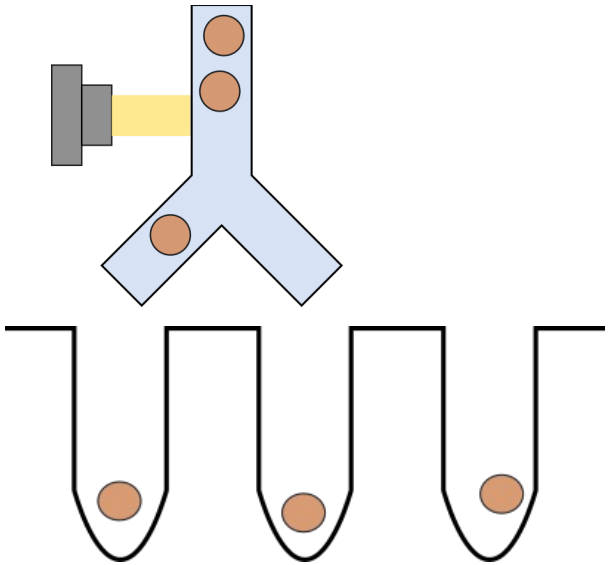


1. Plate separation by FACS: e.g. SMART-seq
2. Droplet-based: e.g. 10x genomics (3’ kit)
3. Combinatorial indexing: e.g. SPLiT-seq
4. Microwell-based: e.g. BD Rhapsody (CytoSeq)

Svensson V et al., Exponential scaling of single-cell RNA-seq in the past decade. Nat Protoc. 2018;13:599–604.

SMART-seq

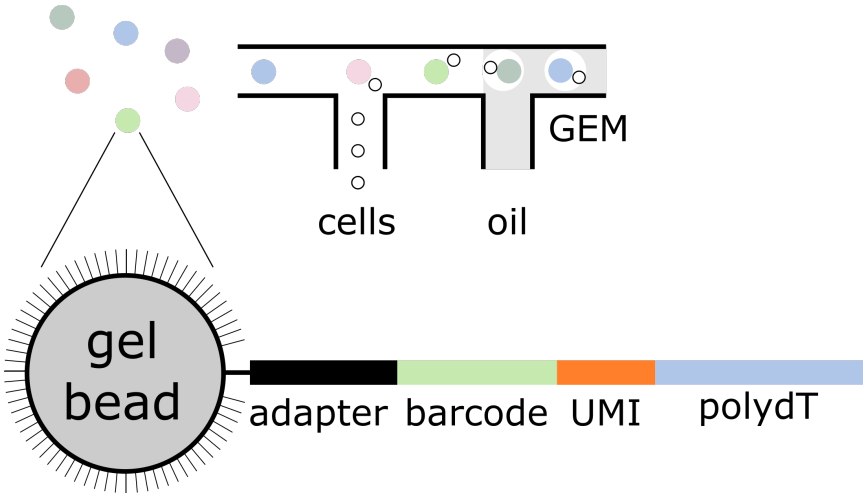
- Use FACS for: 1 well = 1 cell
- Library preparation per cell
- Whole gene can be sequenced



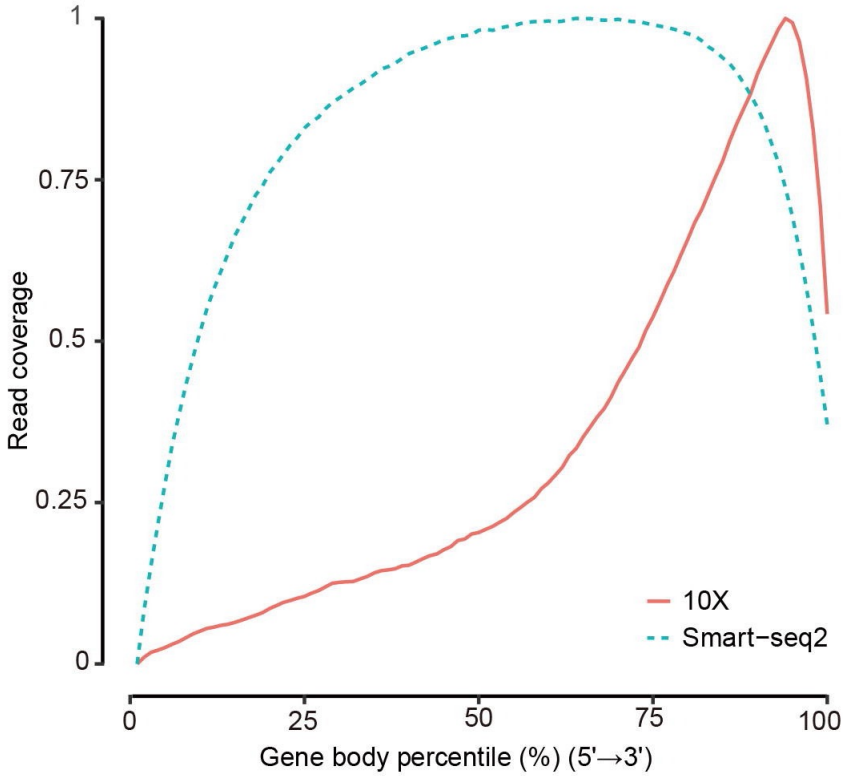
Hagemann-Jensen M et al. Single-cell RNA counting at allele and isoform resolution using Smart-seq3. Nat Biotechnol. 2020;38:708–14.

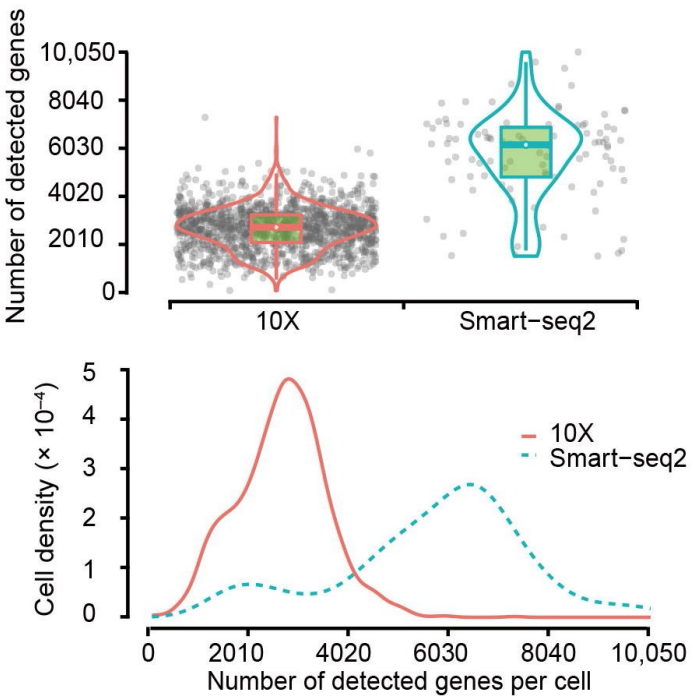
10x genomics (3’ kit)

- Cell isolation within oil droplet
- RNA capture with 1 bead/cell
- Sequencing from 3’ end
- Only expression



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



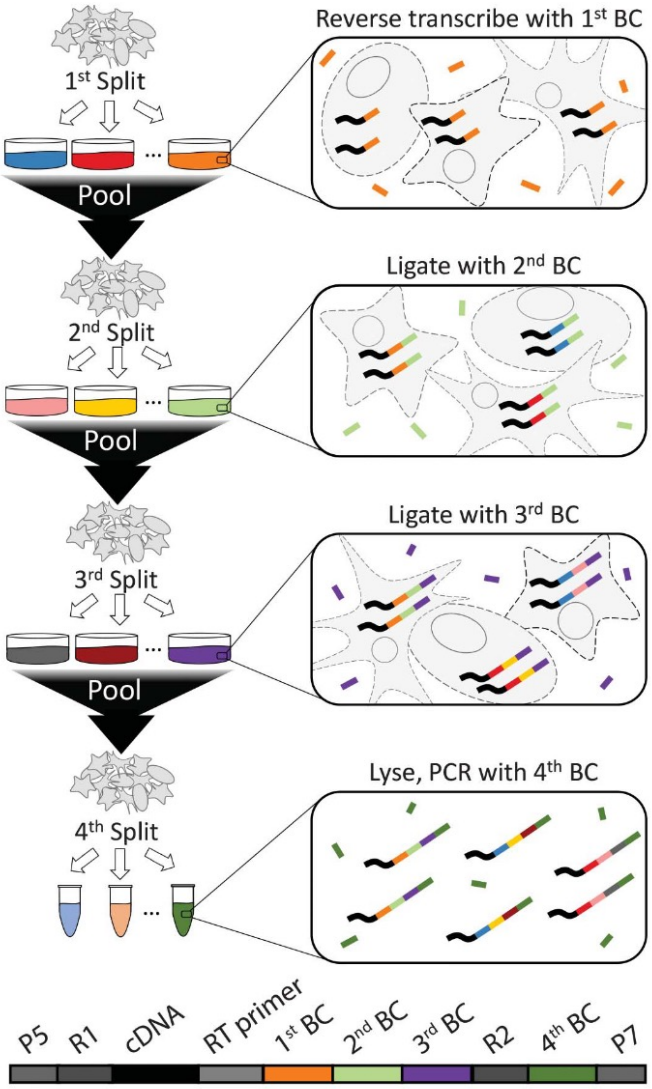


Wang X, et al. Direct Comparative Analyses of 10X Genomics Chromium and Smart-seq2. Genomics Proteomics Bioinformatics; 2021

Droplet (10x genomics)	**SMART-seq**
3’ poly-A bead capture	polydT reverse transcription
Strong bias 3’ end	Whole transcript coverage
Expression analysis	Expression + isoform analysis
Low # transcripts/cell	High # transcripts/cell
Investment (cell sorter)	Only FACS needed
10-100k cells	up to 1k cells
1 sample ≈ 1 library	1 cell = 1 library
Isolation by droplets - doublets	Isolation by FACS – bias to large cells
Low cost/cell	High cost/cell

SPLiT-seq

- Based on:
  - formaldehyde fixation
  - in-cell RT + ligation
- 4 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.

BD rhapsody

- Sparsely loads cells on a microwell array
- 1 cell + 1 bead / microwell -> can be visualized as QC
- Sequences only 3’ end

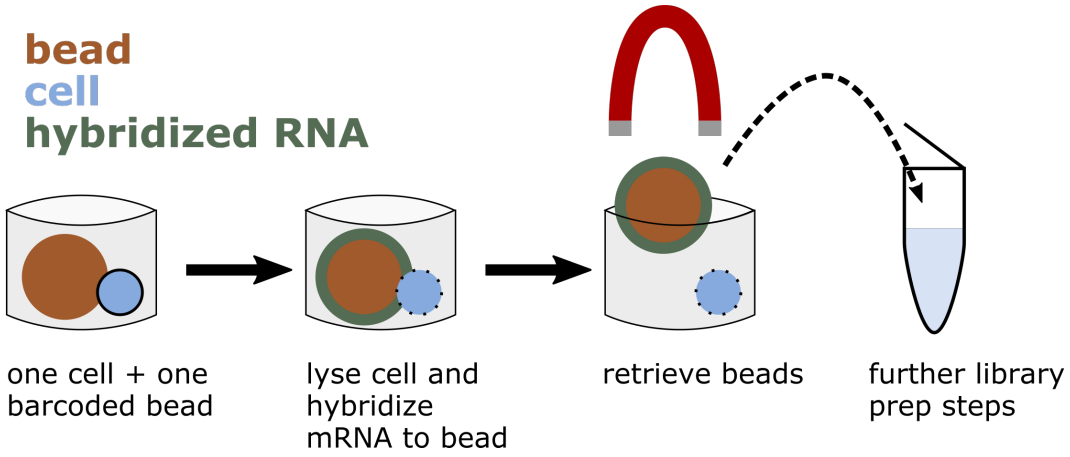


Image: Geert van Geest, CC-BY 4.0, DOI: 10.5281/zenodo.6759810

Fan HC, Fu GK, Fodor SPA. Combinatorial labeling of single cells for gene expression cytometry. Science 2015;347

Quiz Question 2

Experimental design

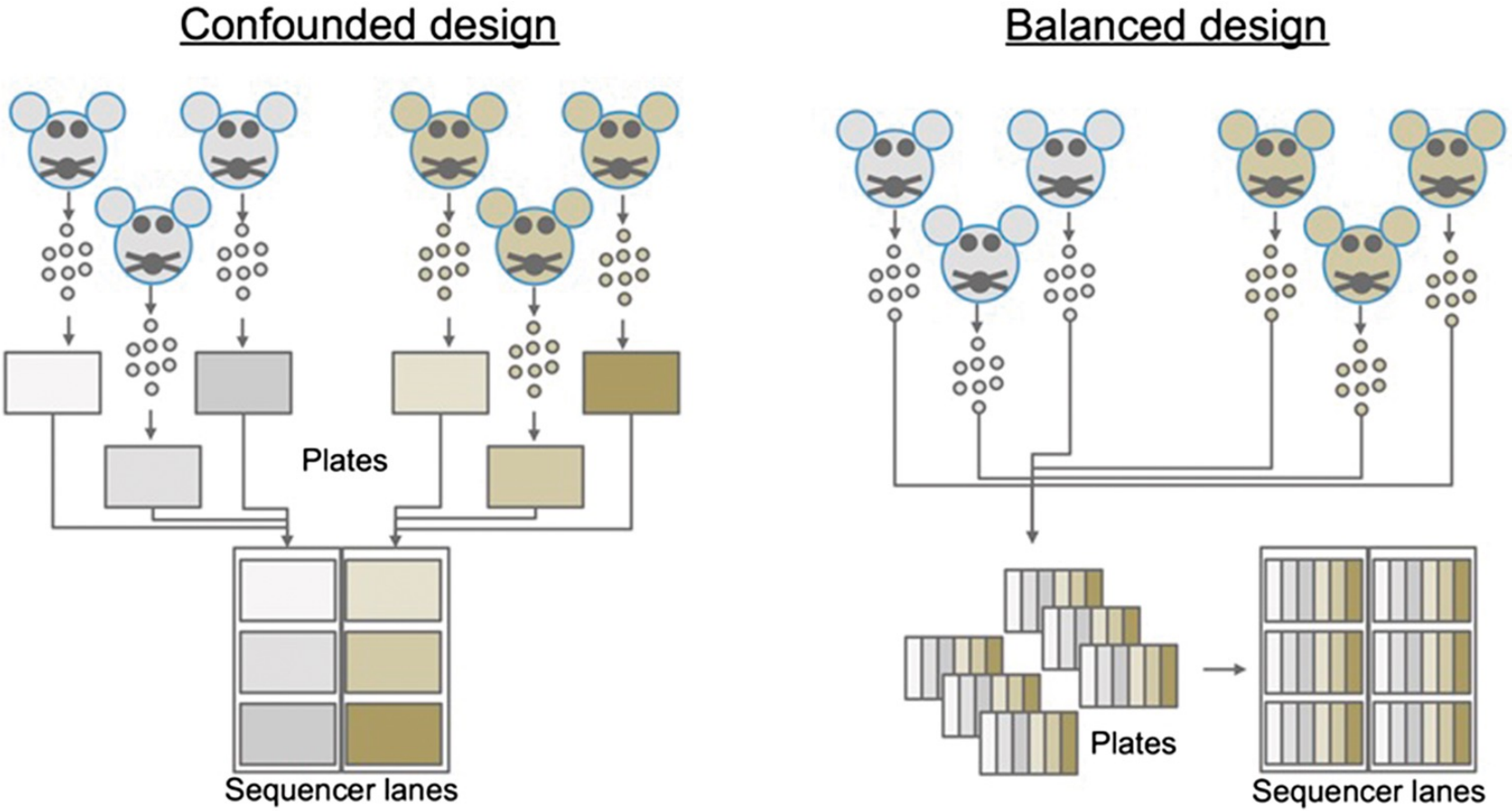
- Replication, randomization and blocking
- Be aware of confounding factors, e.g.:
  - Person performing handling
  - Reagents
  - Sequencing lane/library

- Record any factor for downstream correction

Further reading:

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

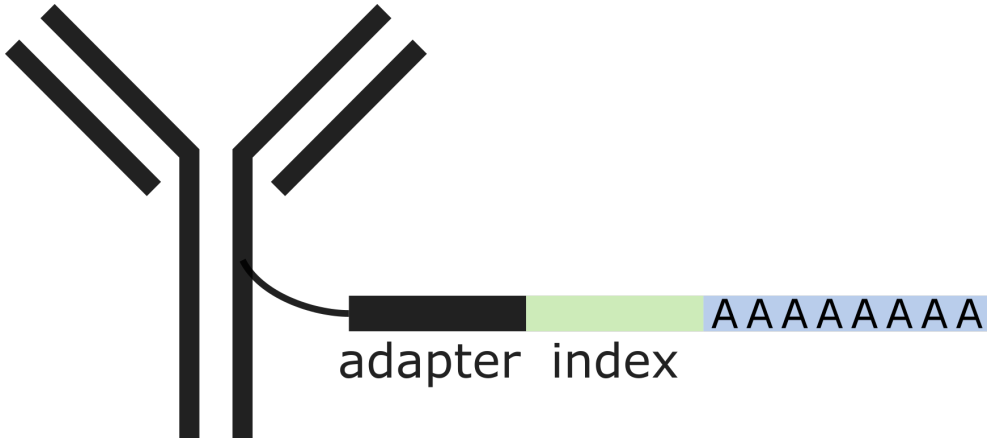
Experimental design



Baran-Gale J, et al. Experimental design for single-cell RNA sequencing. Brief Funct Genomics. 2018;17:233–9.

Question 3

Quantify proteins

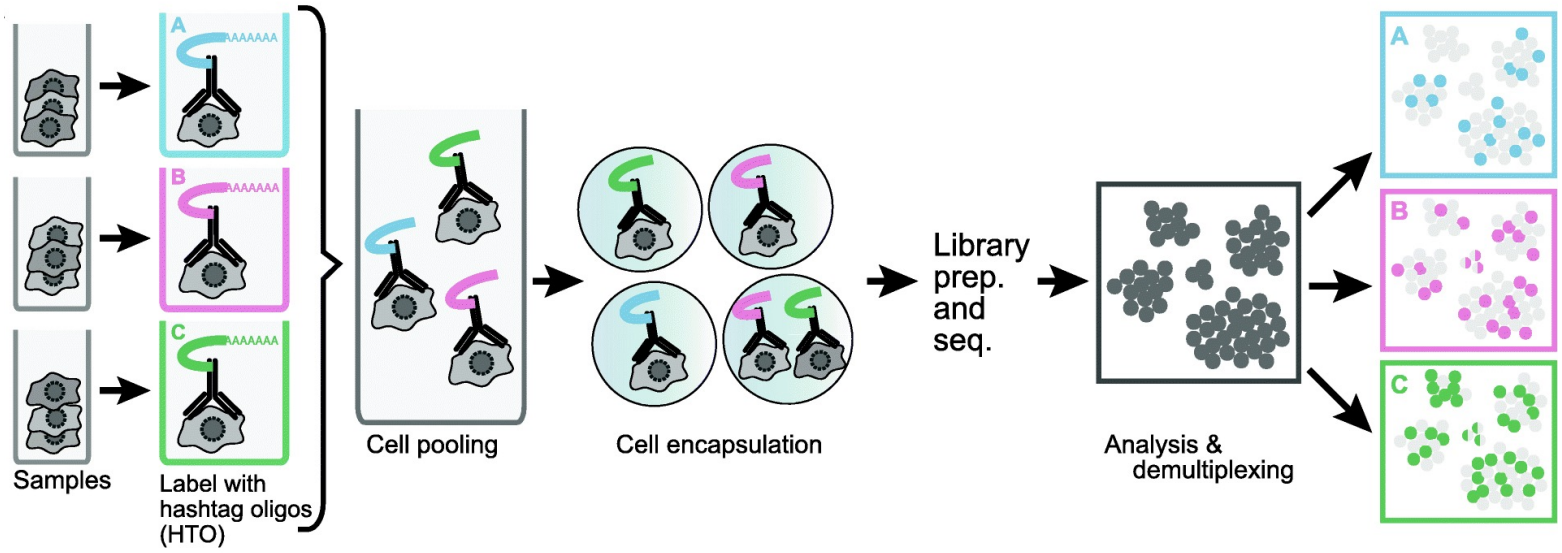


- Quantification of (cell-surface) proteins
- Together with transcriptome

Stoeckius M et al. Simultaneous epitope and transcriptome measurement in single cells. Nat Methods. 2017;14:865–8.

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.

Question 4

single-nucleus RNA-seq

- Alternative to scRNA-seq
- For tissues difficult to dissociate
- No ribosomes -> no translation of transcription factors during processing
- 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.