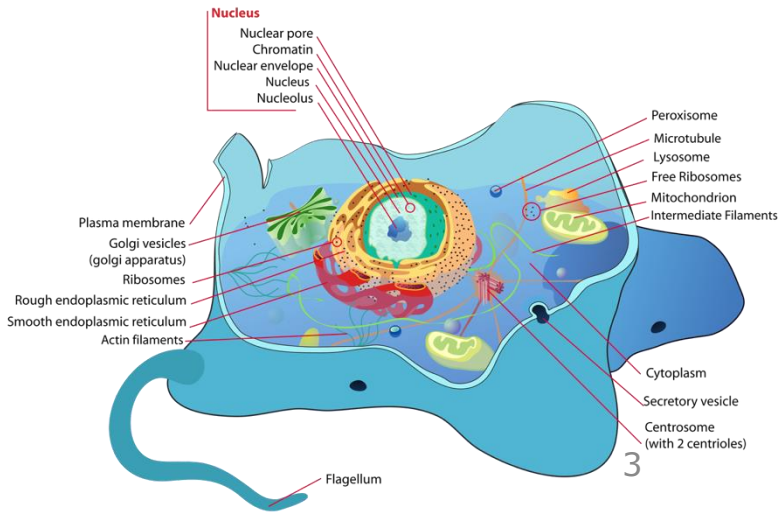
Single cell transcriptomics

Introduction to single cell RNA-seq

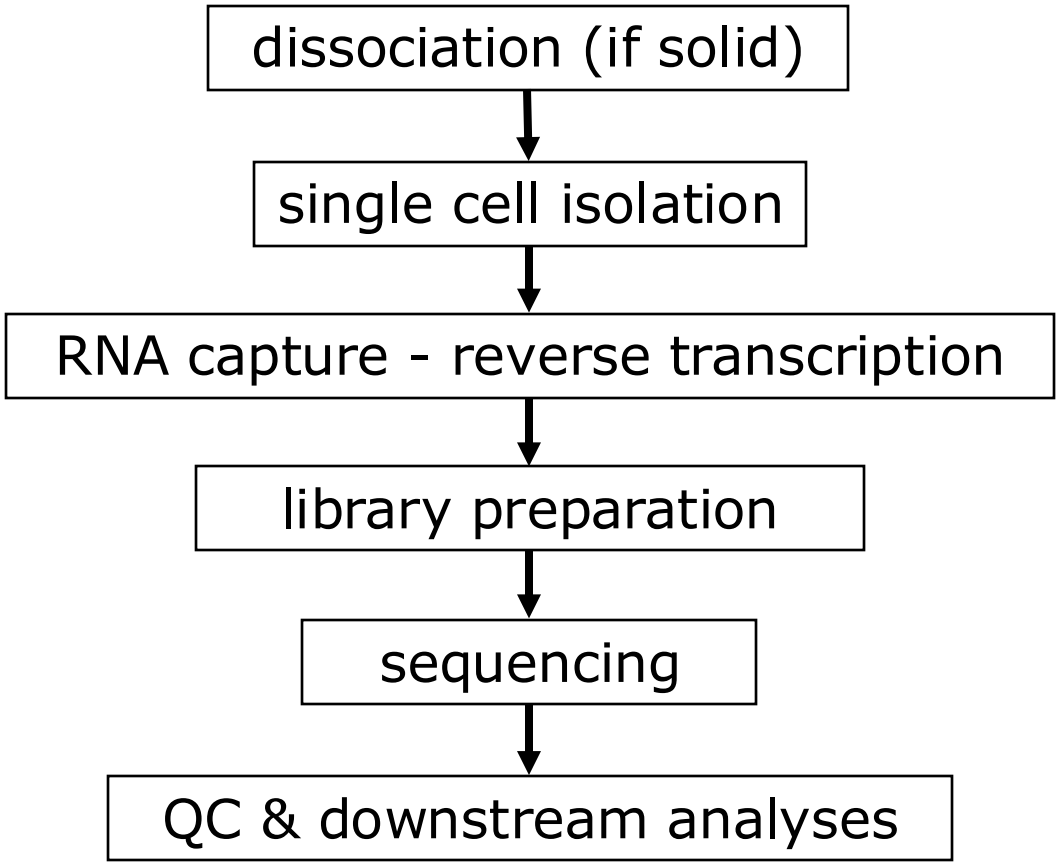
Why single cell RNAseq?

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

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



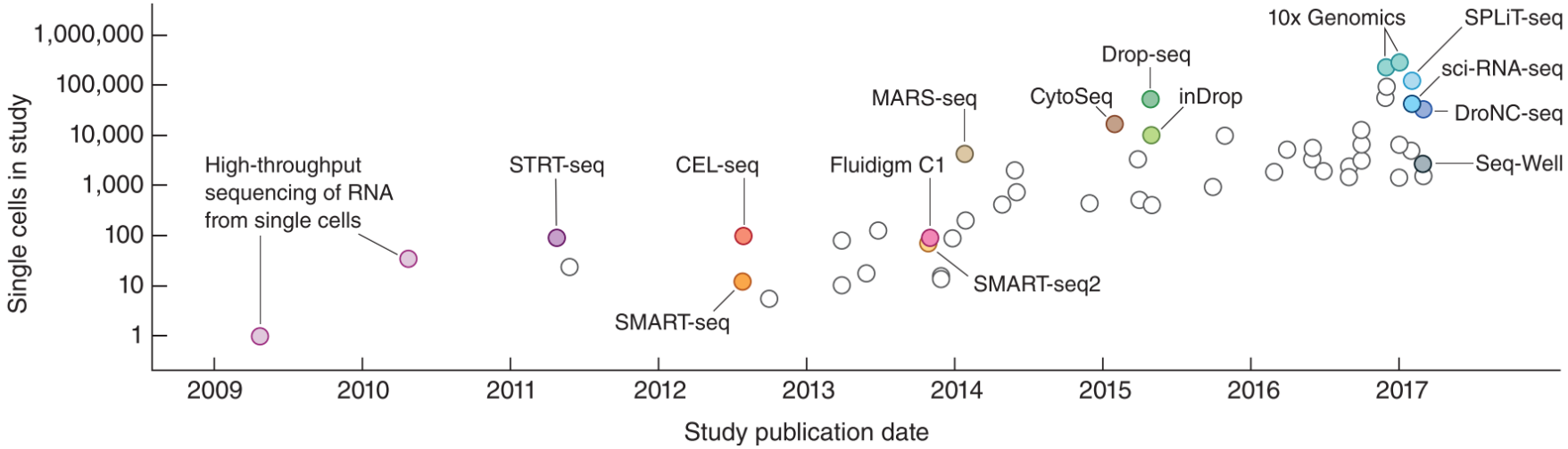
scRNA-seq workflow



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

Technologies

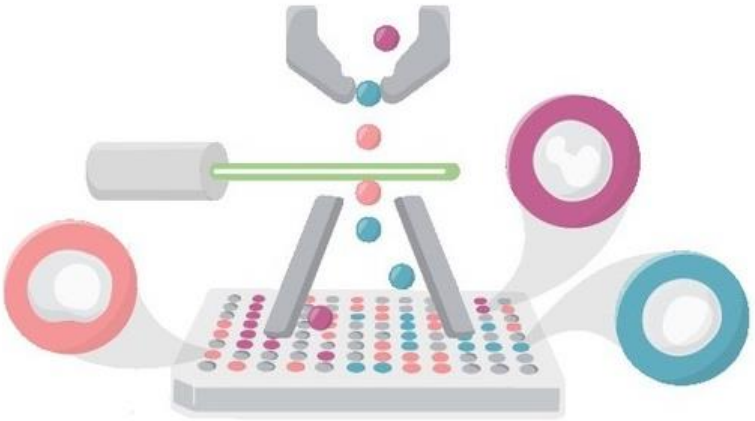


1. Plate separation by FACS: e.g. SMART-seq
2. Droplet-based: e.g. 10x genomics (3’ kit)
3. Combinatorial indexing: e.g. SPLiT-seq
4. Microwell-based: e.g. BD Rhapsody (CytoSeq)

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

SMART-seq

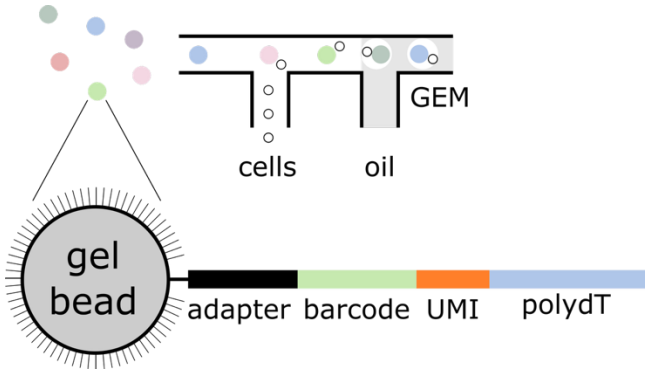
- Use FACS for: 1 well = 1 cell
- Library preparation per cell
- Whole gene can be sequenced



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

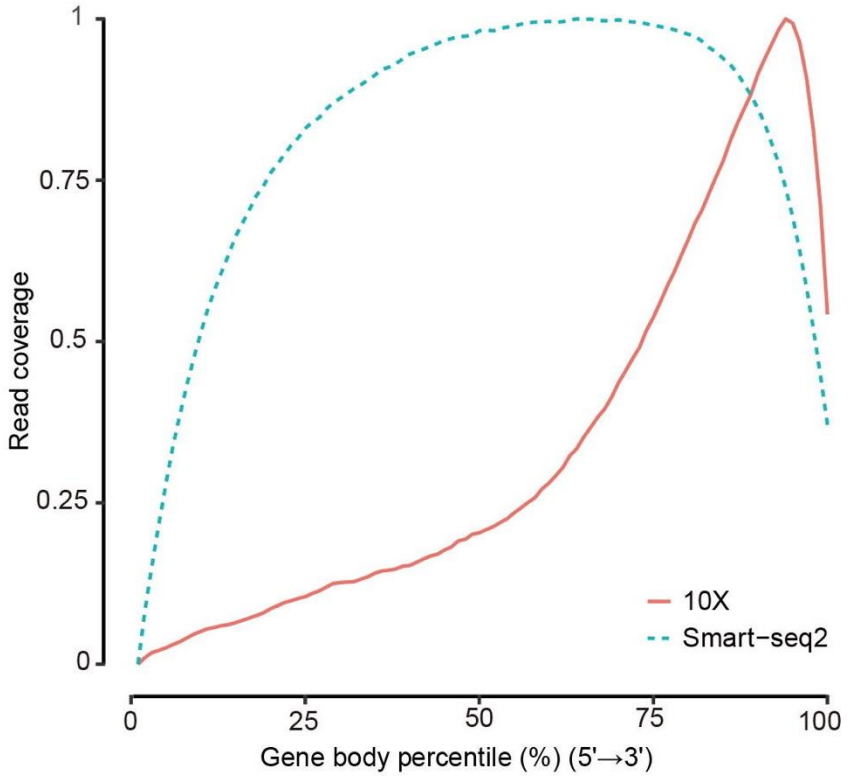
10x genomics (3’ kit)

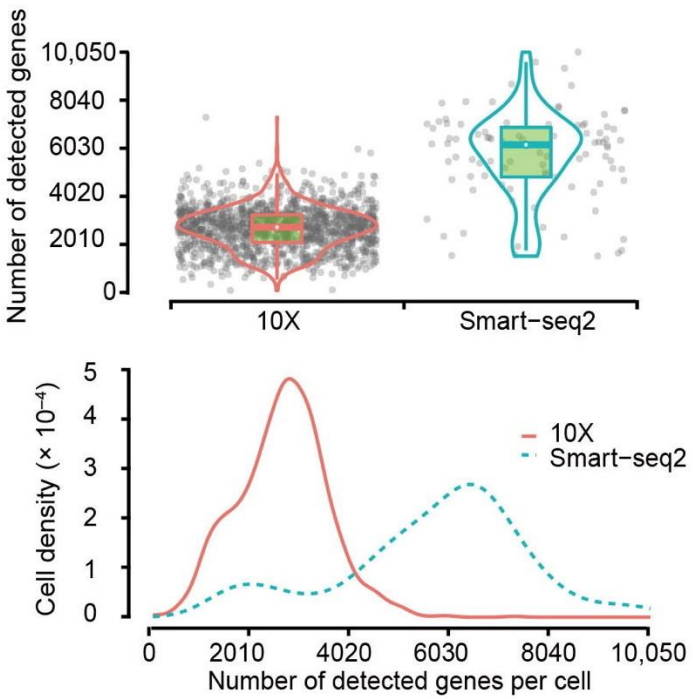
- Cell isolation within GEM (**G**el Bead-In **EM**ulsions)
- RNA capture with 1 bead/cell
- Sequencing from 3’ end
- Only expression
- Up to ~1 million cells/run



10x GENOMICS®

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

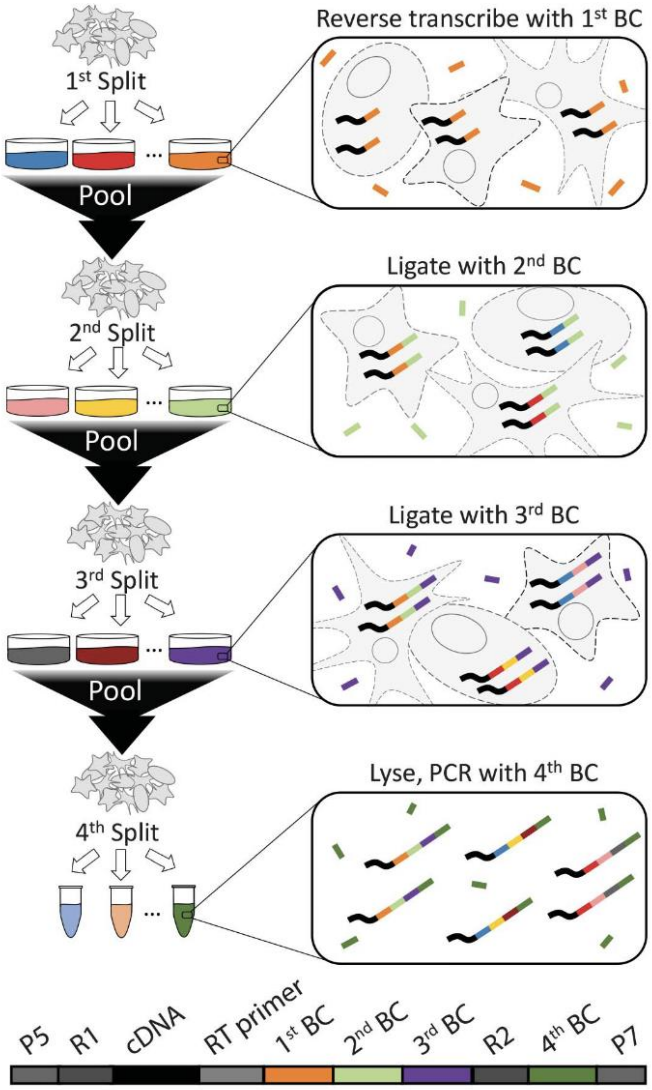




Wang X, et al. Direct Comparative Analyses of 10X Genomics Chromium and Smart-seq2. Genomics Proteomics Bioinformatics; 2021

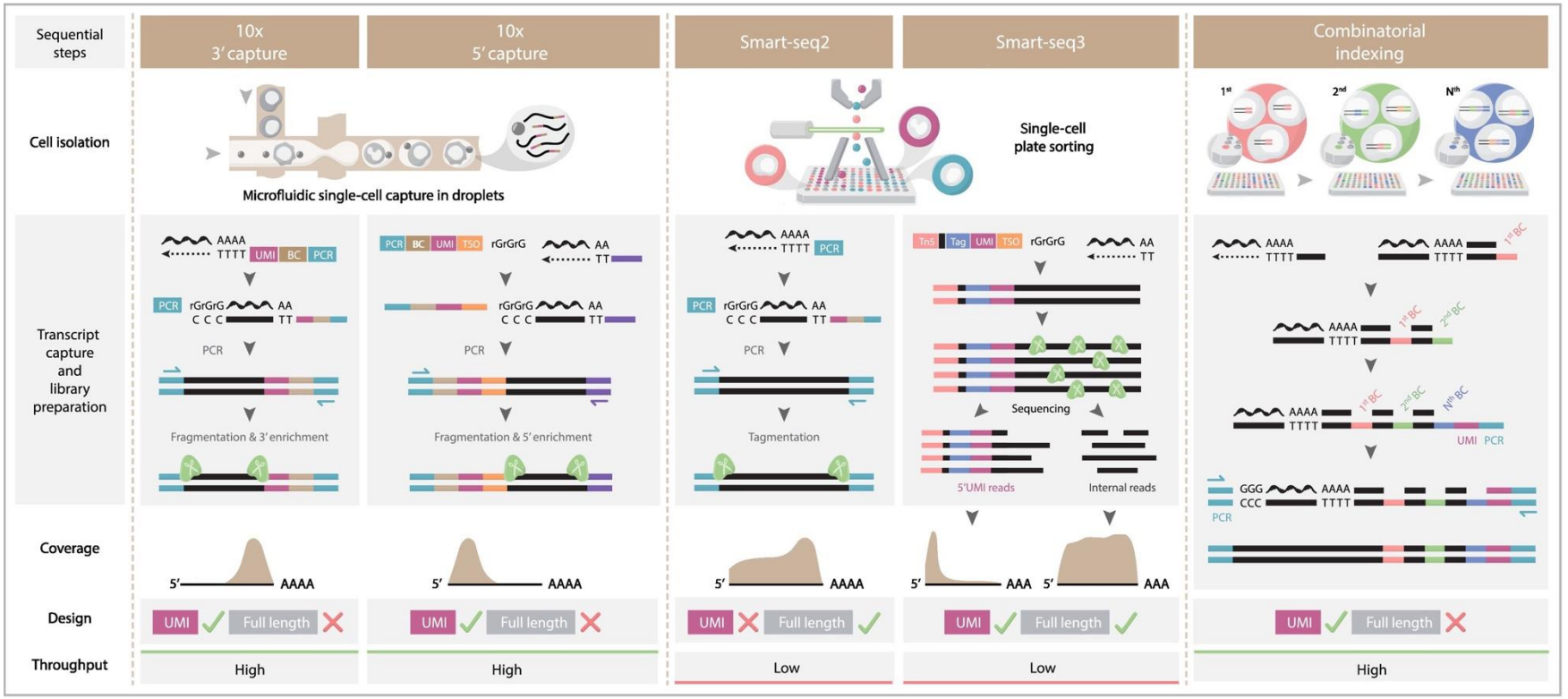
SPLiT-seq

- Based on:
  - formaldehyde fixation
  - in-cell RT + ligation
- 4 rounds of pooling-splitting and barcoding
- Up to ~1 million cells/run
- 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



Trends in Genetics

BD rhapsody

- Sparsely loads cells on a microwell array
- 1 cell + 1 bead / microwell -> can be visualized as QC
- Sequences only 3’ end
- ~20,000 cells/lane (8 lanes/cartridge)

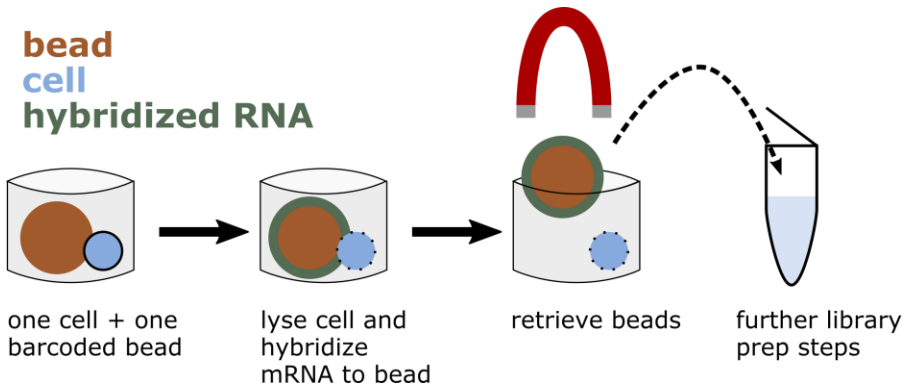


Image: Geert van Geest, CC-BY 4.0, DOI: 10.5281/zenodo.6759810

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

Quiz Question

Experimental design

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

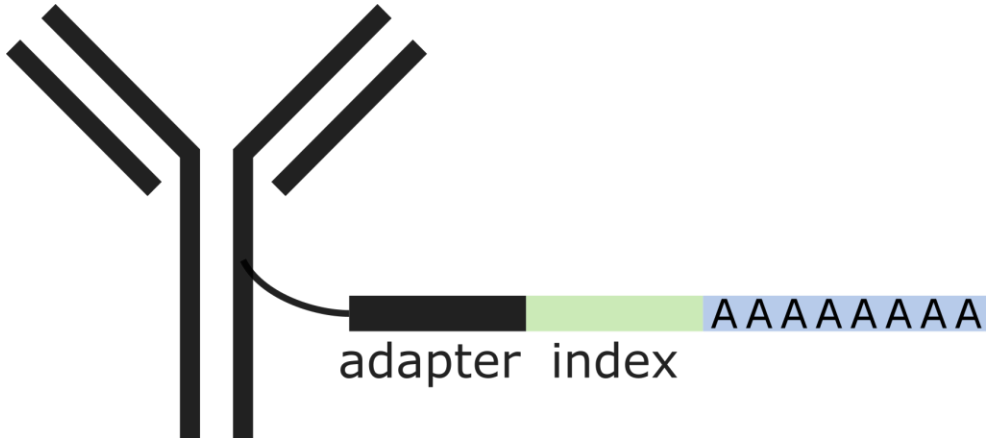
- Record any factor for downstream correction: Taking notes = the best QC
- Different cells of one replicate ≠ replicates!

Further reading:

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

Question 3

Quantify surface proteins (CITE-seq/TotalSeq)



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

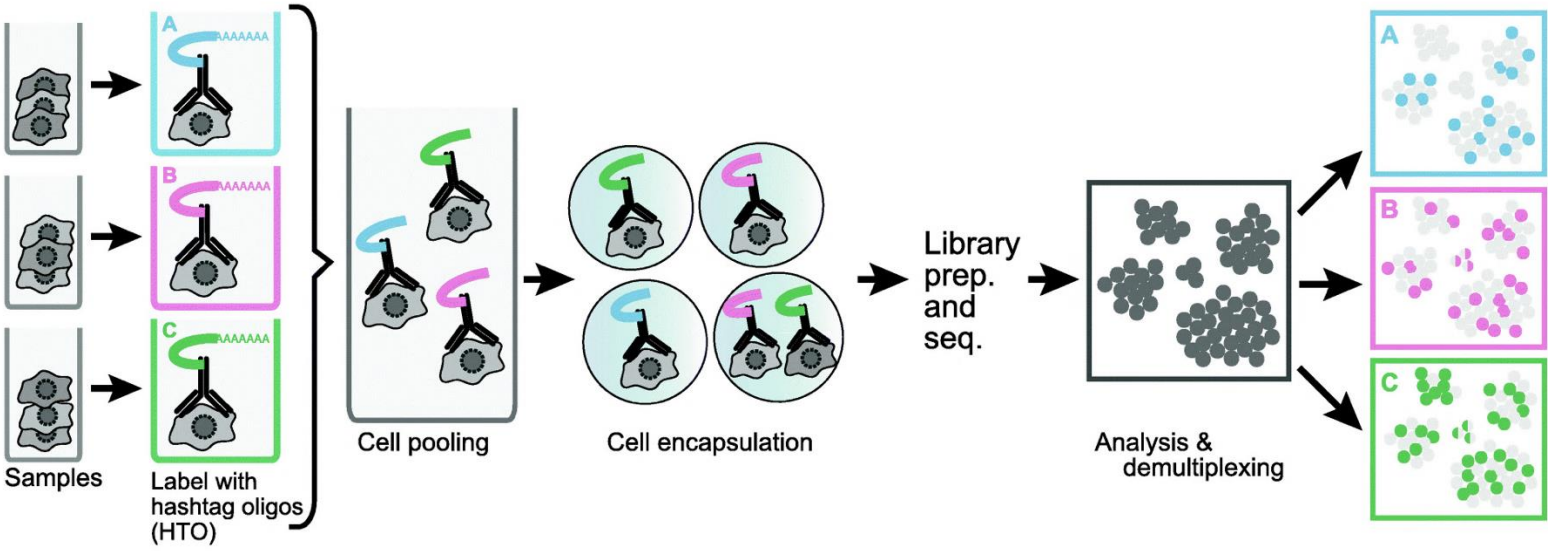
Stoeckius M et al. Simultaneous epitope and transcriptome measurement in single cells. Nat Methods. 2017;14:865–8.

Sample multiplexing

- **Cell hashing** with antibodies (hashtag oligonucleotides)
- **Lipid-based** e.g. 10x CellPlex
- **SNP-based** e.g. demuxlet. Each sample different genotype – requires WES data
- **Probe-based** e.g. 10x Flex

Sample multiplexing

Additional advantage: better doublet detection = more cells!



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
- Required for scATAC-seq
- Also for tissues difficult to dissociate
- No ribosomes -> no translation of transcription factors during processing
- Lower RNA capture

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.