

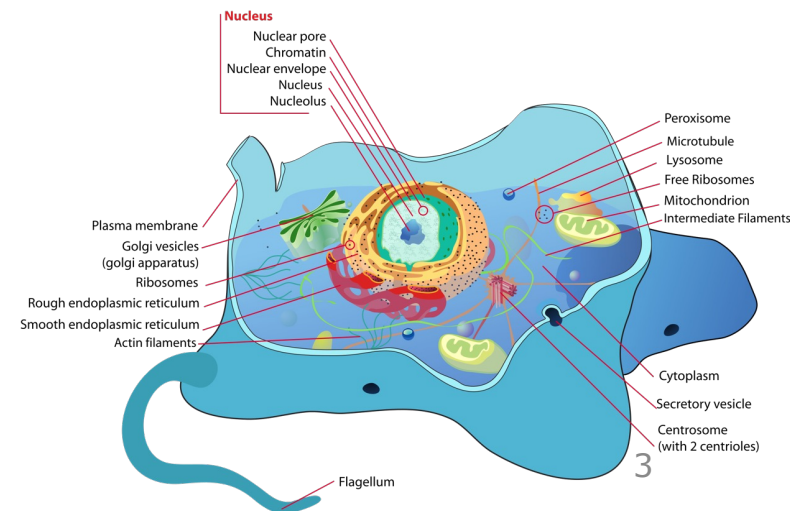
Single cell transcriptomics

Introduction to single cell RNA-seq

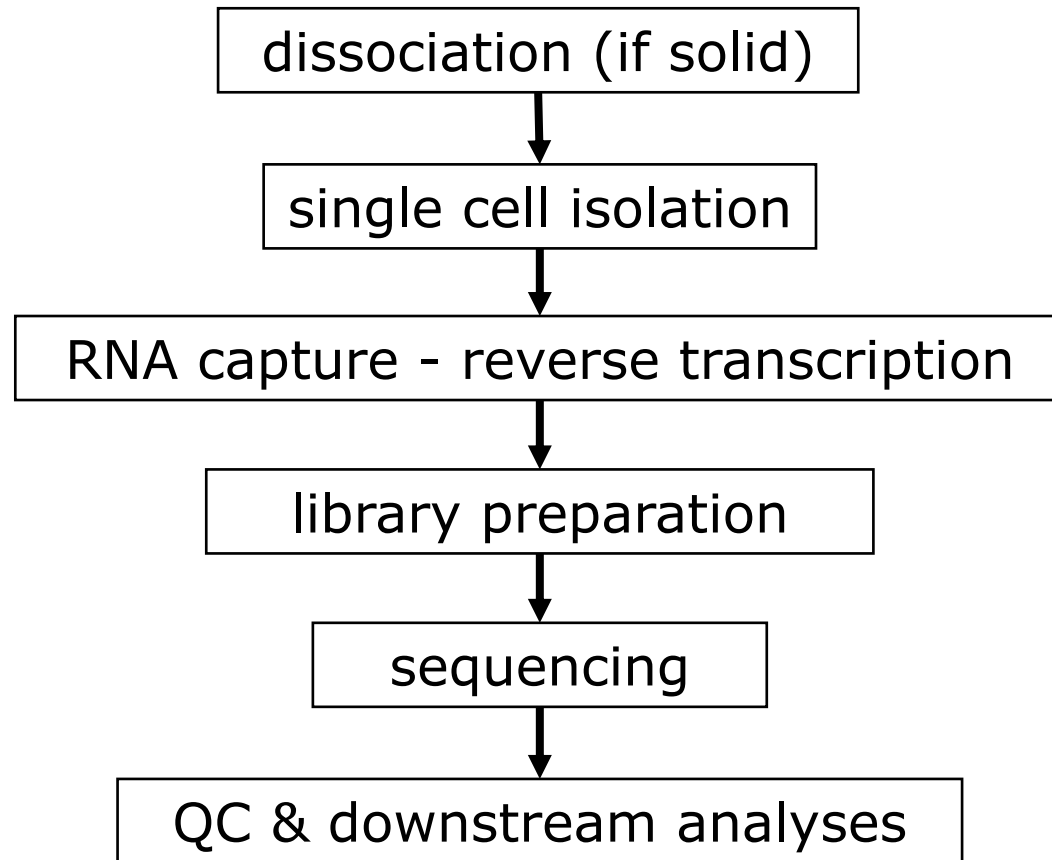


Why single cell RNAseq?

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



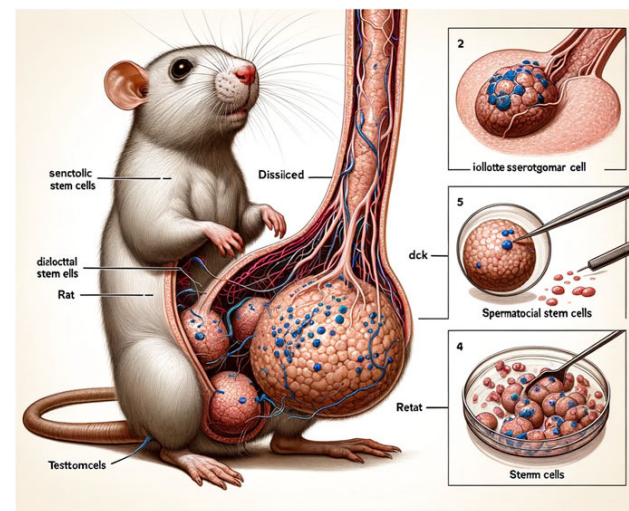
scRNA-seq workflow



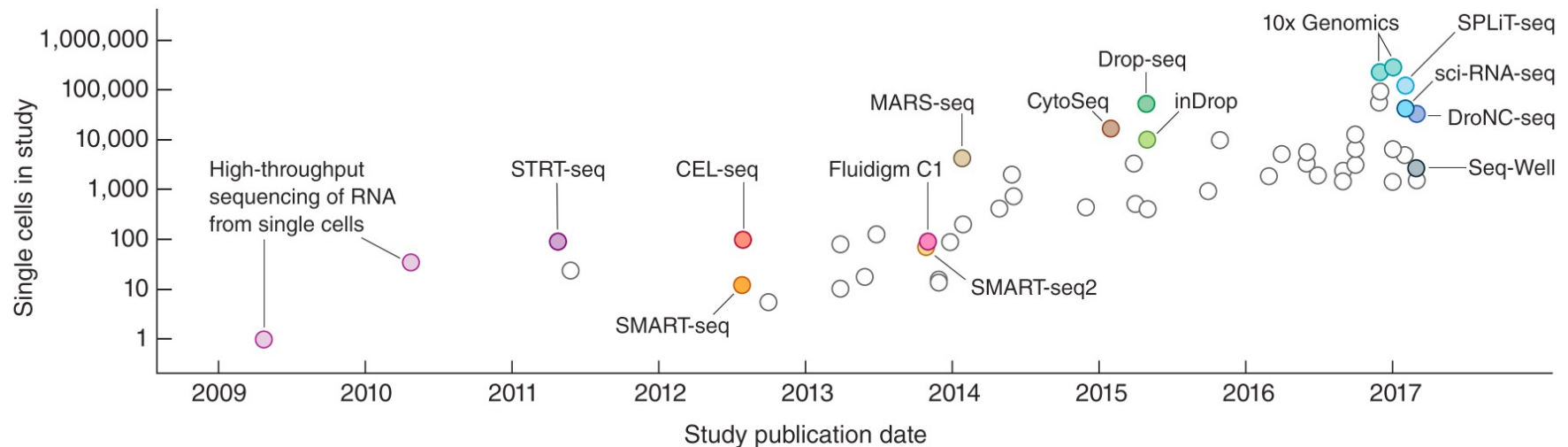
Obtaining cells

More about this at
day3 from Andreia
Gouvêa (10x)

- 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

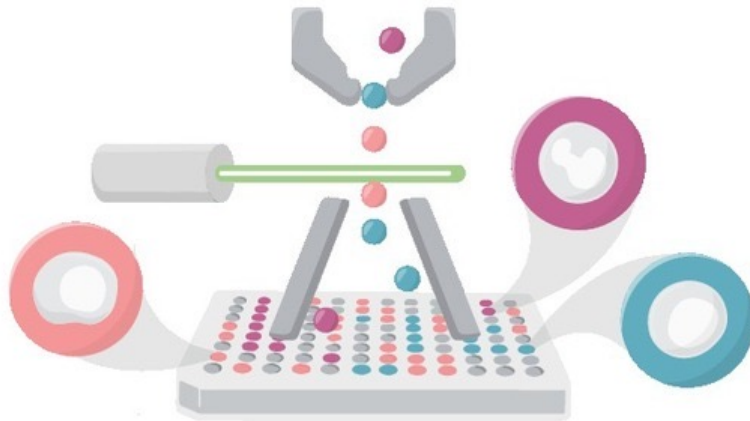


1. Plate separation by FACS: e.g. SMART-seq
2. Droplet-based: e.g. 10x genomics (3' kit)
3. Combinatorial indexing: e.g. SPLiT-seq
4. Microwell-based: e.g. BD Rhapsody (CytoSeq)

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

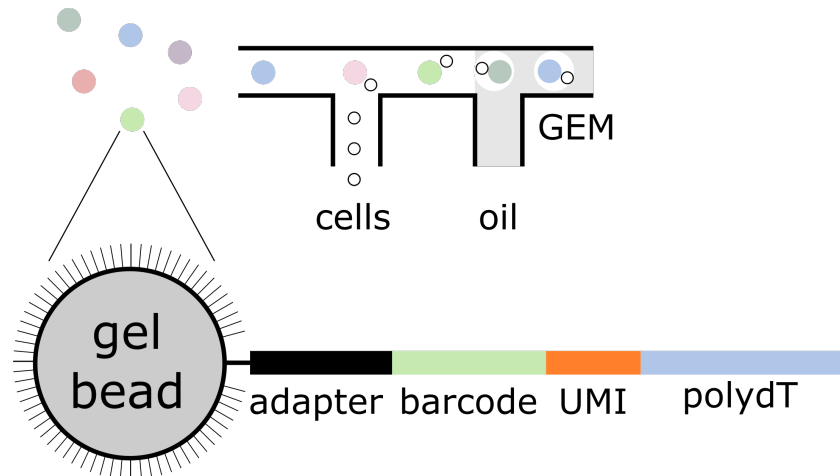


Hagemann-Jensen M et al. Single-cell RNA counting at allele and isoform resolution using Smart-seq3. Nat Biotechnol. 2020;38:708–14.

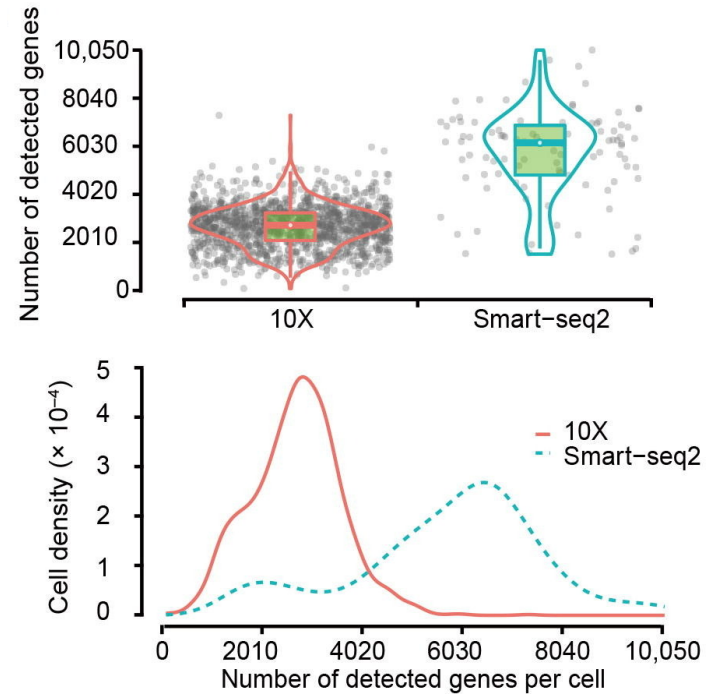
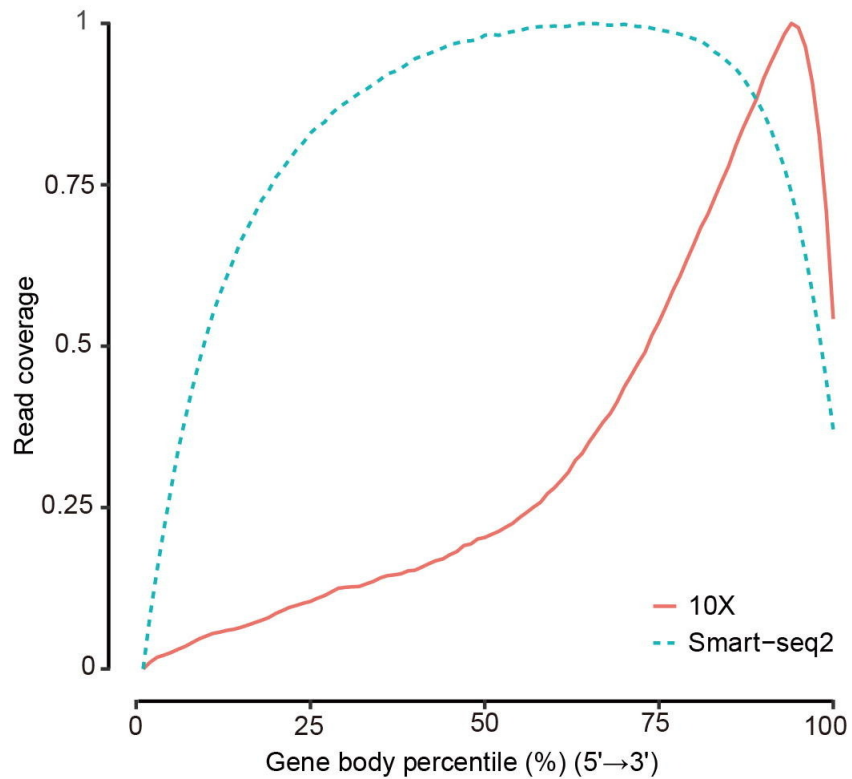
Image: *Trends in Genetics* DOI: (10.1016/j.tig.2023.10.003)

10x genomics (3' kit)

- Cell isolation within GEM (**G**el Bead-In **E**mulsions)
- RNA capture with 1 bead/cell
- Sequencing from 3' end
- Only expression



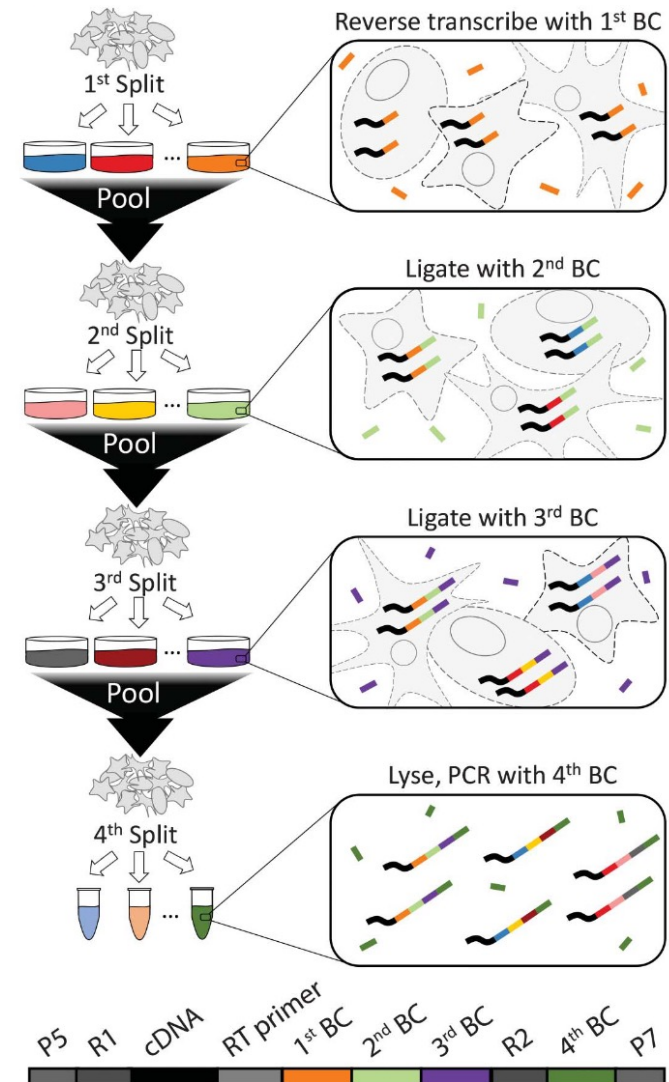
Zheng GXY et al. Massively parallel digital transcriptional profiling of single cells. Nat Commun; 2017;8.



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

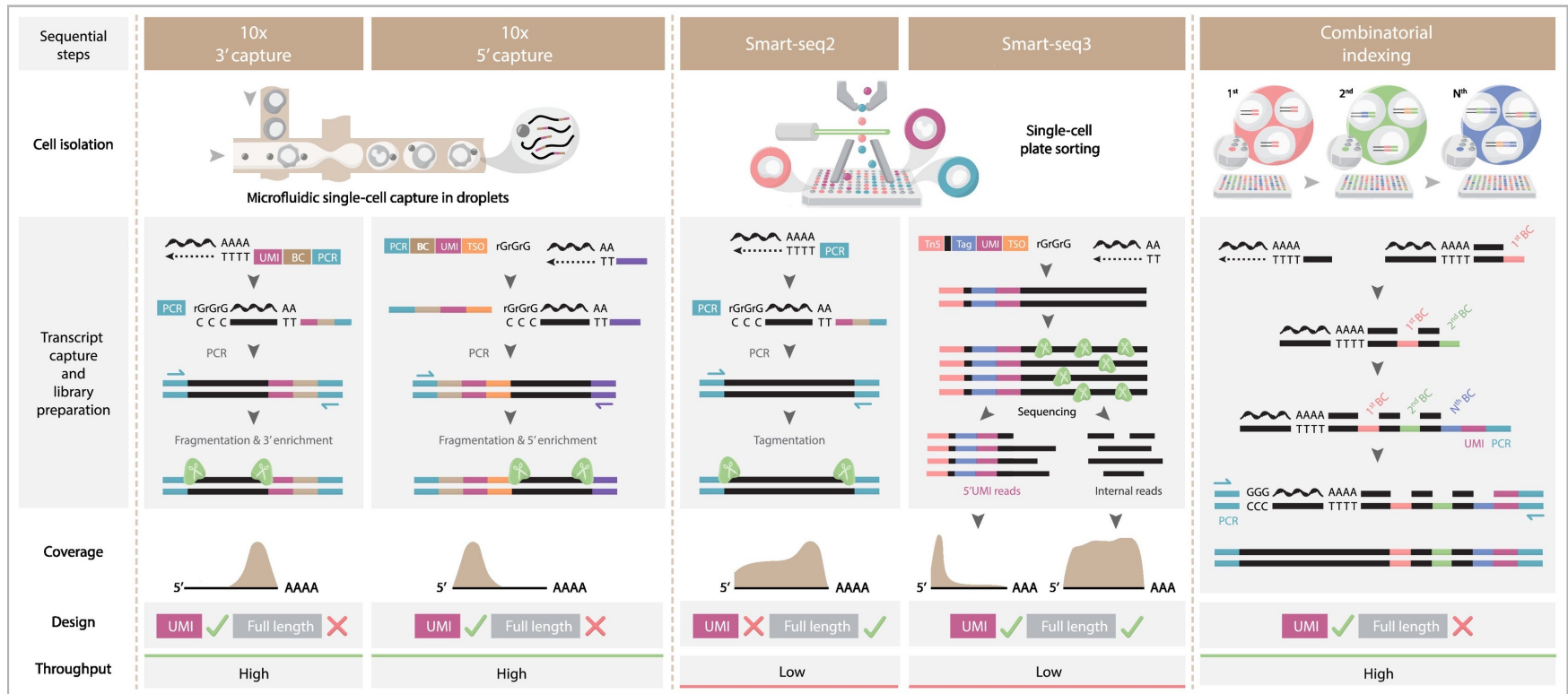
SPLiT-seq

- 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.

Overview

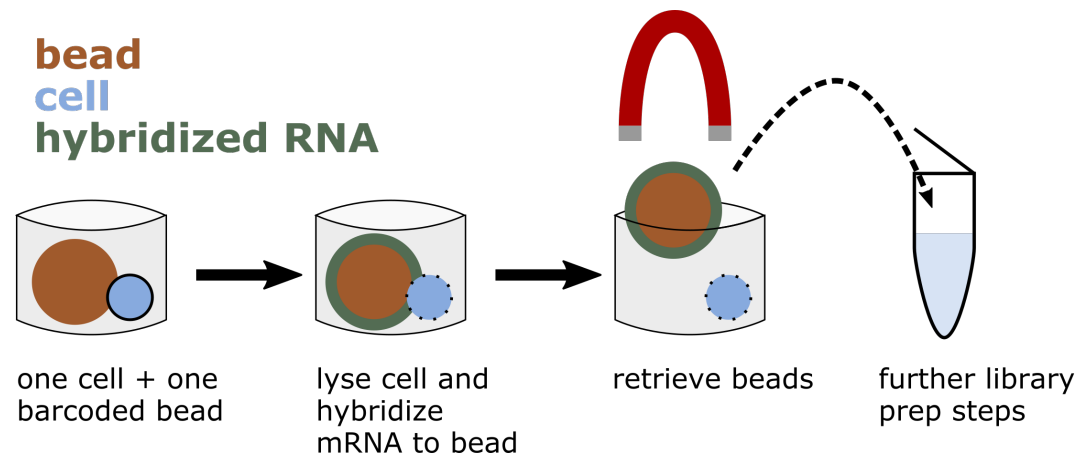


Trends in Genetics

BD rhapsody



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



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 \neq replicates!

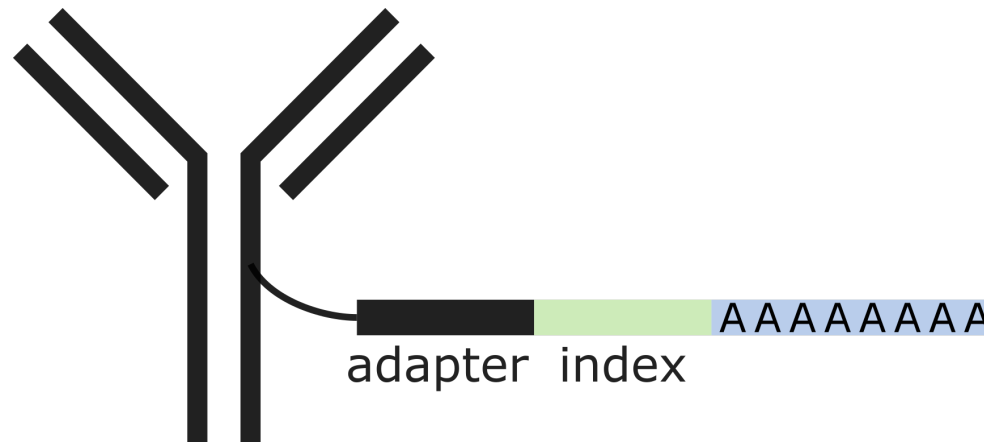


Further reading:

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

Question 3

Quantify proteins

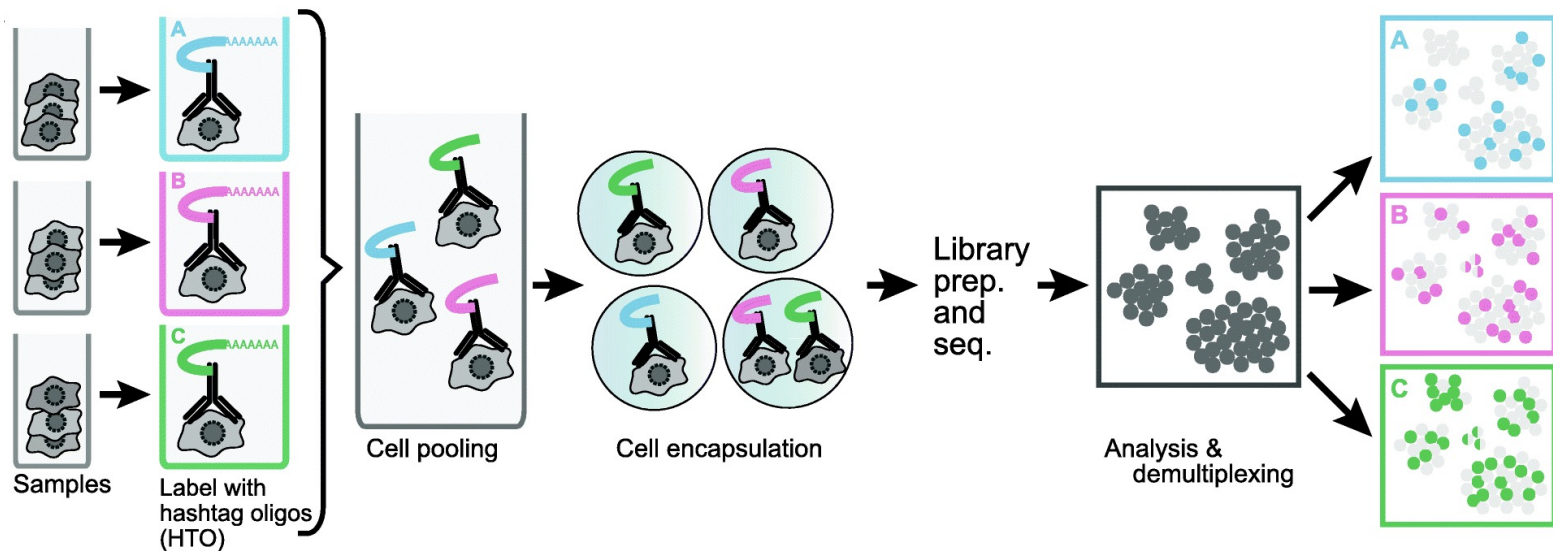


- Quantification of (cell-surface) proteins
- Together with transcriptome

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
- Required for scATAC-seq
- Also for tissues difficult to dissociate
- No ribosomes -> no translation of transcription factors during processing
- Lower representation of immune cells + surface proteins