Introduction to Single-Cell RNA-seq

The CCDL

What can bulk RNA-seq vs single-cell RNA-seq help us determine?

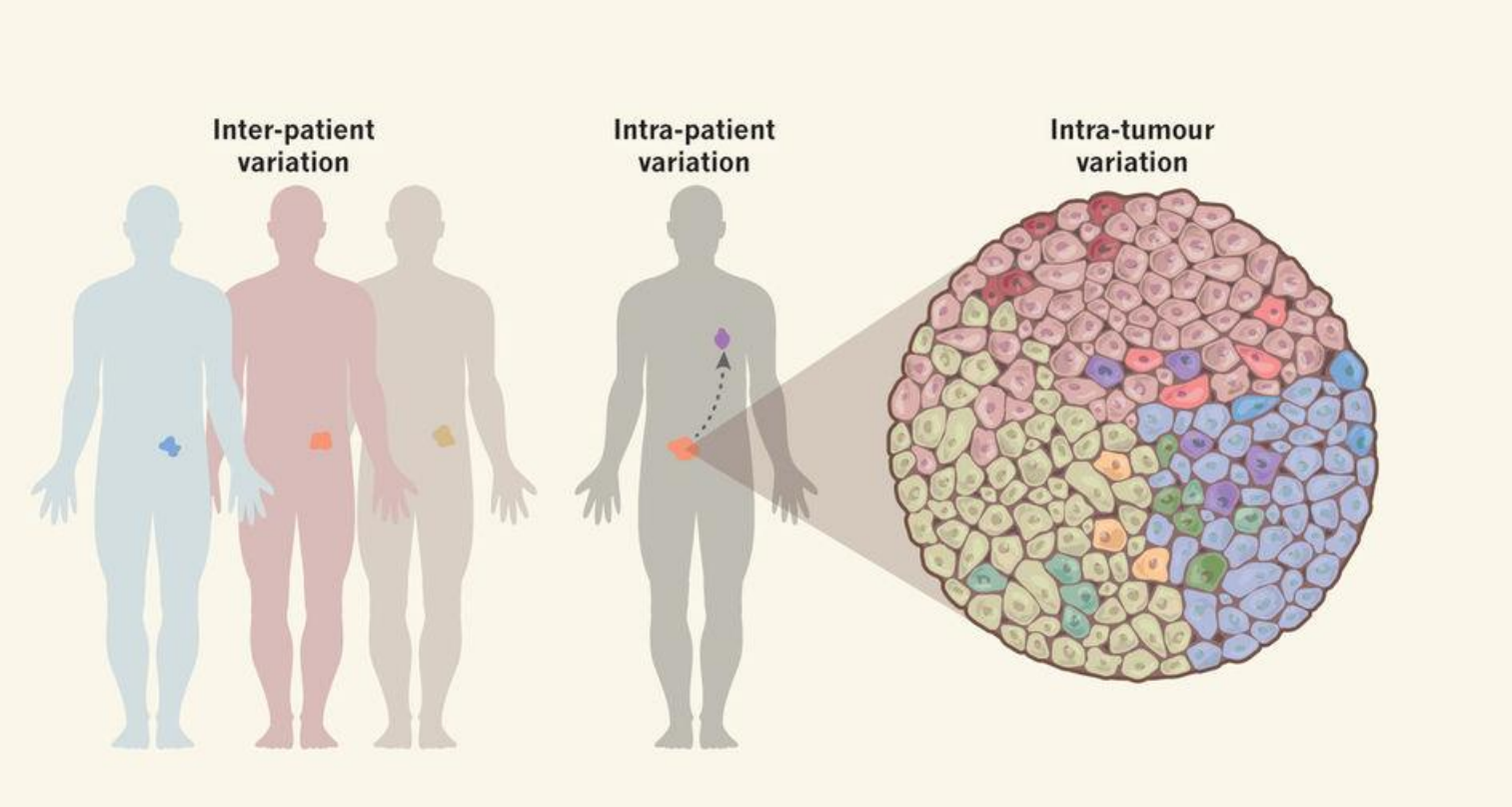

Image from Tanaka *et al*. 2018 https://doi.org/10.1038/s41551-017-0162-1.

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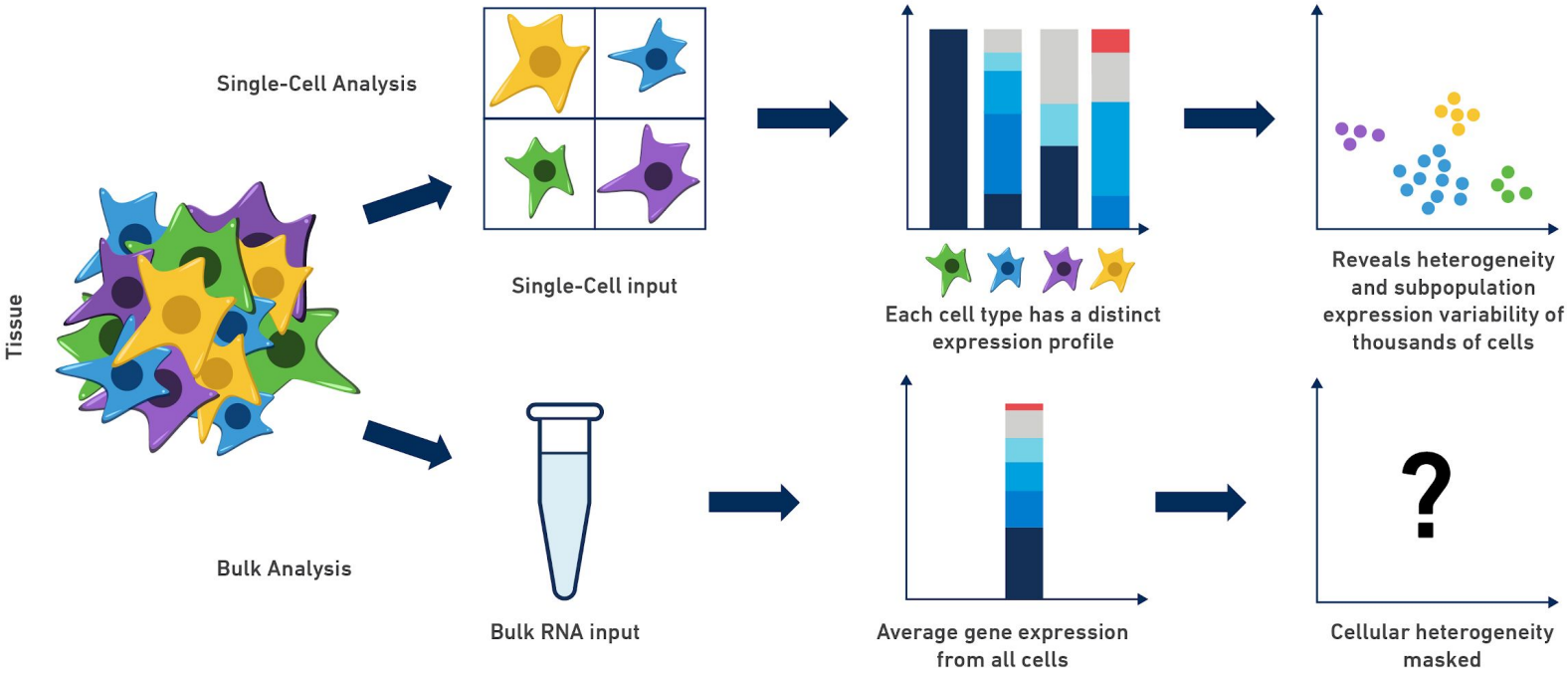


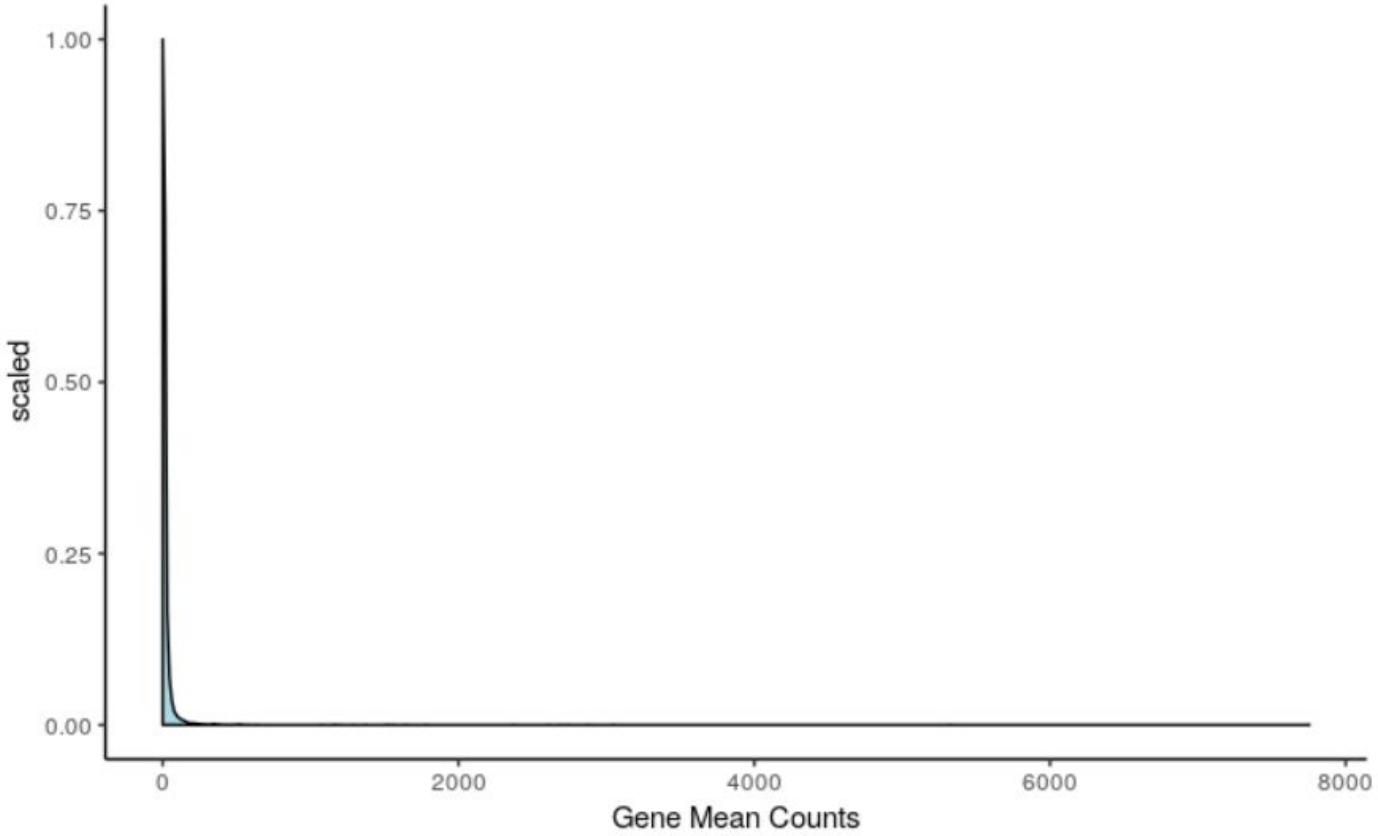
Image from 10X Genomics blog:
https://community.10xgenomics.com/t5/10x-Blog/Single-Cell-RNA-Seq-An-Introductory-Overview-and-Tools-for/ba-p/547

Single-cell RNA-seq quirks

Less starting material means:

- More PCR amplification (*and its associated biases*)
- More zero counts (*but this is probably biology!*)
    - Choi *et al.* (Preprint) https://www.biorxiv.org/content/10.1101/2020.03.03.974808v1

Single-cell gene mean density graph



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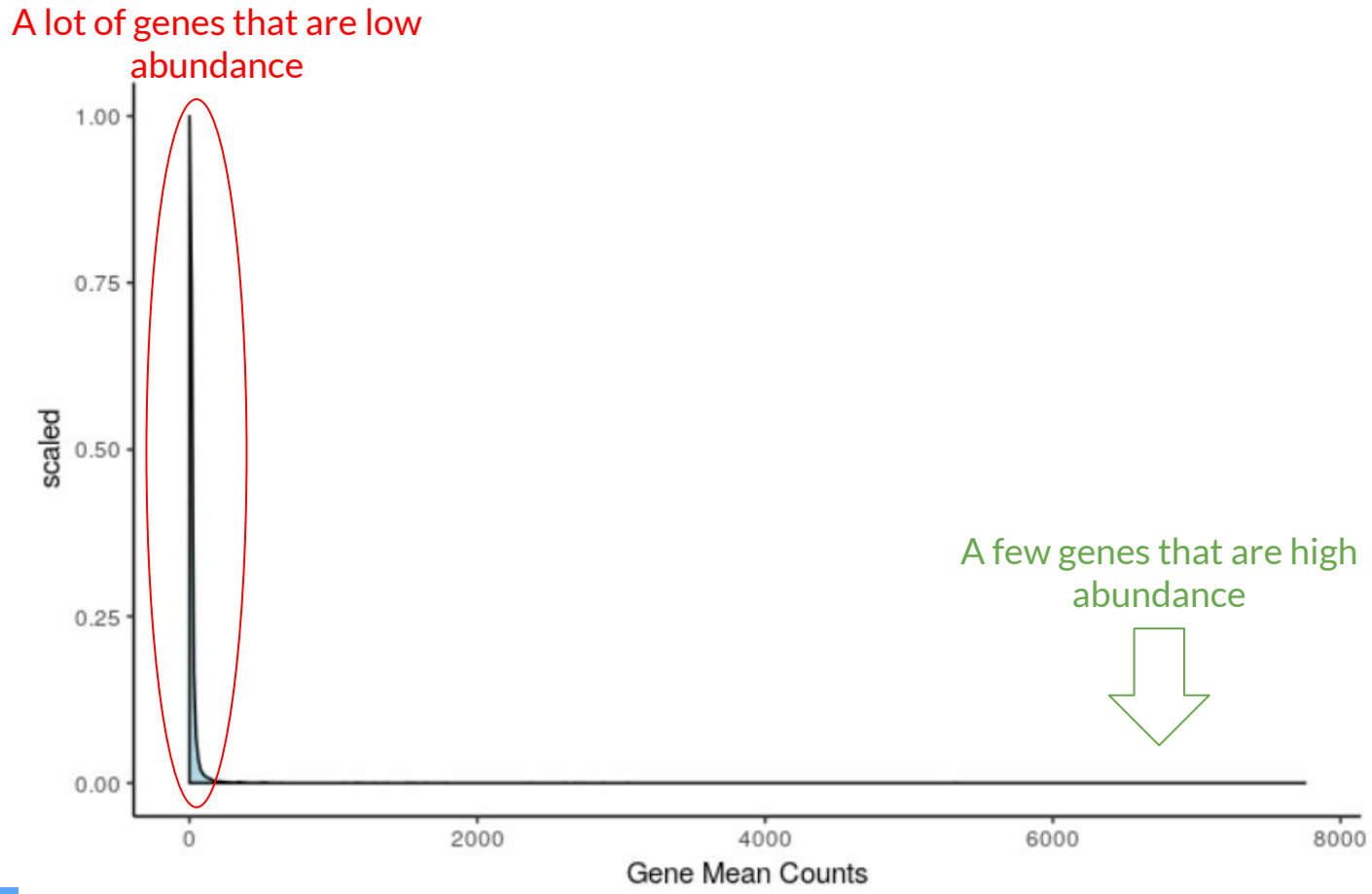
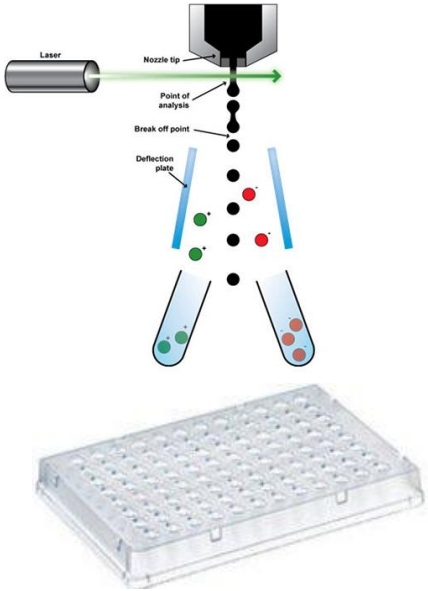


Single Cell Basic Set-ups

1. Full-length scRNA-seq



Physical separation of cells *before* sequencing

Example: Smart-seq2

Picelli et al. Nature Protocols. 2014 https://www.nature.com/articles/nprot.2014.006
Zheng et al. Nat Commun. 2017 https://www.ncbi.nlm.nih.gov/pubmed/28091601

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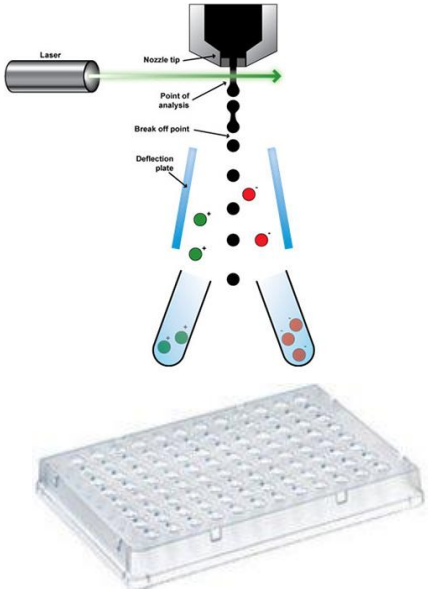

Physical separation of cells *before* sequencing

Example: Smart-seq2

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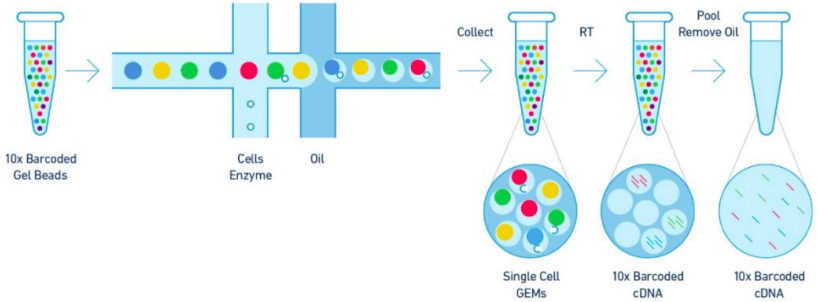
2. Tag-based scRNA-seq



Tag-based separation of cells' data *after* sequencing

Example: 10X Genomics Chromium

Zheng *et al.* 2017
https://www.ncbi.nlm.nih.gov/pubmed/28091601

Full-length scRNA-seq

Pros:

- Can be paired-end sequencing which has less risk for 3' bias.
- More complete coverage of transcripts, which may be better for transcript discovery purposes.

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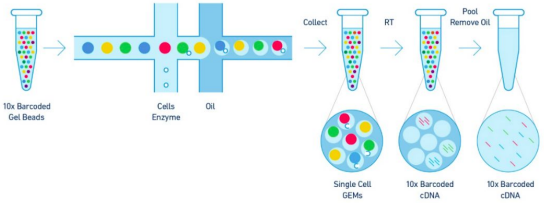
- Can be paired-end sequencing which has less risk for 3' bias.
- More complete coverage of transcripts, which may be better for transcript discovery purposes.

Cons:

- Is not very efficient (generally 96 cells per plate).
- Takes much longer to run (days/weeks depending on sample size).
- Expensive.

Pre-processing: Very similar to bulk RNA-seq

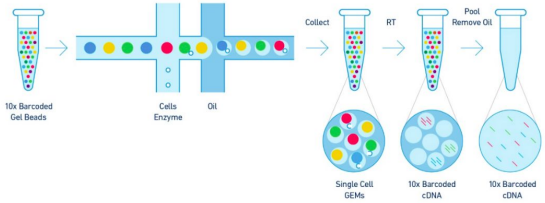
Tag-Based scRNA-seq



Pros:

- Can profile up to millions of cells.
- Takes less computing power.
- File storage requirements are smaller.
- Much less expensive.

Tag-Based scRNA-seq



Pros:

- Can profile up to millions of cells.
- Takes less computing power.
- File storage requirements are smaller.
- Much less expensive.

Cons:

- More intense 3' bias because sequencing is not bidirectional.
- Coverage is generally not as deep as full-length scRNA-seq.

Processing: use Alevin (a Salmon tool) to separate cells’ data using the cell barcodes

Unique Molecular Identifiers (UMIs):

a '*snapshot*' of the original molecules in the *pre-amplified* cell

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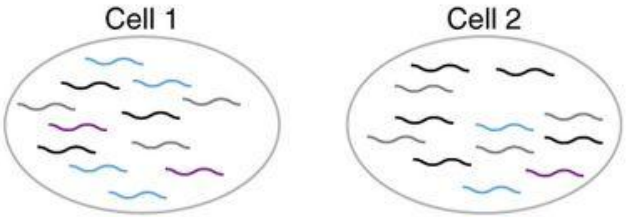

Original image from: Islam *et al.* 2014 https://doi.org/10.1038/nmeth.2772

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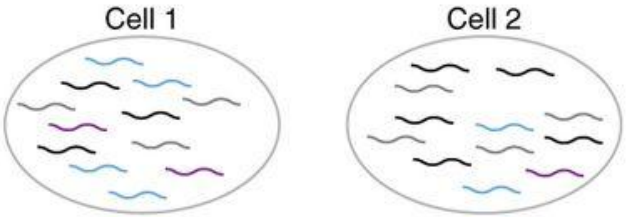

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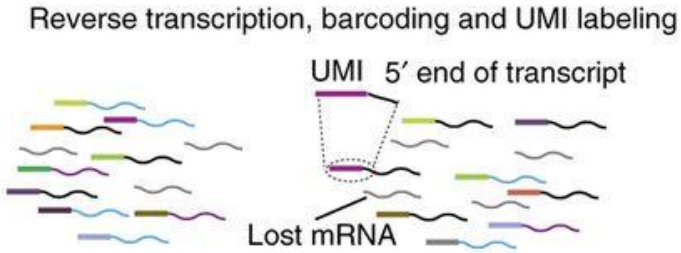

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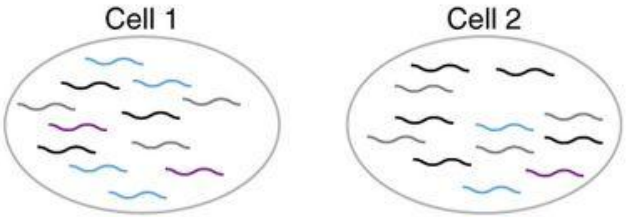

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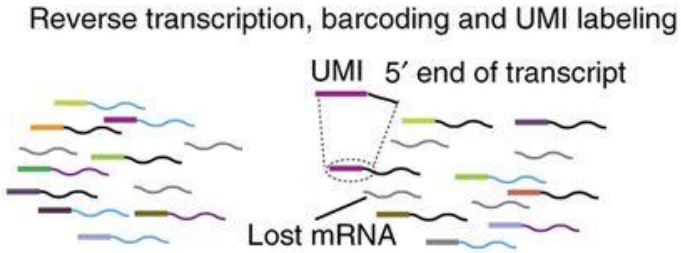

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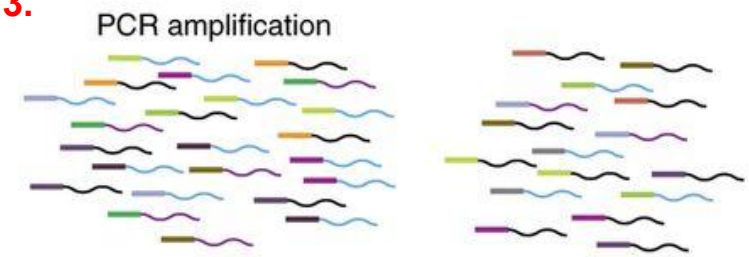

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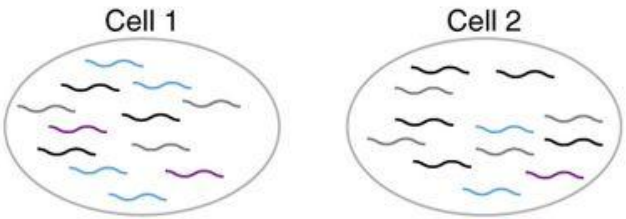

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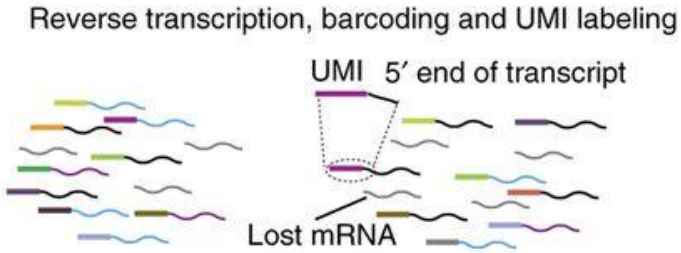

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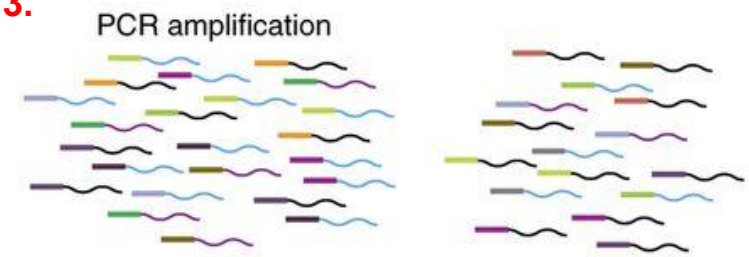

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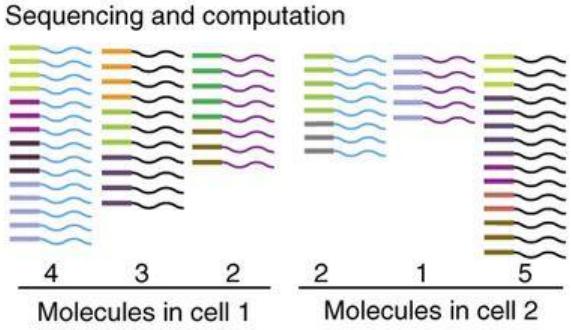

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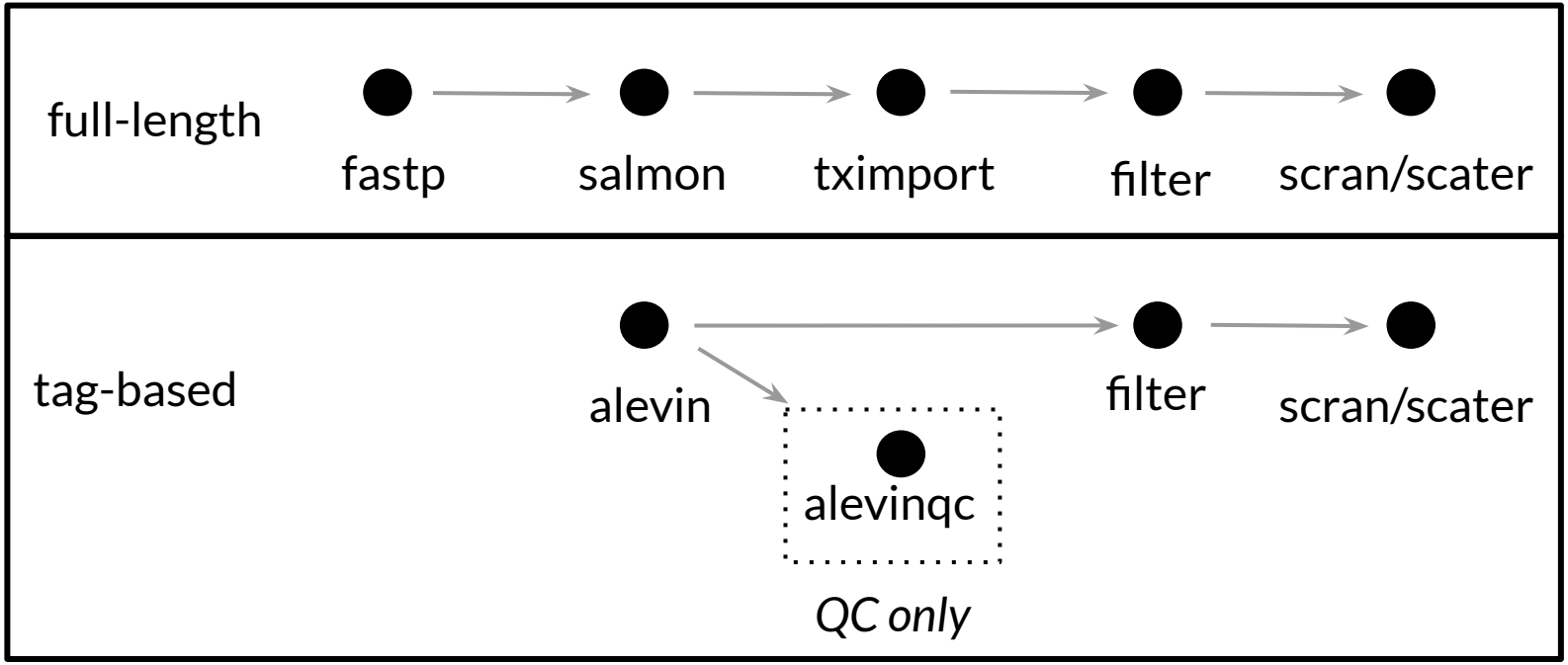
full-length
fastp
salmon
tximport
filter
scran/scater
tag-based
alevin
filter
scran/scater
alevinqc
QC only

Resources for you in `00-scRNA-seq_introduction.md`

- Hemburg lab scRNA-seq training course
- ASAP: Automated Single-cell Analysis Pipeline is a web server that allows you to process scRNA-seq data.
- Smith. Unique Molecular Identifiers – the problem, the solution and the proof - article on background of UMIs
- Literature on technologies

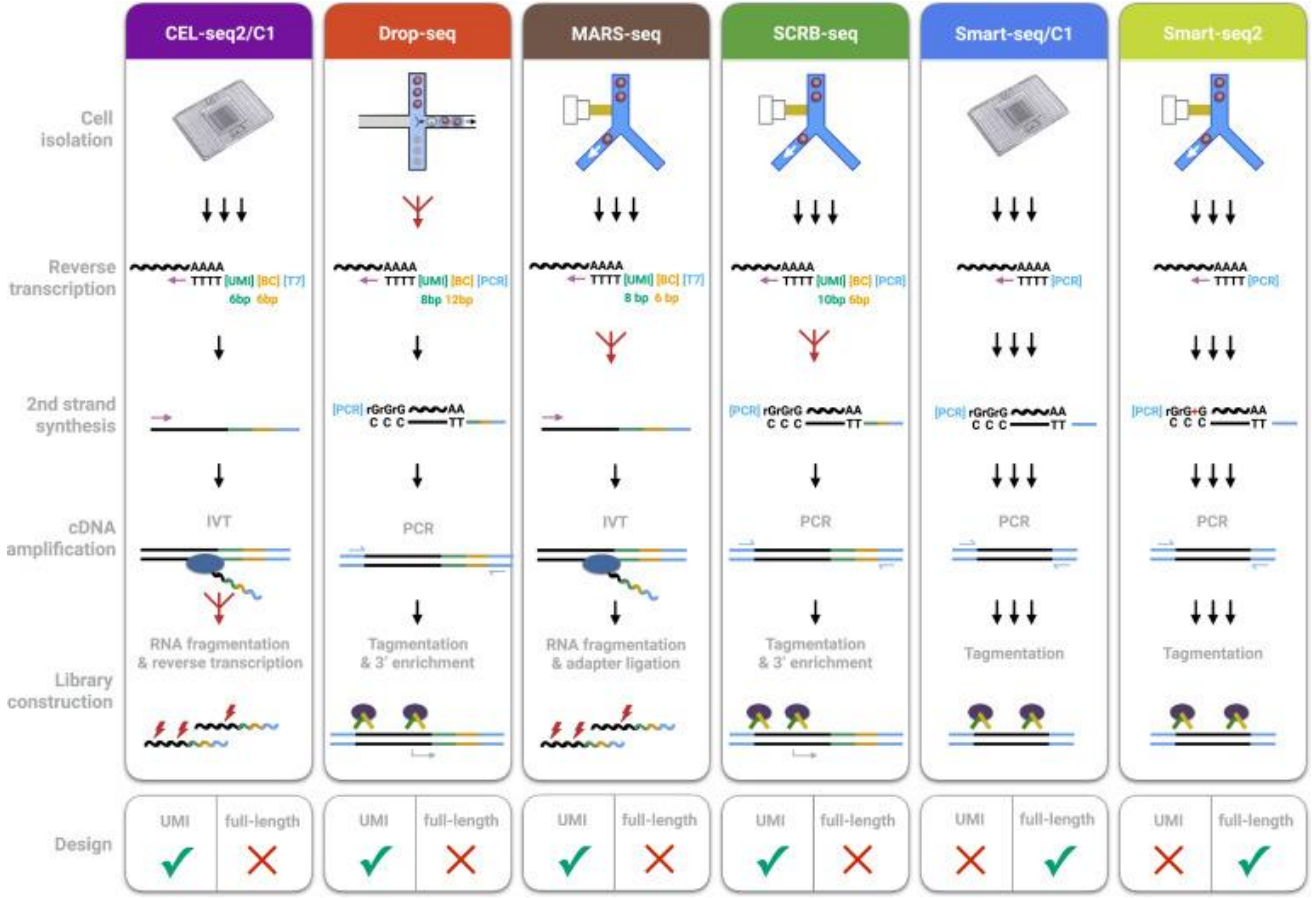

Image from: Zeigenhain et al. Mol Cell. 2018 (http://dx.doi.org/10.1016/j.molcel.2017.01.023)

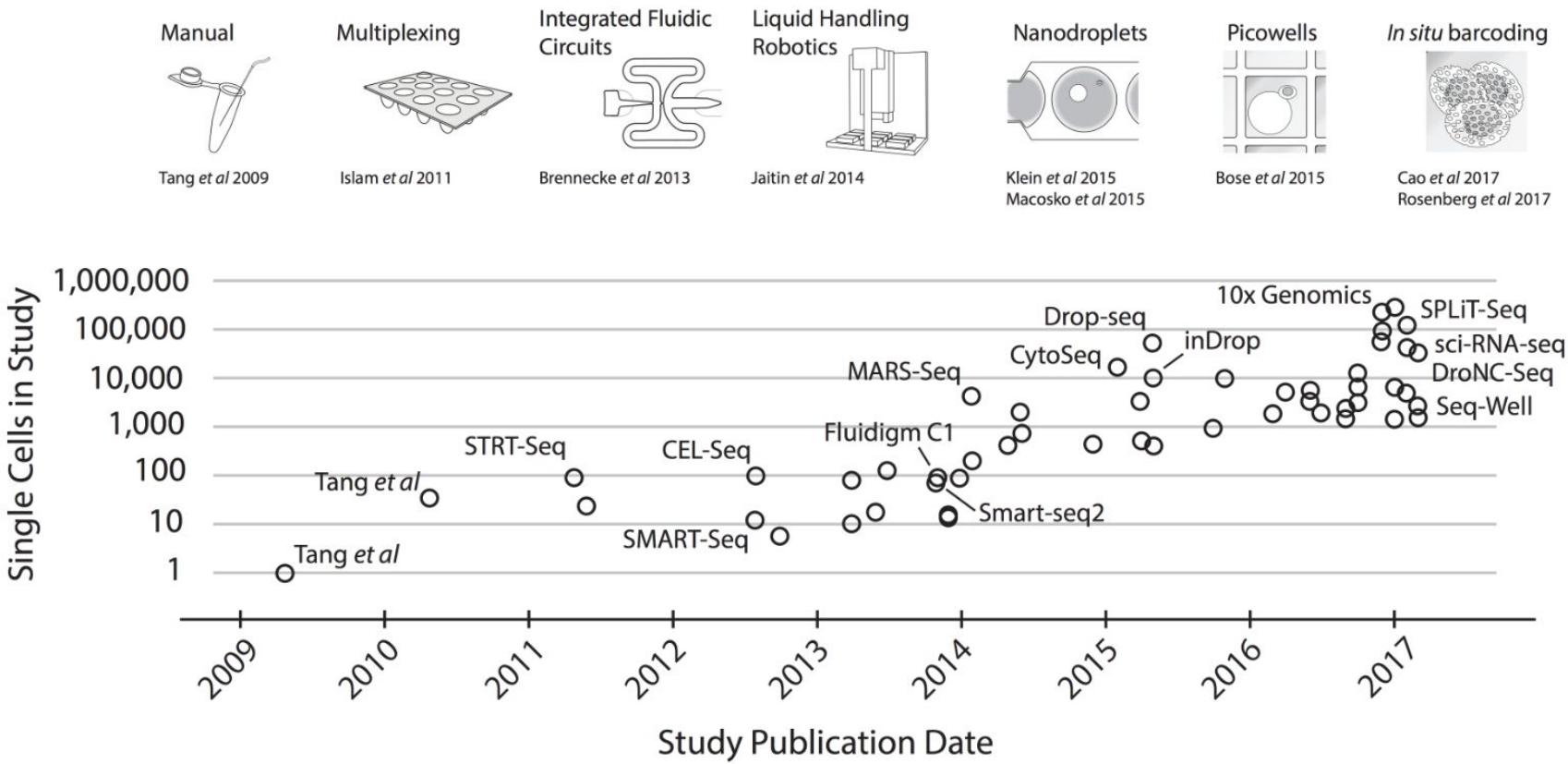

Image from Svensson et al: https://arxiv.org/abs/1704.01379