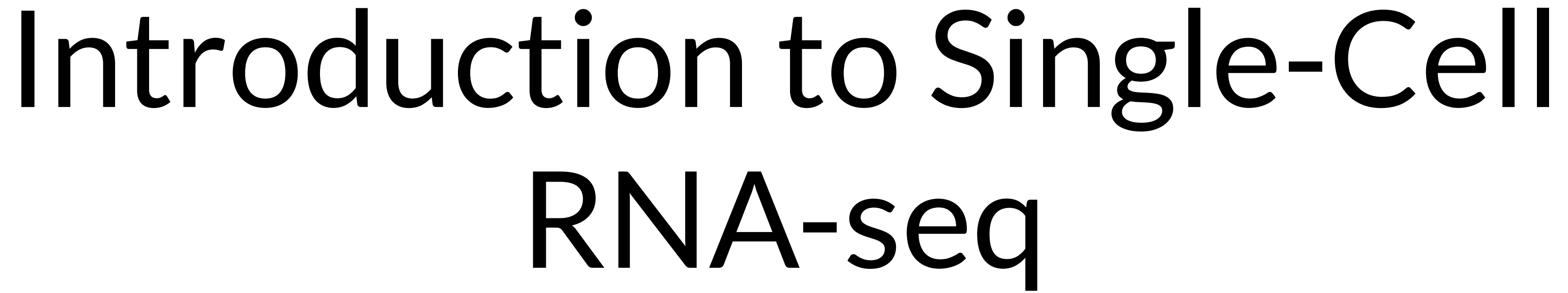


Introduction to Single-Cell RNA-seq

The Data Lab

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

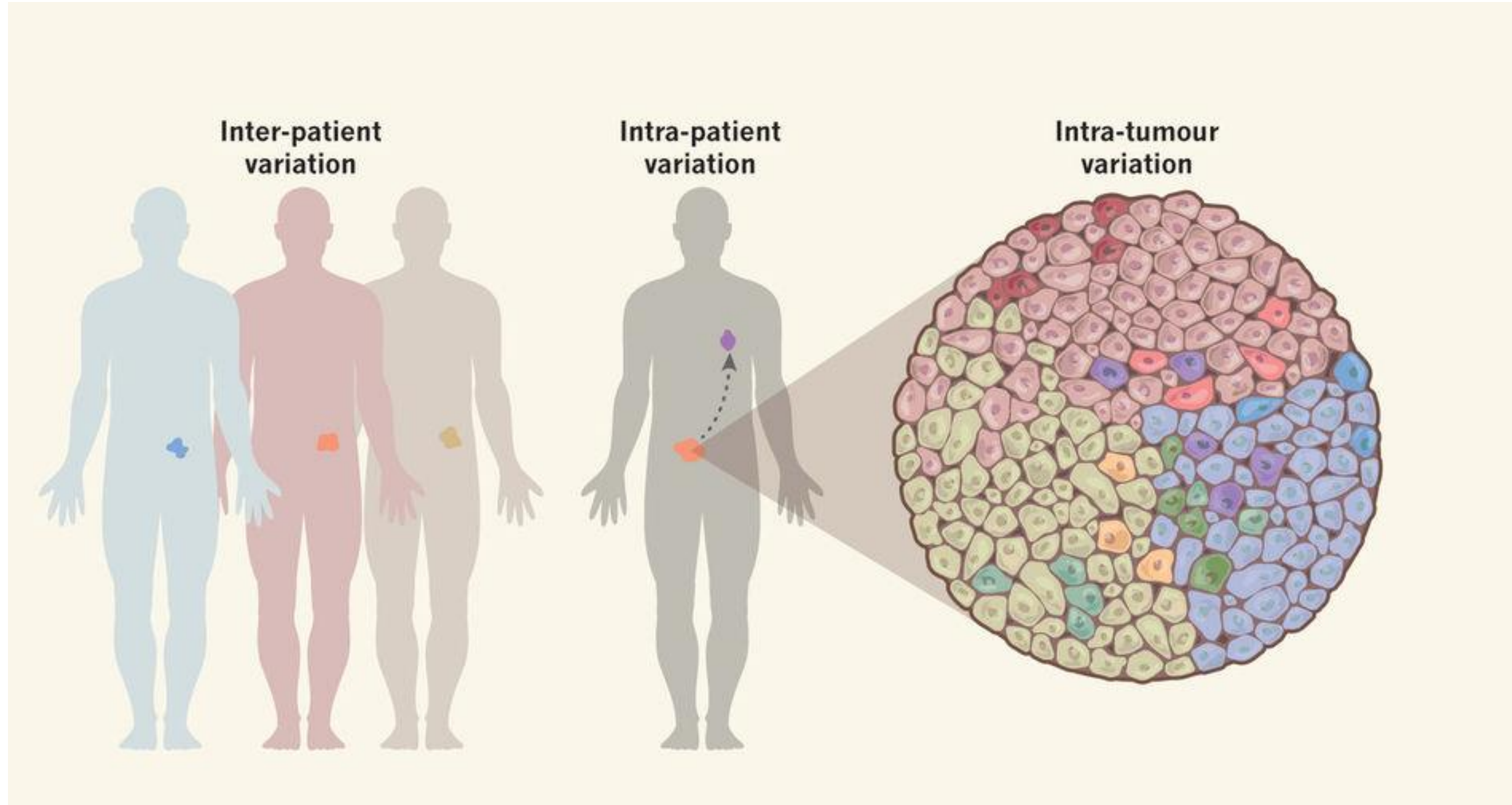


Image from Tanaka *et al.* 2018 https://doi.org/10.1038/s41551-017-0162-1.

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

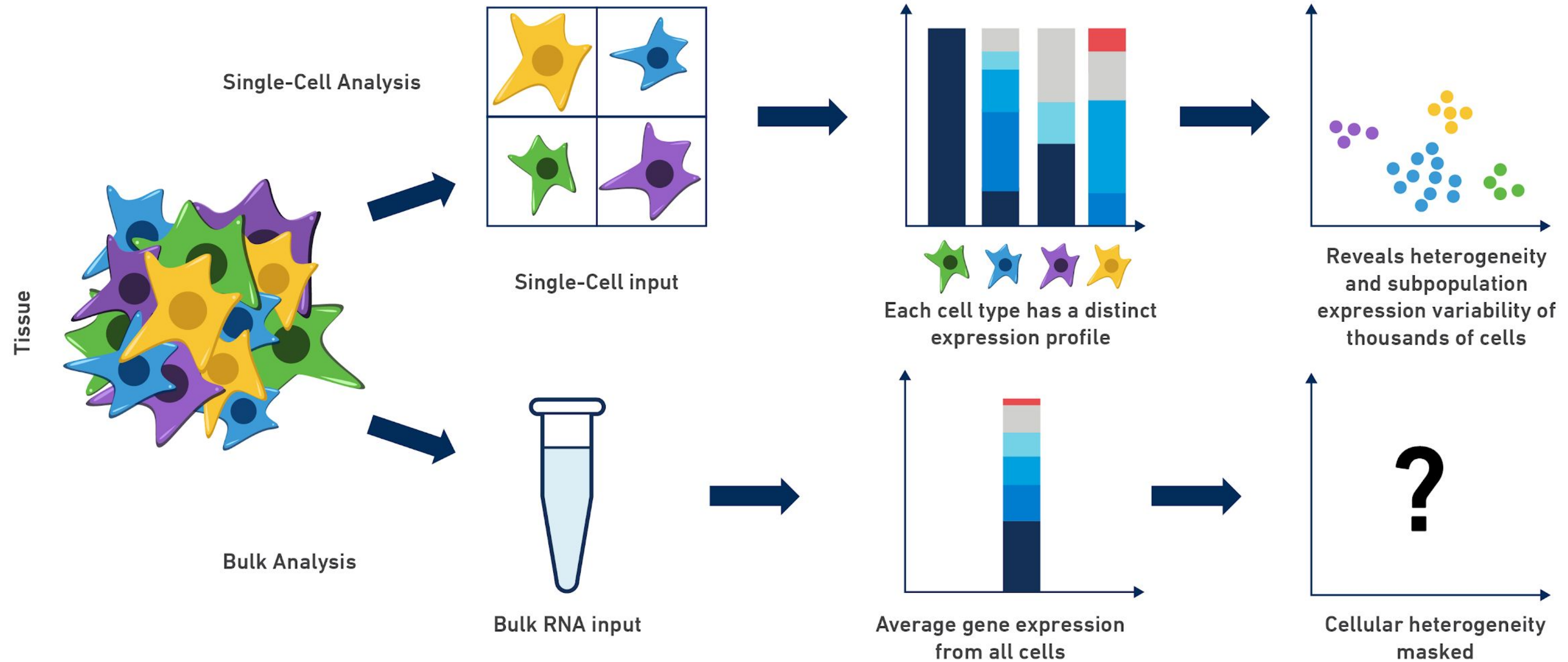


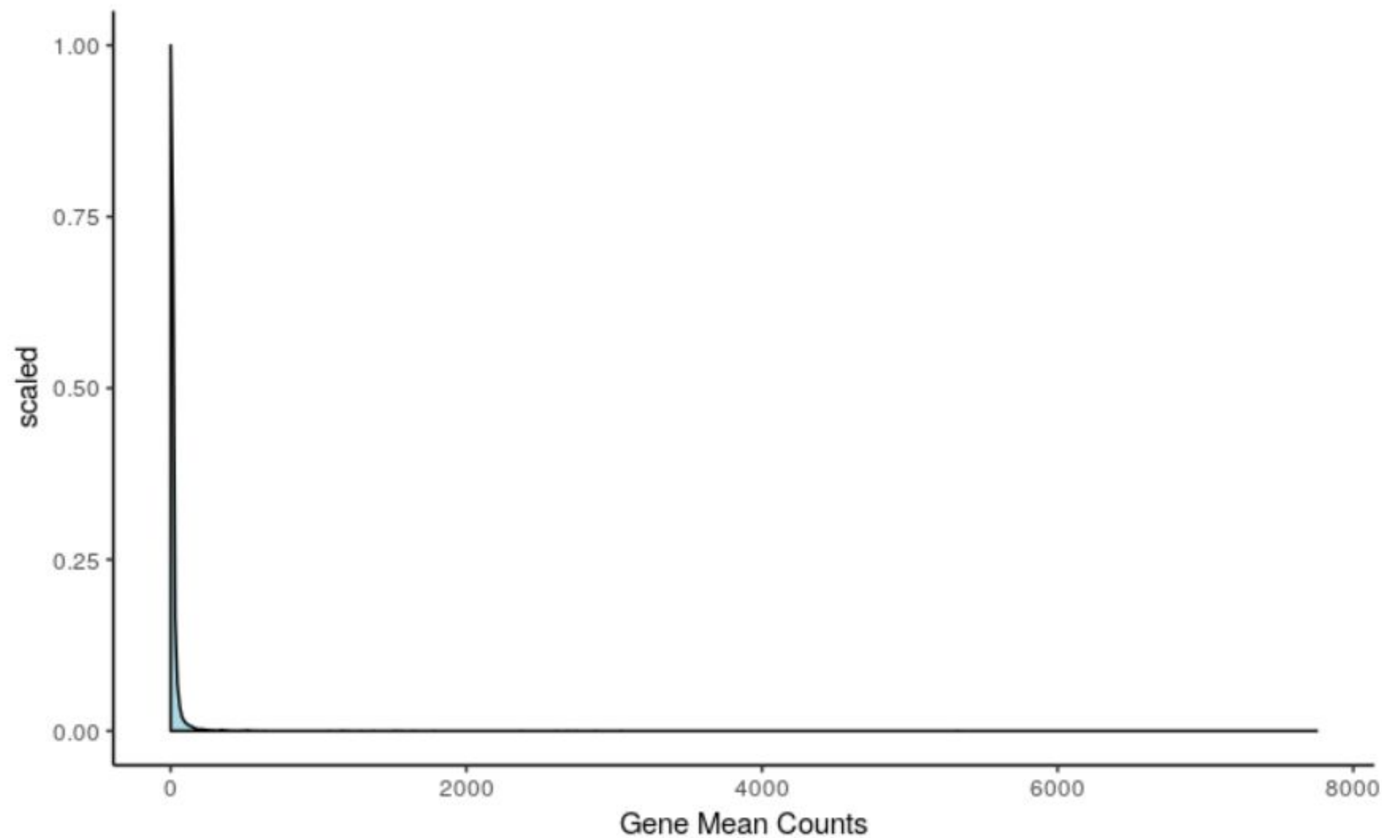
Image from 10X Genomics blog: https://www.10xgenomics.com/blog/single-cell-rna-seq-an-introductory-overview-and-tools-for-getting-started

Single-cell RNA-seq quirks

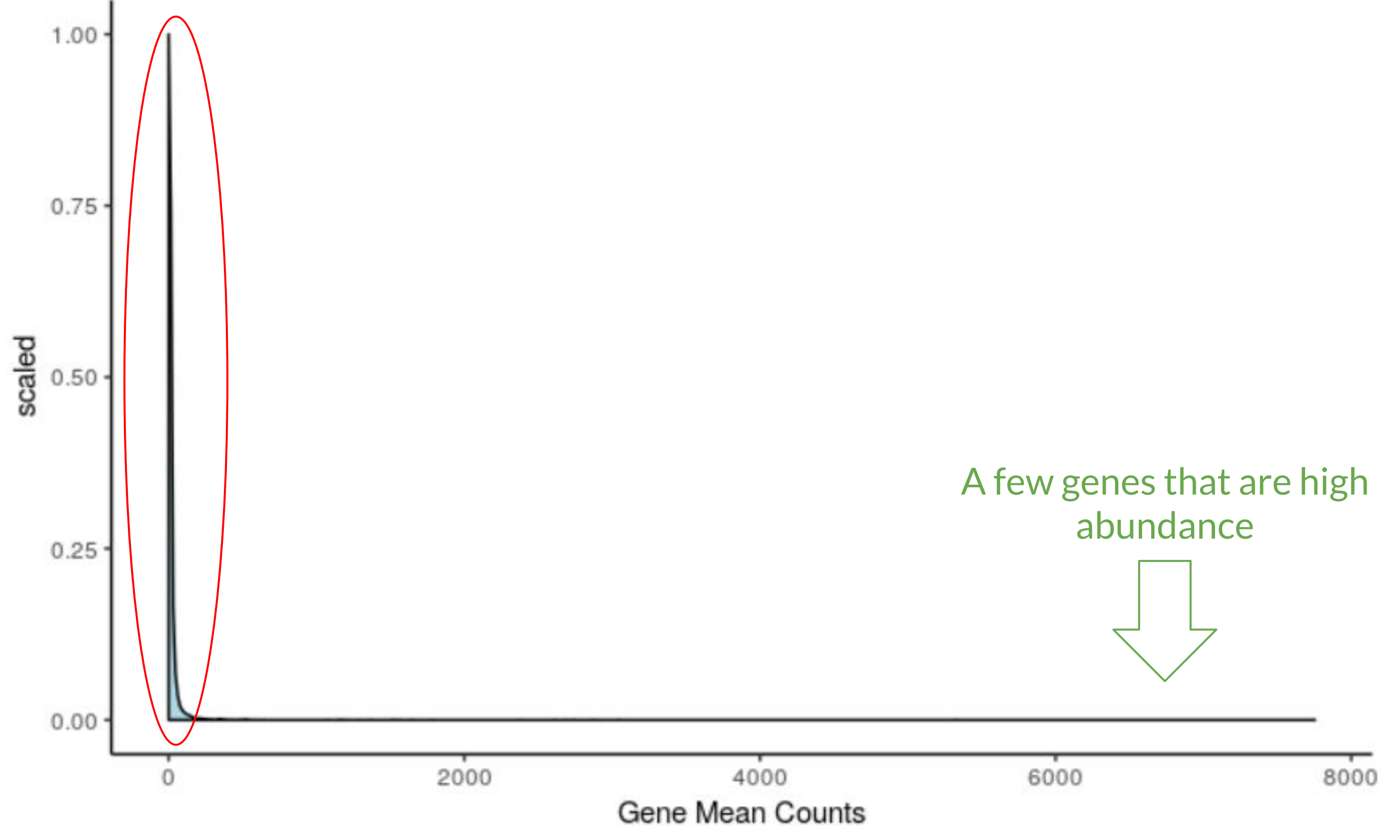
Less starting material means:

- More PCR amplification (*and its associated biases*)
- More zero counts
    - Biology - Not every gene is expressed in every cell
    - Technical - Biased capture methods, Sequencing every RNA in every cell requires a lot more sequencing

Single-cell gene mean density graph

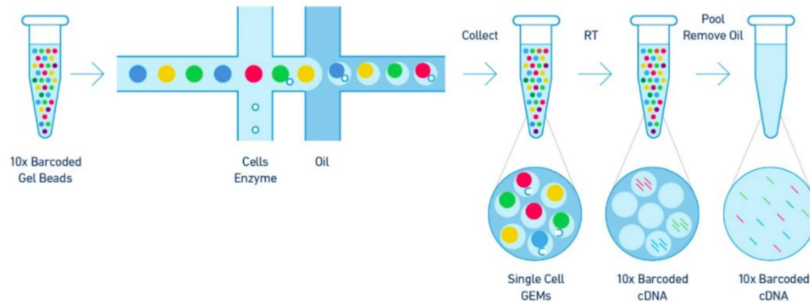


Single-cell gene mean density graph



Single Cell Basic Set-ups

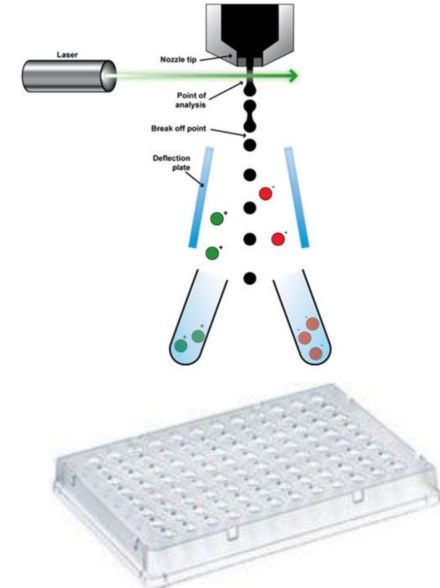
1. Tag-based scRNA-seq



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

Example: 10X Genomics Chromium
Zheng *et al.* 2017
https://www.ncbi.nlm.nih.gov/pubmed/28091601

2. Full-length scRNA-seq



Physical separation of cells *before* sequencing

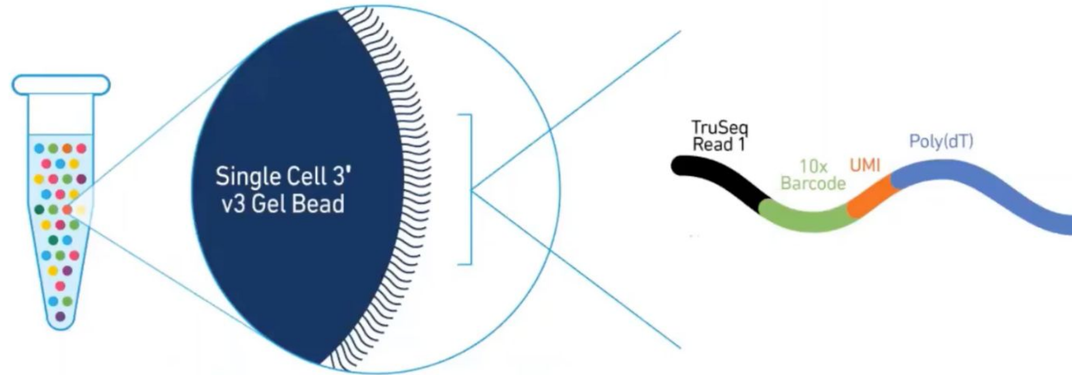
Example: Smart-seq2
Picelli *et al.* 2014
https://www.nature.com/articles/nprot.2014.006

Cell Barcodes + Unique Molecular Identifiers (UMIs) are used to label individual transcripts

Each droplet contains 1 cell, all with the same cell barcode

Within each droplet, each bead contains millions of distinct UMIs

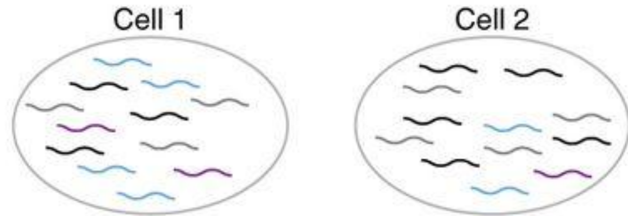
Each transcript within a cell is tagged with a cell barcode and unique molecular identifier (UMI)



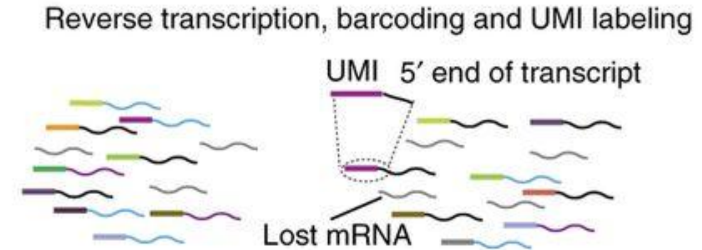
Unique Molecular Identifiers (UMIs):

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

1.



2.



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

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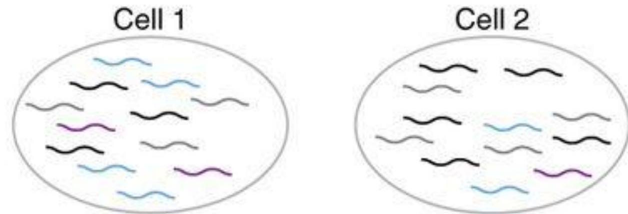

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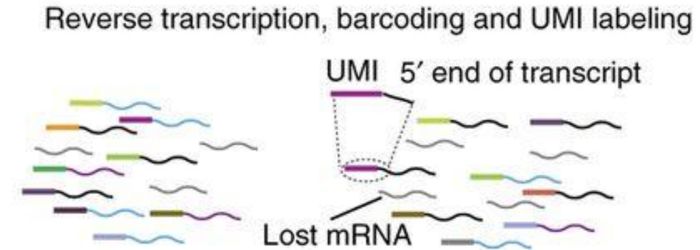
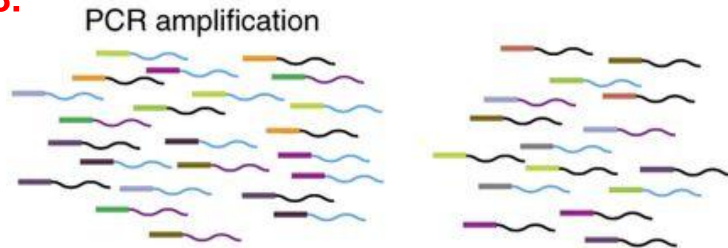


3.



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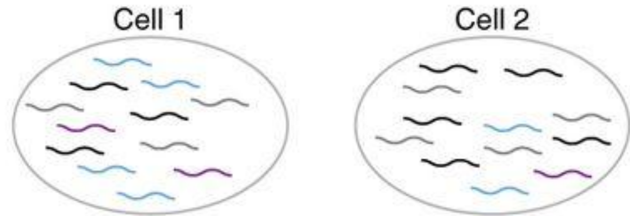

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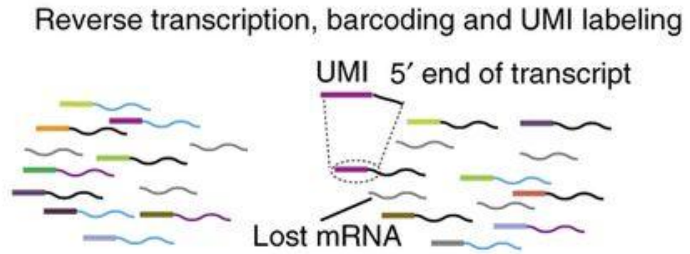

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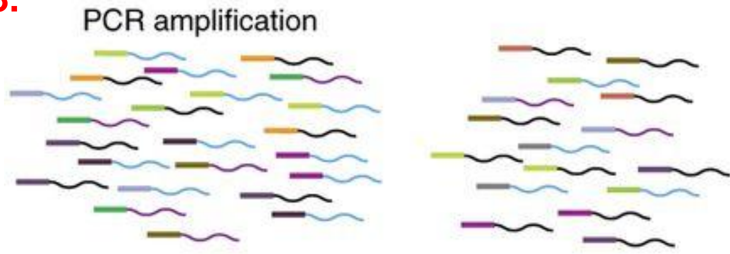
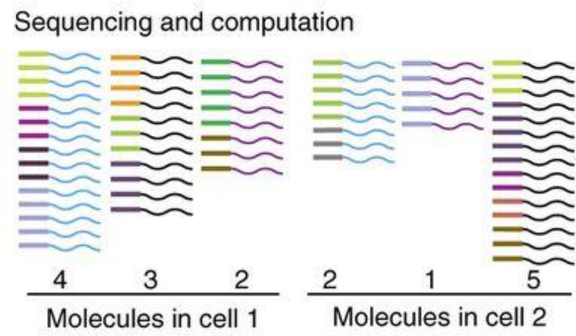


4.



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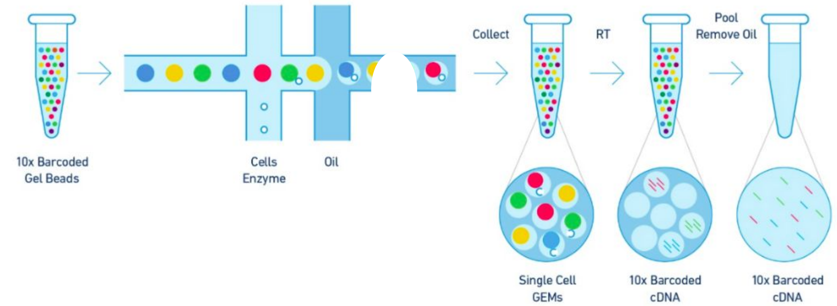
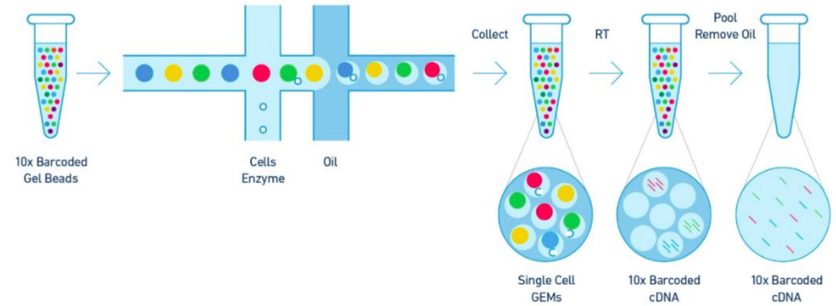
Tag-Based scRNA-seq



Pros:

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

Tag-Based scRNA-seq



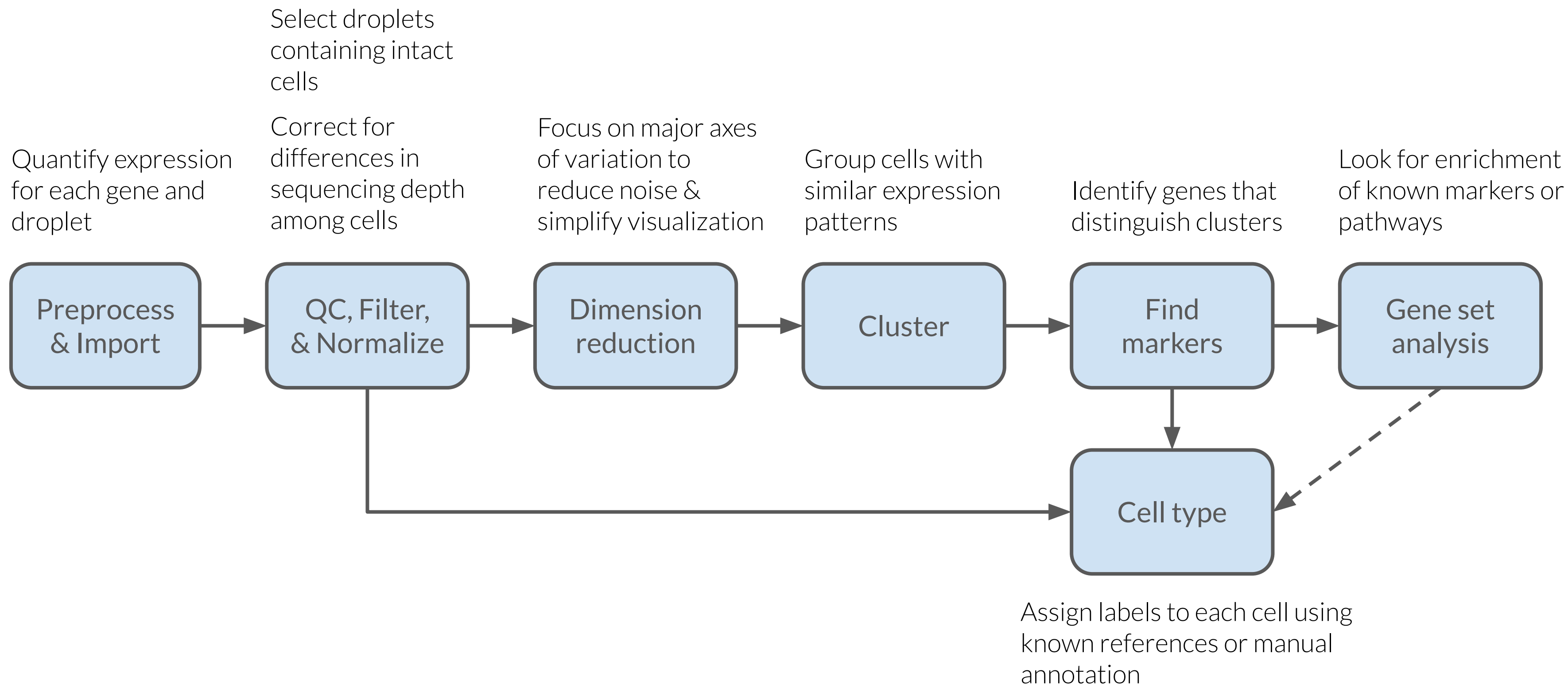
Pros:

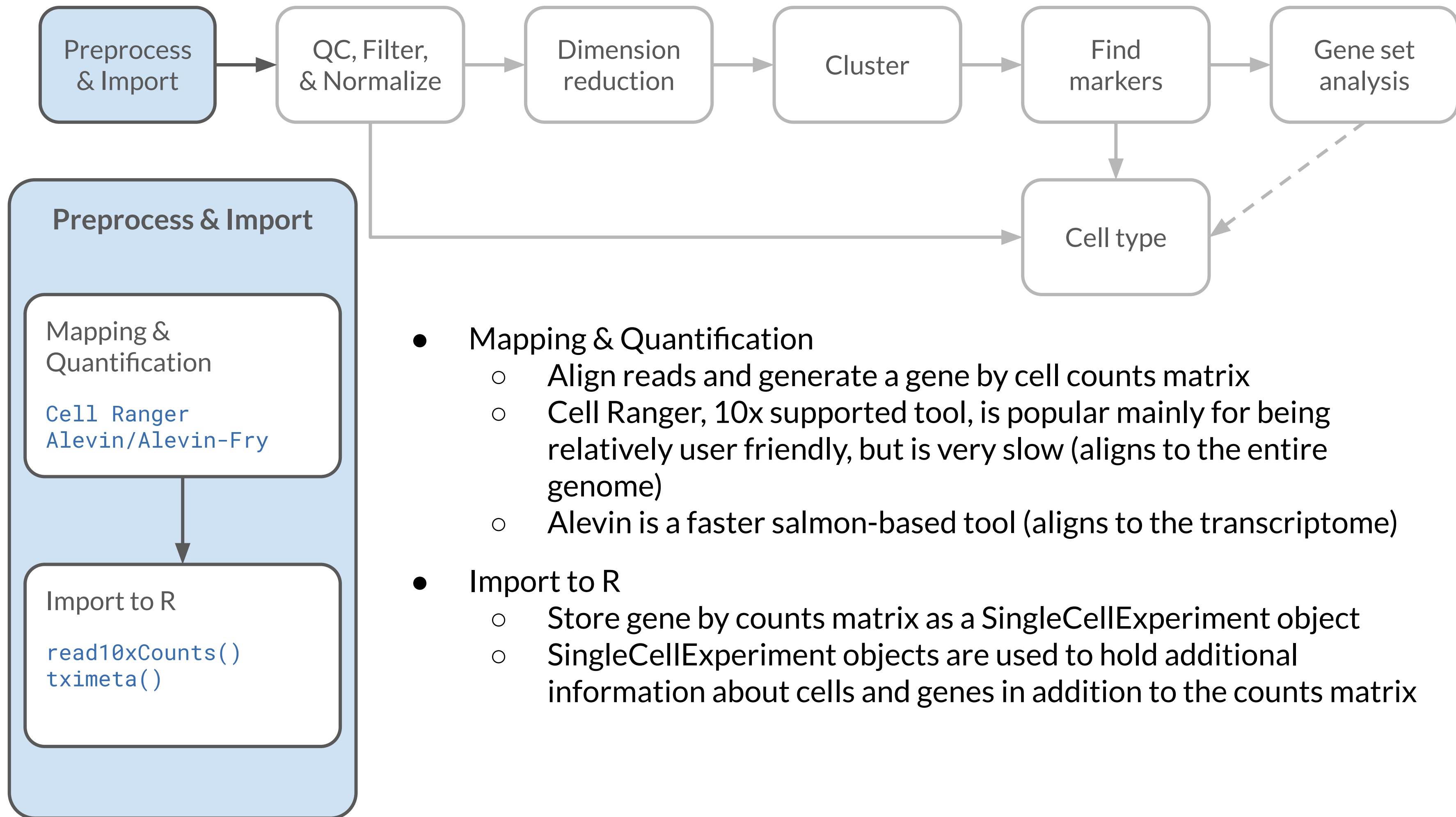
- Can profile up to millions of cells.
- Takes less computing power.
- File storage requirements are smaller.
- Much less expensive.

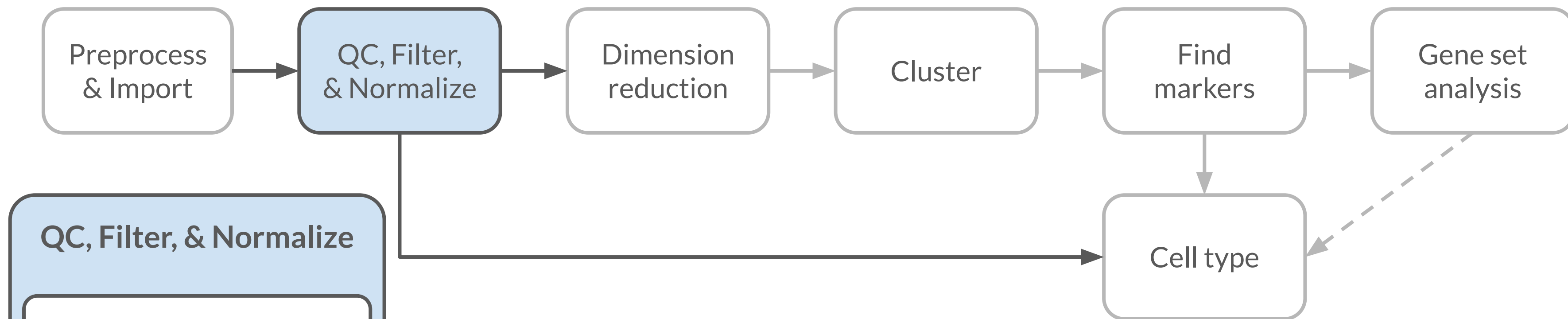
Cons:

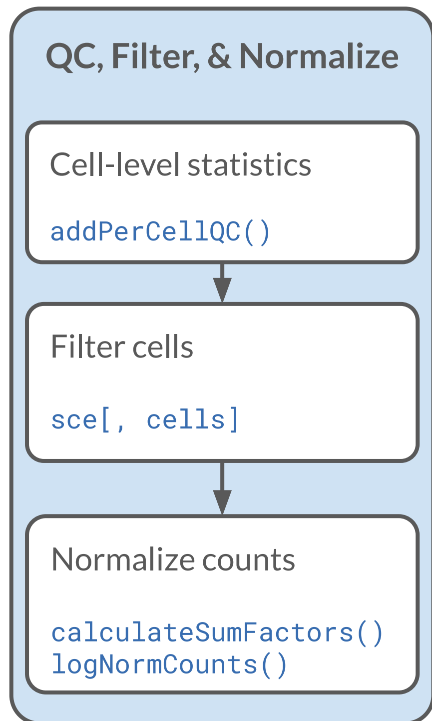
- More intense 3' bias because sequencing is not bidirectional.
- Coverage is generally not as deep as full-length scRNA-seq.

Single sample scRNA-seq overview




Preprocess & Import
QC, Filter, & Normalize
Dimension reduction
Cluster
Find markers
Gene set analysis
Cell type
Preprocess & Import
Mapping & Quantification
Cell Ranger
Alevin/Alevin-Fry
Import to R
read10xCounts()
tximeta()
Mapping & Quantification
Align reads and generate a gene by cell counts matrix
Cell Ranger, 10x supported tool, is popular mainly for being relatively user friendly, but is very slow (aligns to the entire genome)
Alevin is a faster salmon-based tool (aligns to the transcriptome)
Import to R
Store gene by counts matrix as a SingleCellExperiment object
SingleCellExperiment objects are used to hold additional information about cells and genes in addition to the counts matrix





- Cell-level statistics
  - Calculate quality metrics for each cell
    - total UMI count
    - number of detected genes
    - mitochondrial content

- Filter cells
  - Remove low quality cells from counts matrix
- Normalize counts
  - Use size factors to minimize technical differences and maximize biological differences across cells
  - Log transform counts data

Some of the many resources for you in `00-scRNA_introduction.Rmd`

- 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