

Introduction to Single-Cell RNA-seq

The CCDL

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

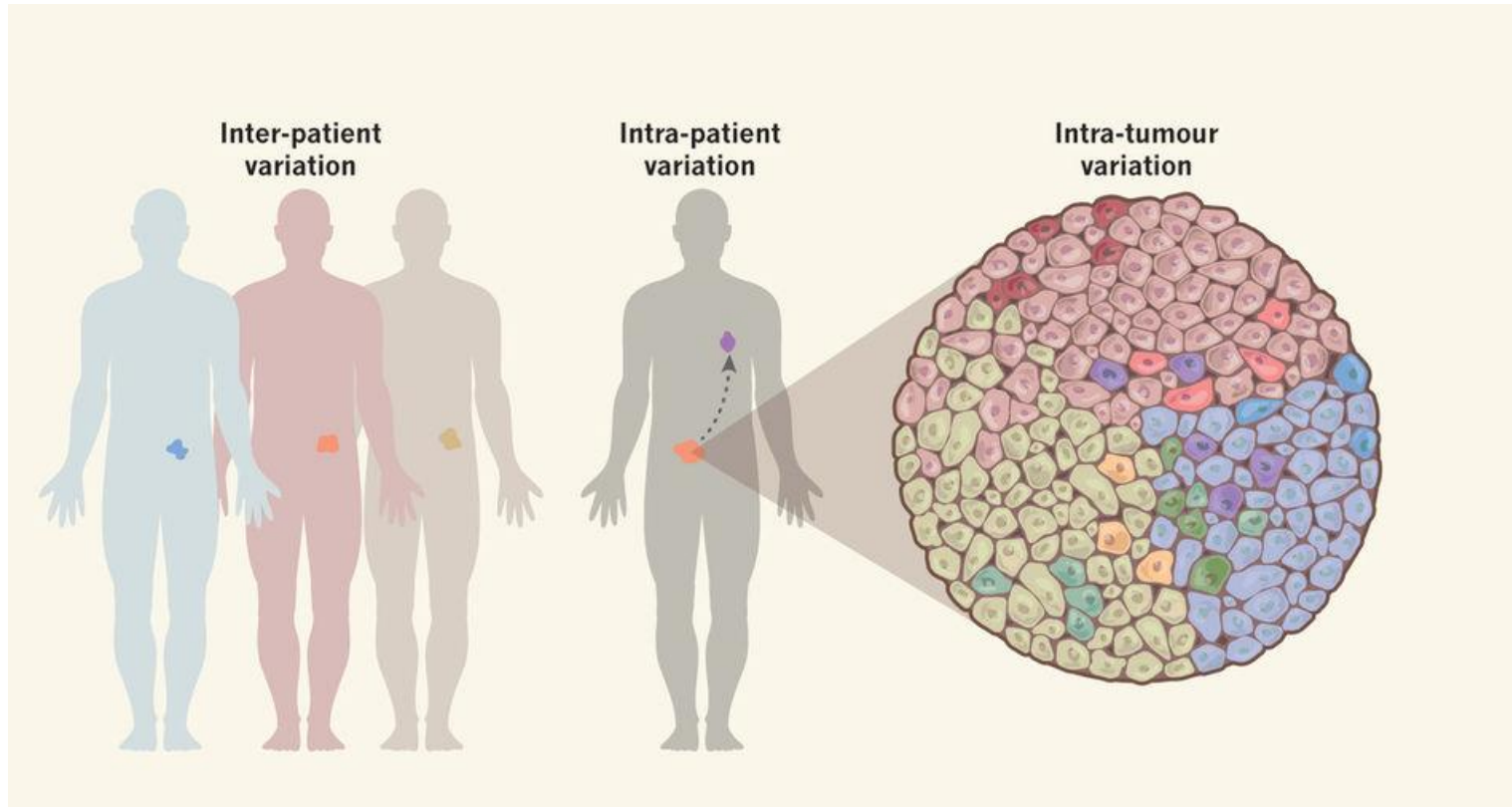


Image from Tanaka *et al.* 2018 <https://doi.org/10.1038/s41551-017-0162-1>.

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

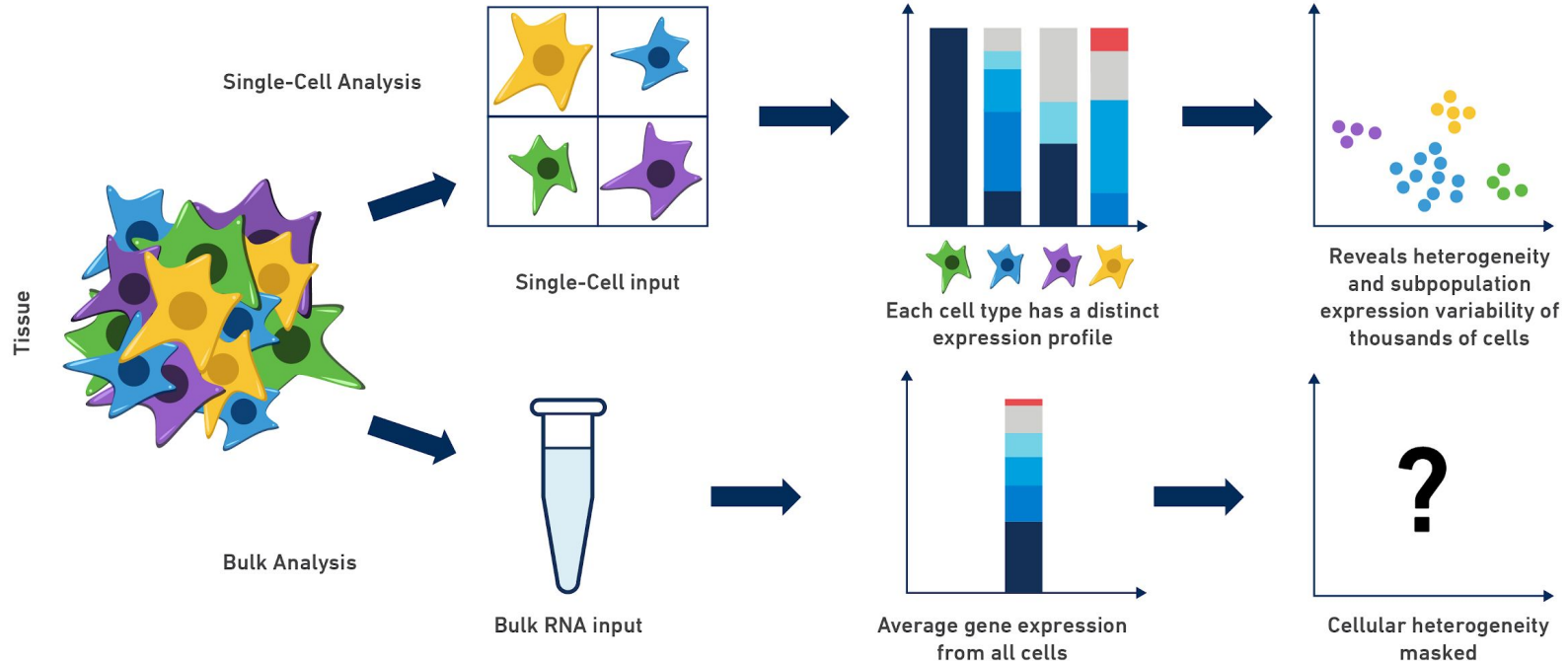


Image from 10X Genomics blog: <https://www.10xgenomics.com/blog/single-cell-rna-seq-an-introductory-overview-and-tools-for-getting-started>

Single-cell RNA-seq quirks

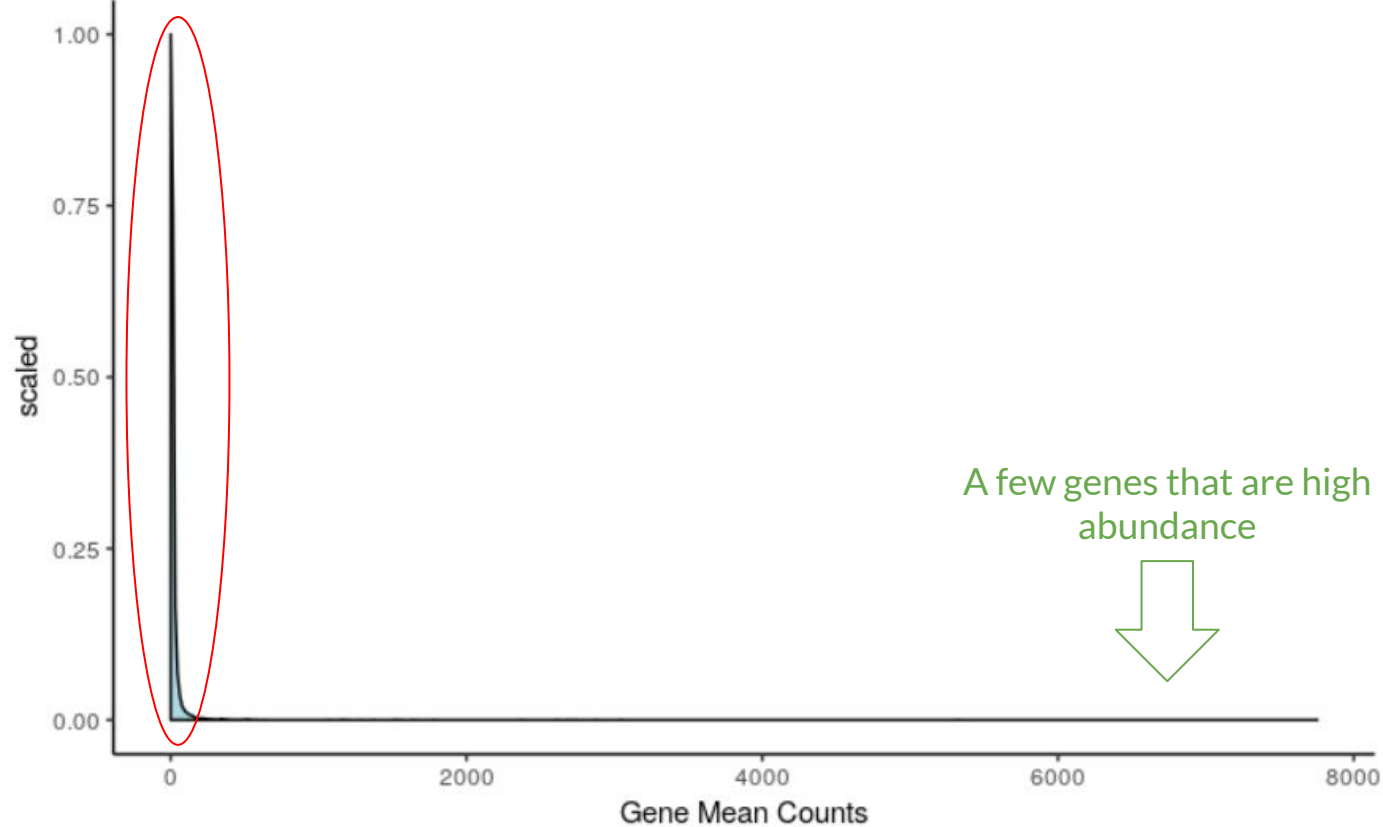
Less starting material means:

- 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

Choi et al. (Genome Biology, 2020) <https://genomebiology.biomedcentral.com/articles/10.1186/s13059-020-02103-2>

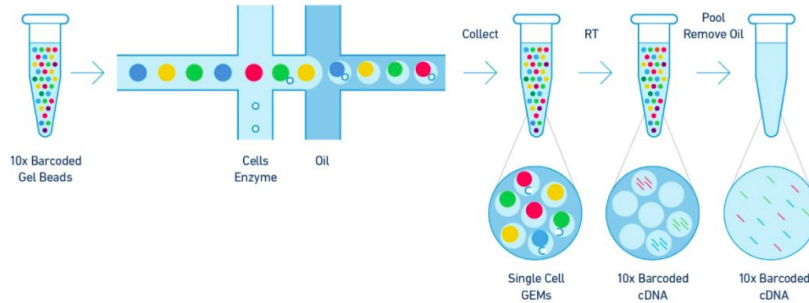
Single-cell gene mean density graph

A lot of genes that are low abundance



Single Cell Basic Set-ups

1. Tag-based scRNA-seq



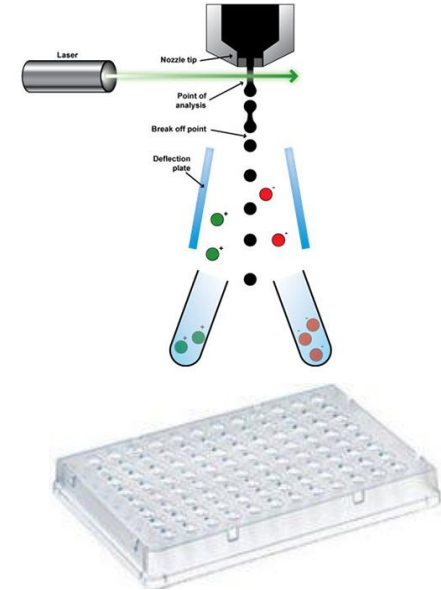
Tag-based
separation
of cells' data *after*
sequencing

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

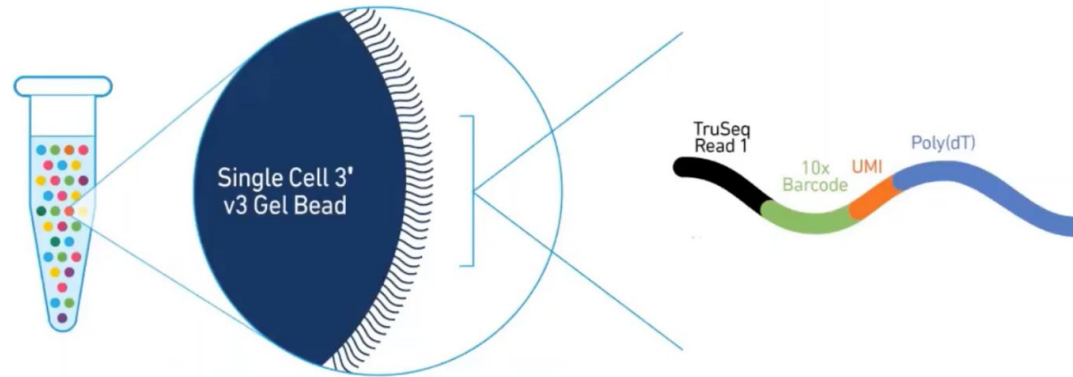
<https://www.nature.com/articles/nprot.2014.006>

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

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

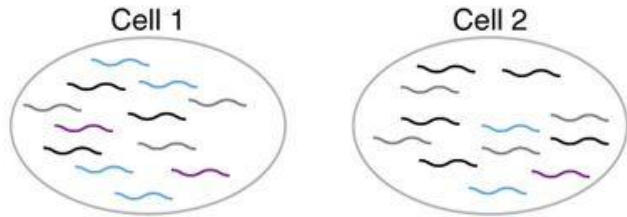
Within each droplet, each bead contains millions of distinct UMIs

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

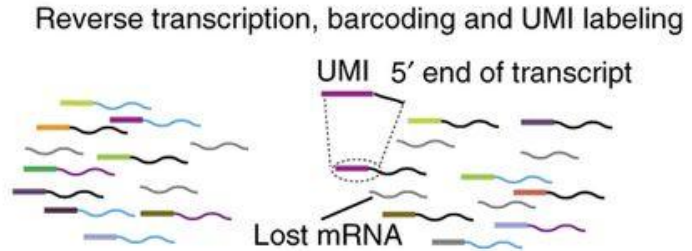


Unique Molecular Identifiers (UMIs): a 'snapshot' of the original molecules in the *pre-amplified* cell

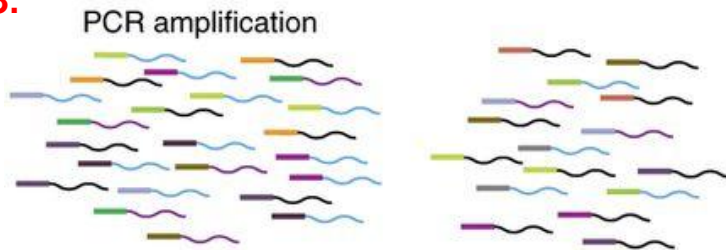
1.



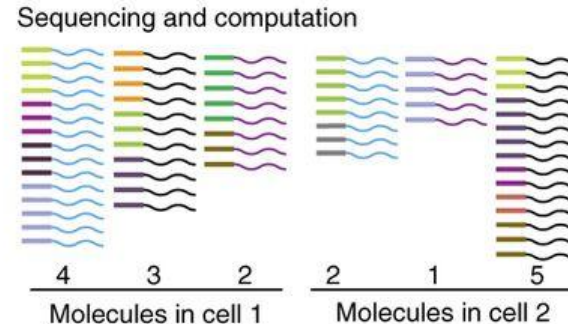
2.



3.



4.



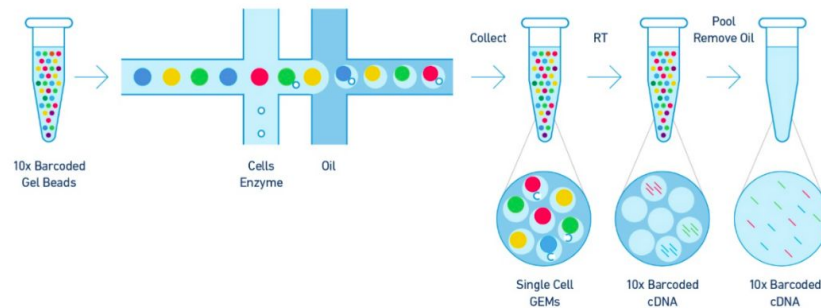
Tag-Based scRNA-seq

Pros:

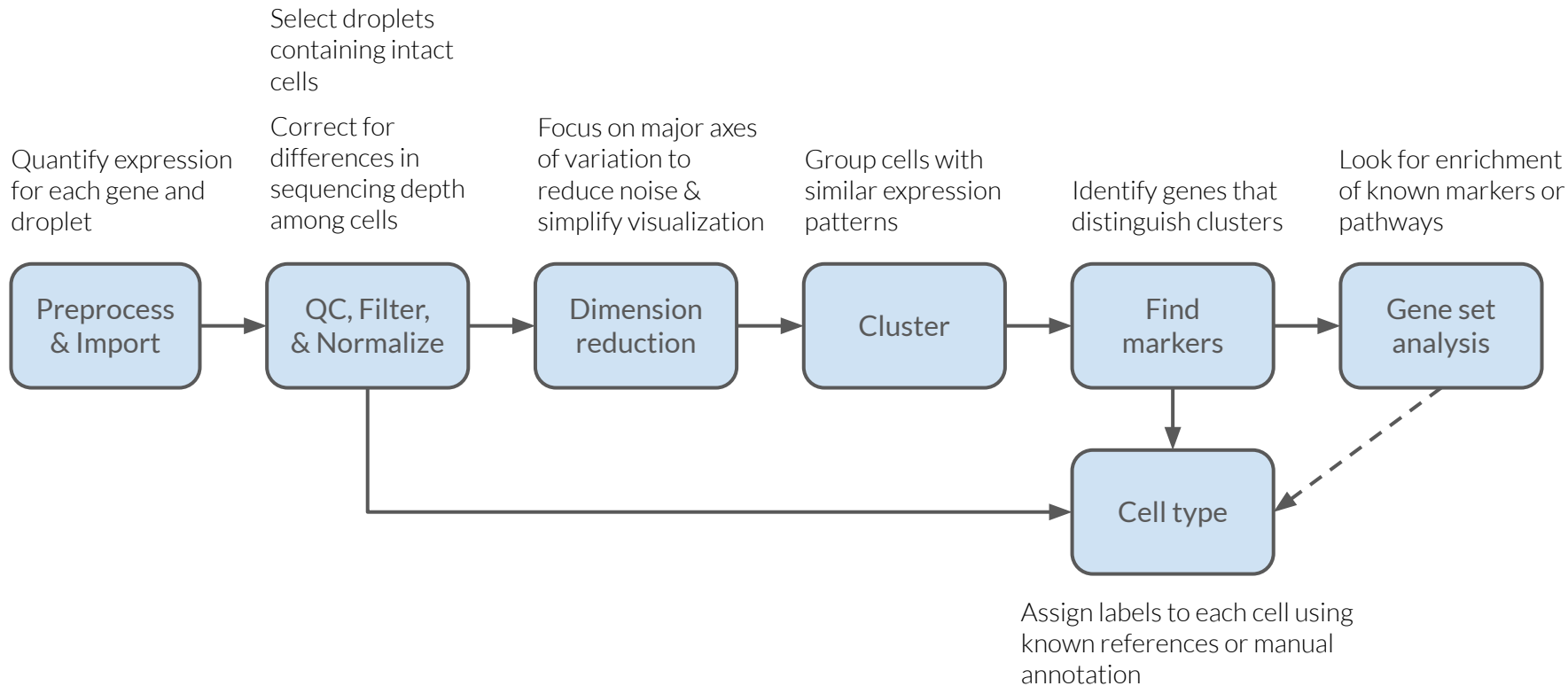
- Can profile up to millions of cells.
- Takes less computing power.
- File storage requirements are smaller.
- Much less expensive.

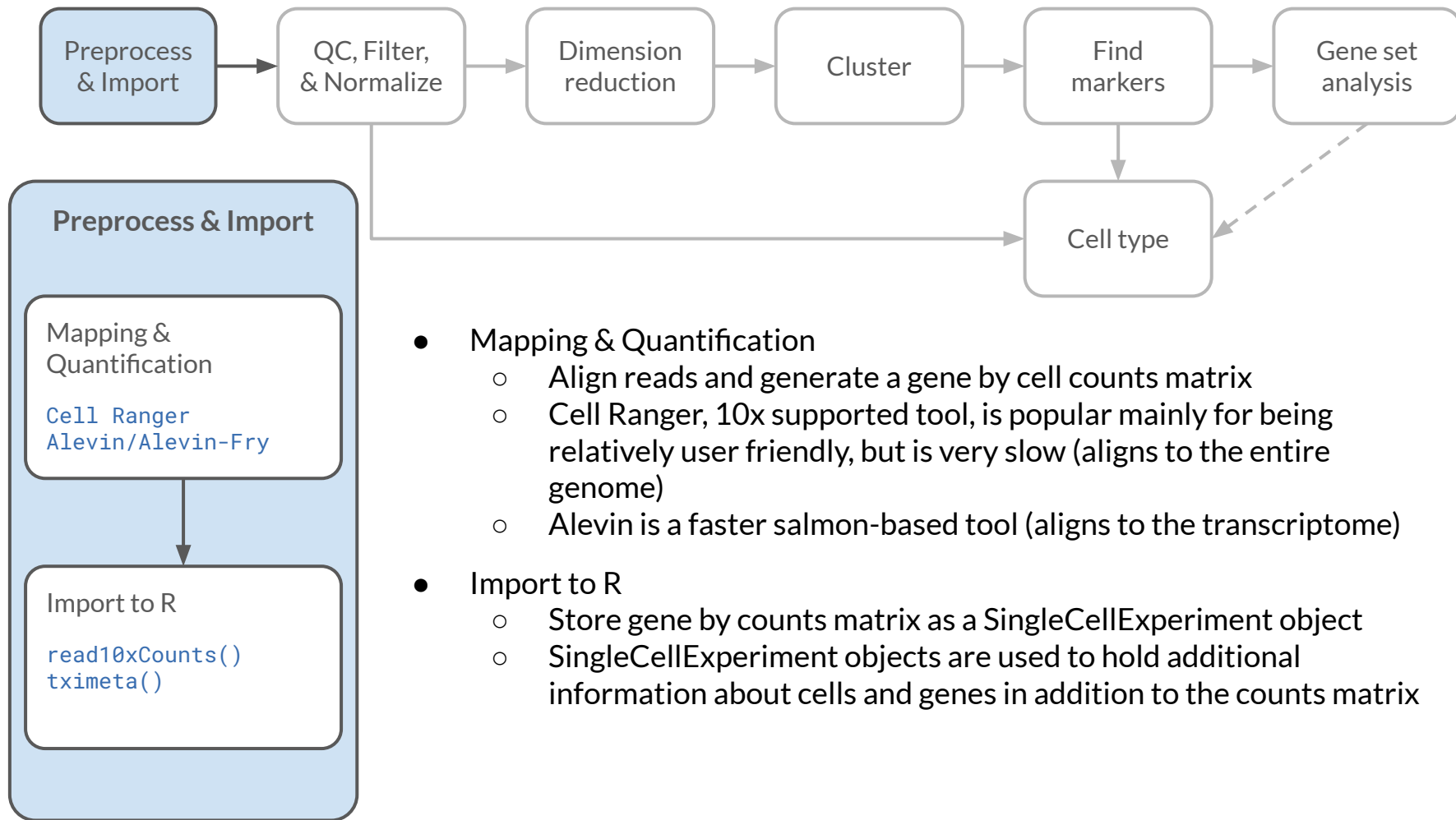
Cons:

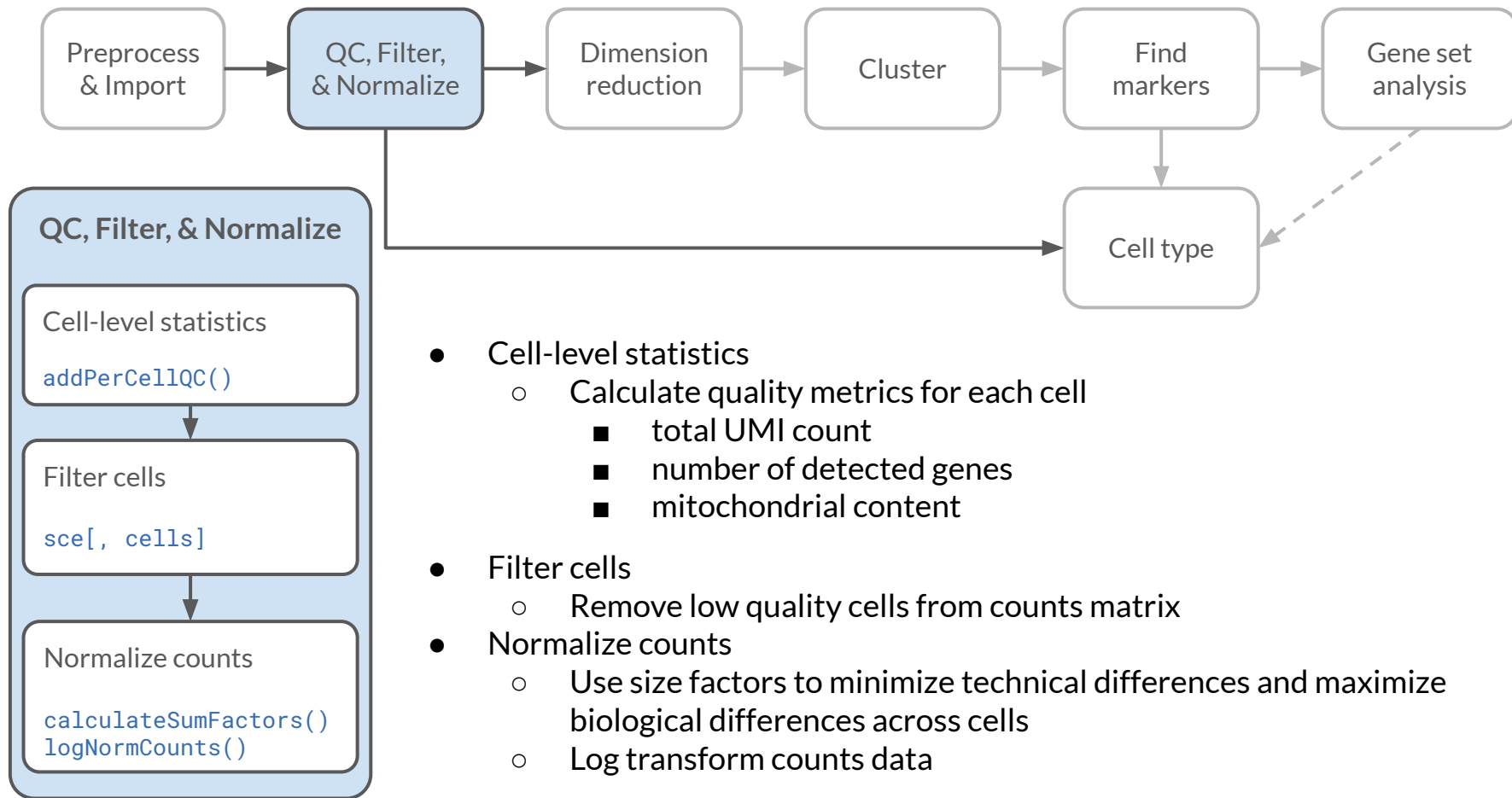
- More intense 3' bias because sequencing is not bidirectional.
- Coverage is generally not as deep as full-length scRNA-seq.



Single sample scRNA-seq overview







Some of the many resources for you in `00-scRNA-seq_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](#)