

Single cell transcriptomics

Introduction to single cell RNA-seq

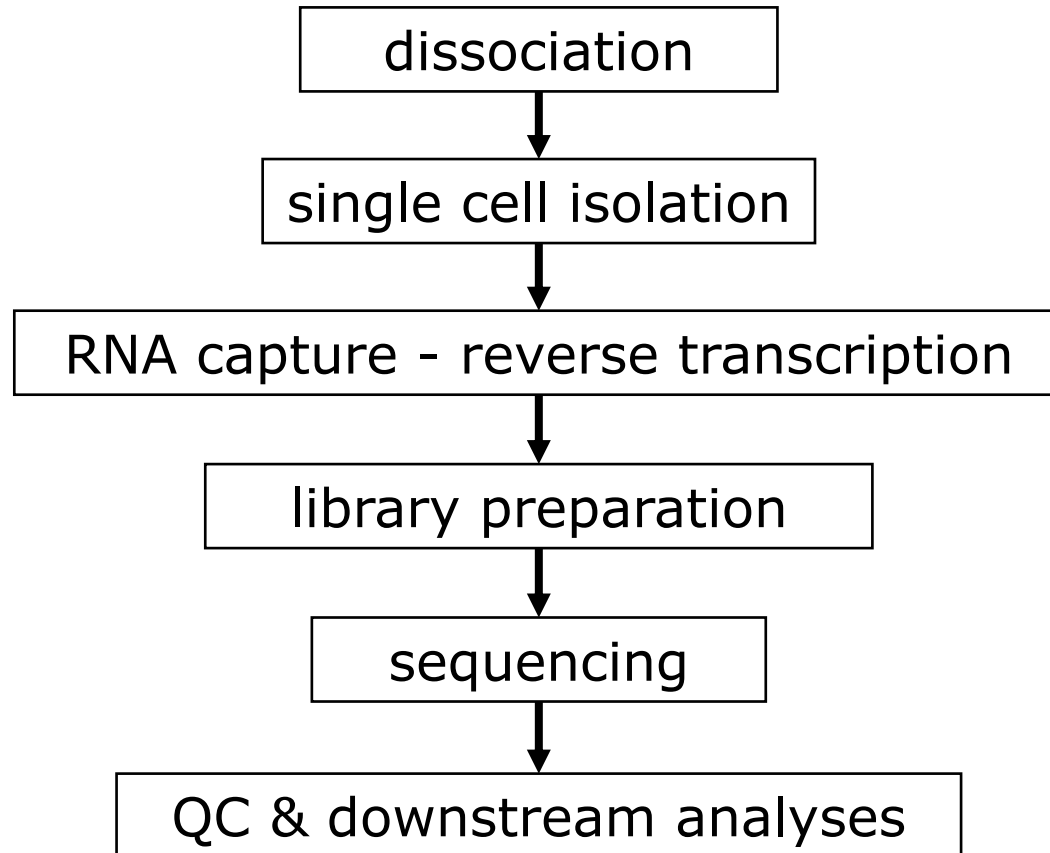


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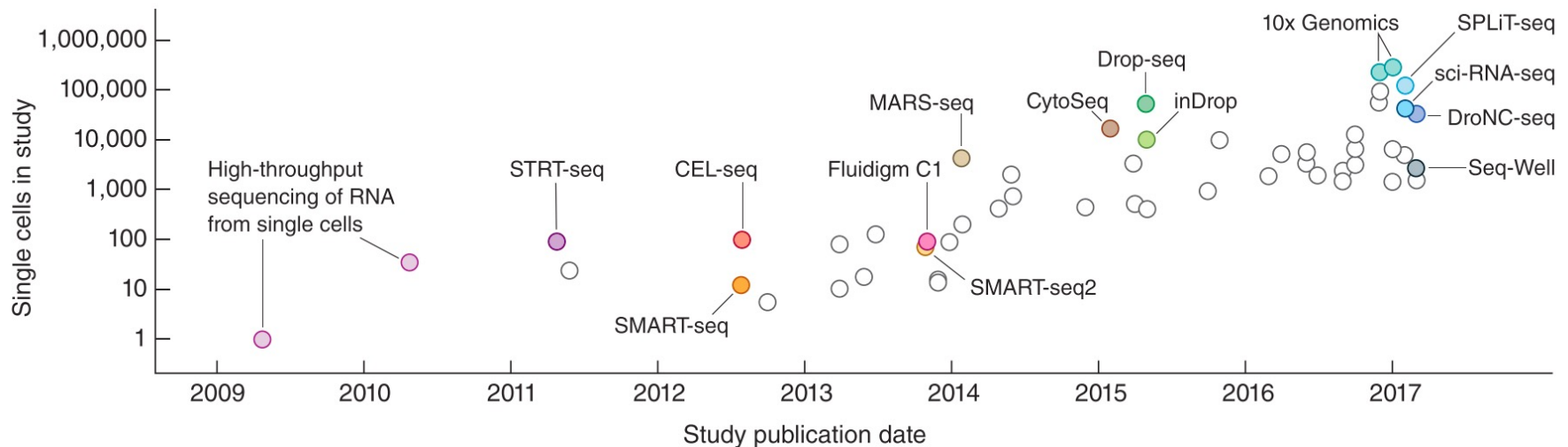
Why single cell RNAseq?

- Cell specific effects of treatments
- Determine gene regulatory relationships
- Understand drug action/delivery
- Understand differences in cell populations
- ...

scRNA-seq workflow



Technologies



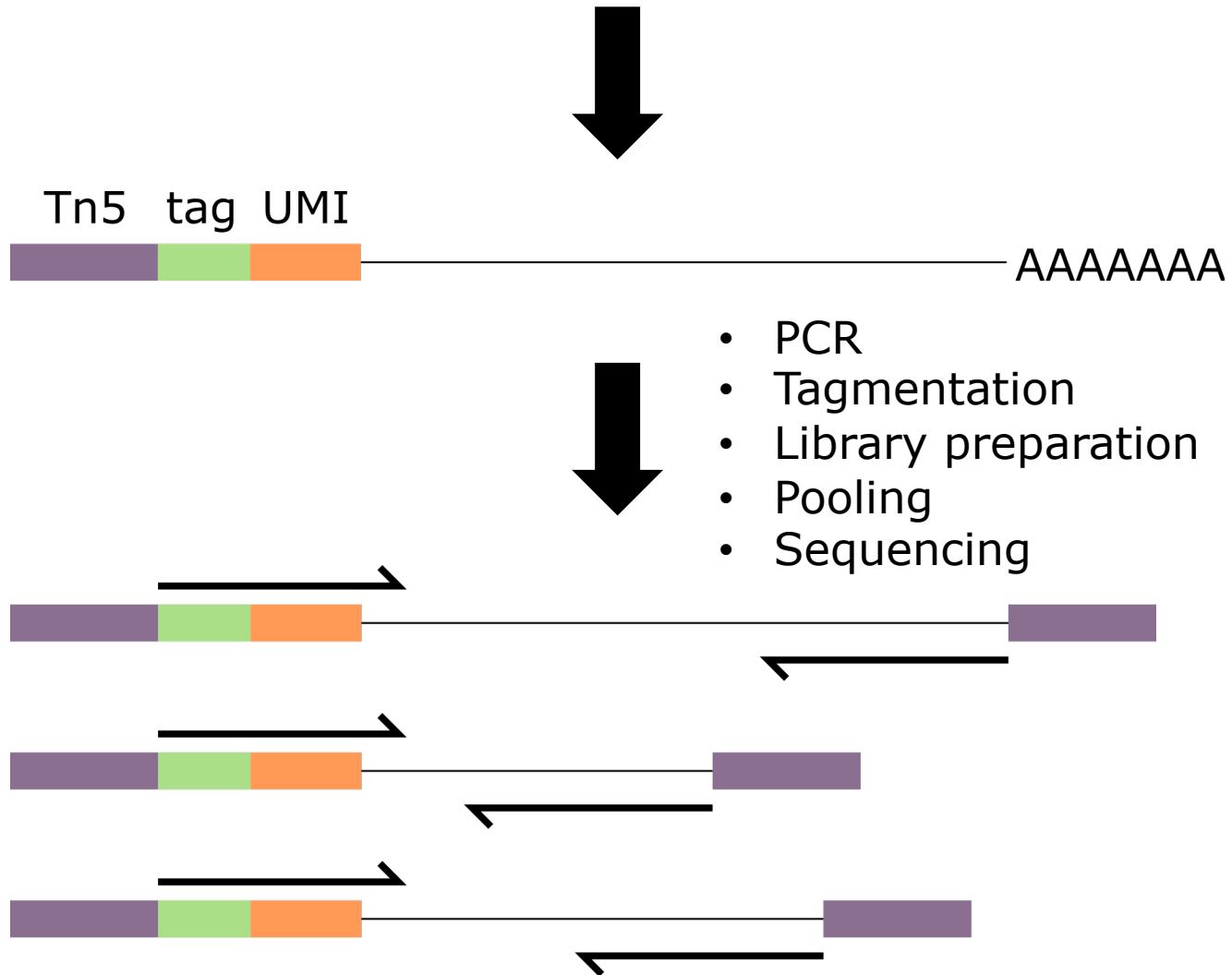
- Plate-based: SMART-seq
- Droplet-based: 10x genomics (3' kit)

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

SMART-seq3

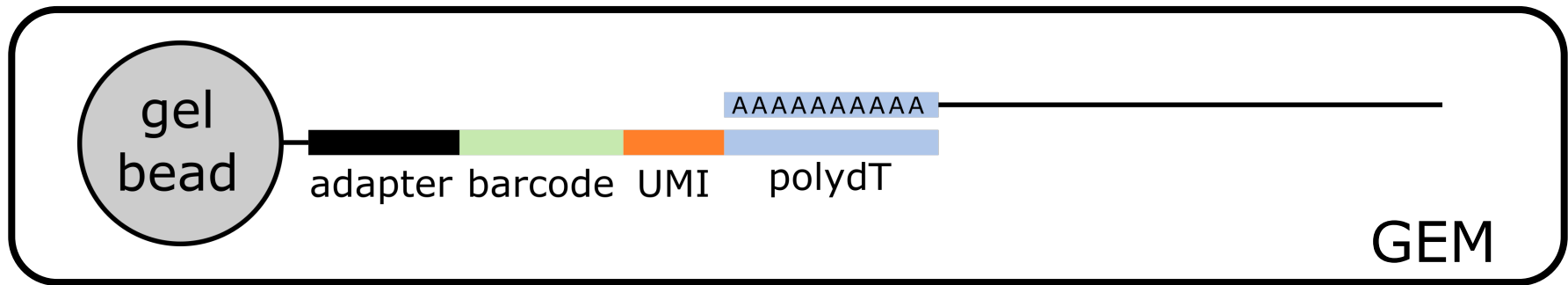
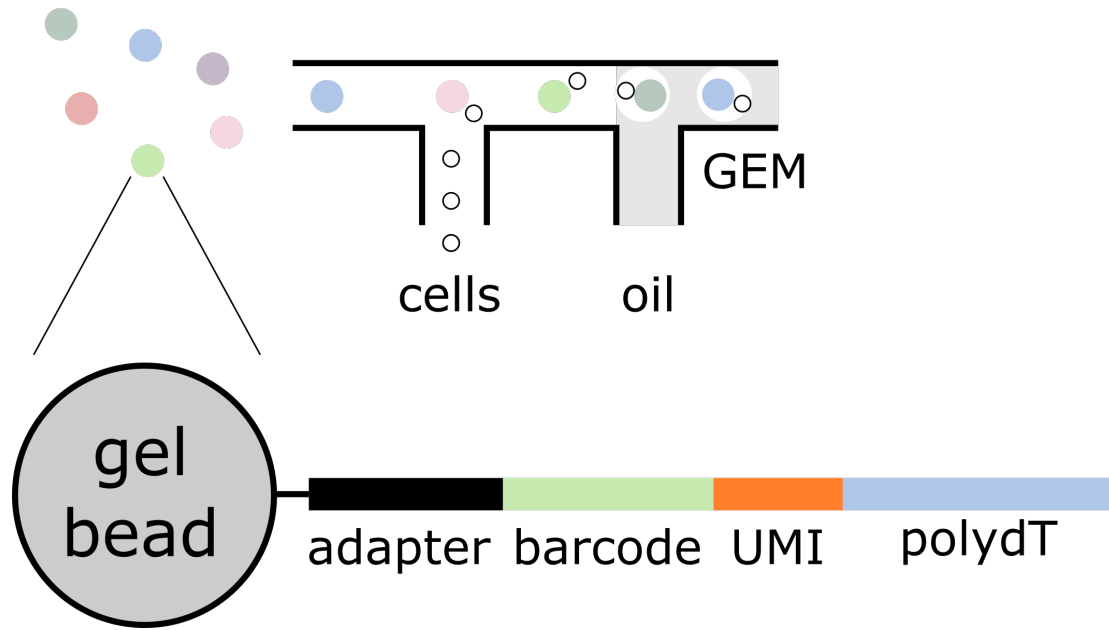
- Use FACS for: 1 well = 1 cell
- Library preparation per cell
- Paired-end sequencing enables isoform reconstruction

Reverse transcription + template switching

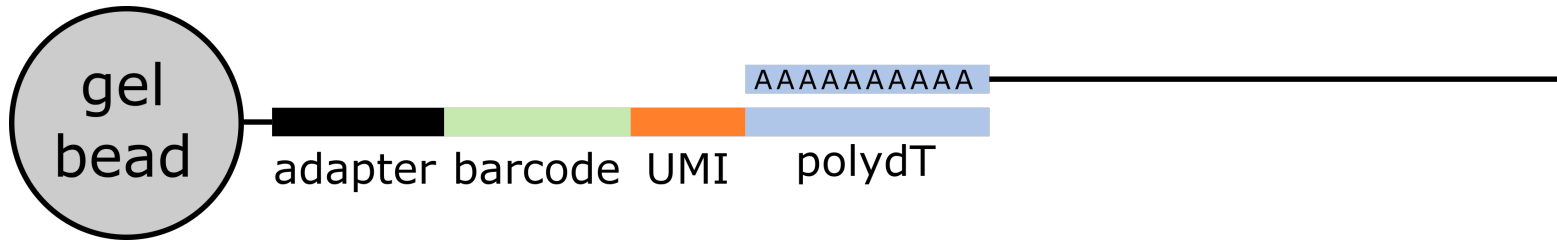


10x genomics (3' kit)

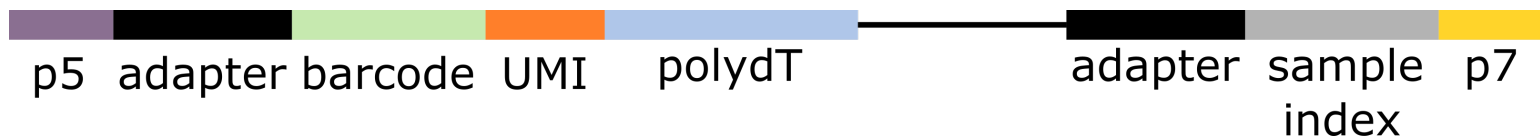
- Cell isolation within oil droplet
- RNA capture with 1 bead/cell
- Sequencing from 3' end
- Only expression



All captured **transcripts** from **single** cell:
identical + unique barcode

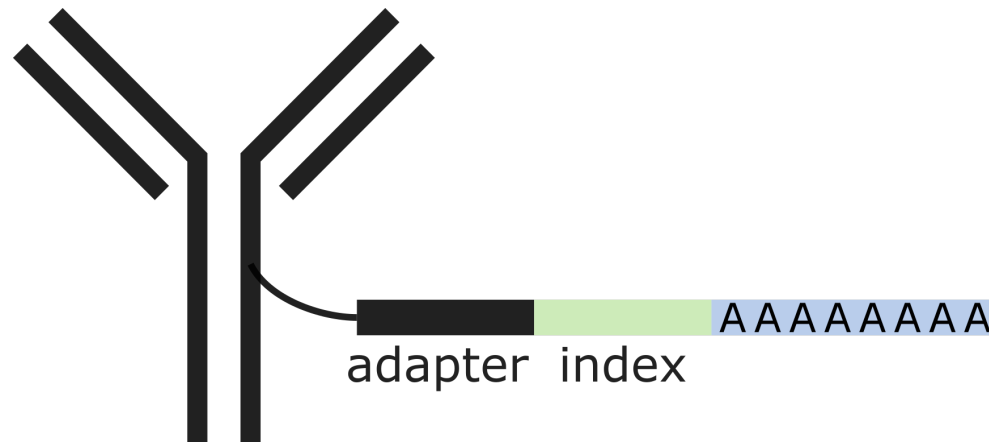


- reverse transcription
- breaking GEMs
- fragmentation
- primer ligation
- index PCR



Droplet (10x genomics)	SMART-seq
3' poly-A bead capture	polydT reverse transcription
Strong bias 3' end	Whole transcript coverage
Expression analysis	Expression + isoform analysis
Low # transcripts/cell	High # transcripts/cell
Investment (cell sorter)	Only FACS needed
10-100k cells	up to 1k cells
1 sample \approx 1 library	1 cell = 1 library
Isolation by droplets - doublets	Isolation by FACS – bias to large cells
Low cost/cell	High cost/cell

Quantify proteins

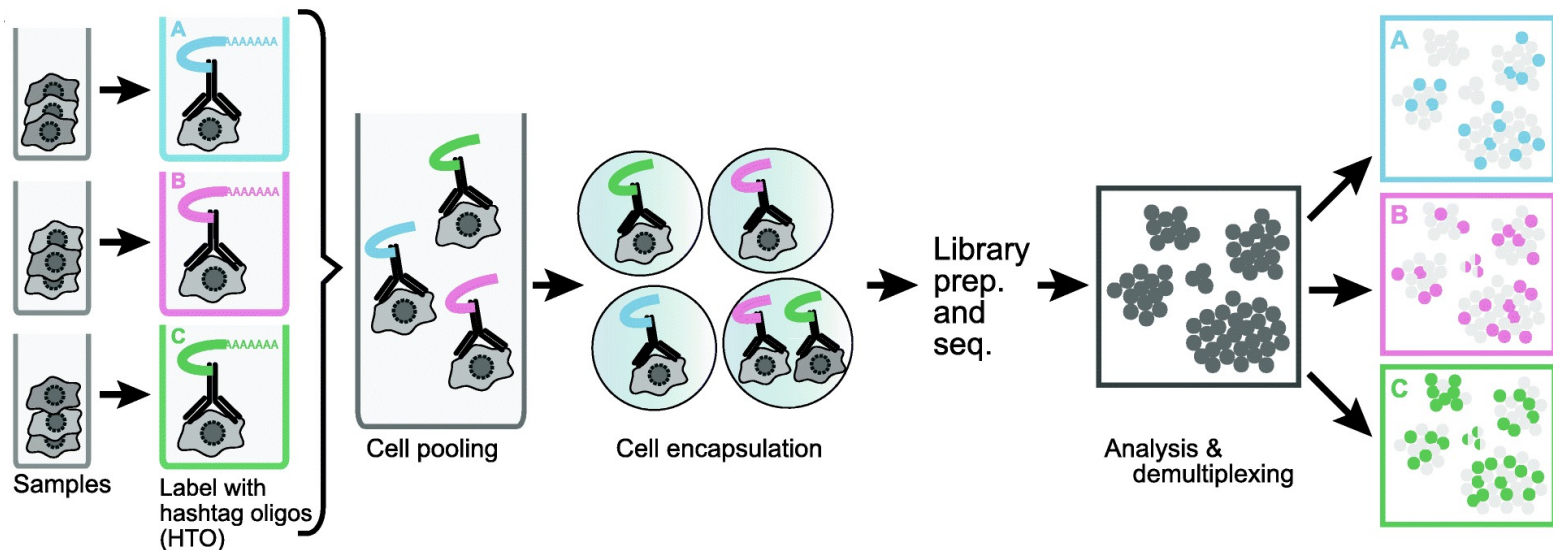


- Quantification of (cell-surface) proteins
- Together with transcriptome

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.

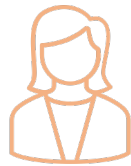
Experimental design

- Replication, randomization and blocking
- Be aware of confounding factors, e.g.:
 - Person performing handling
 - Reagents
 - Sequencing lane/library
- Record any confounding factor for downstream batch correction



Further reading:

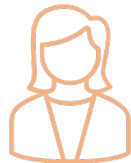
- <https://doi.org/10.3389/fcell.2018.00108>
- <https://doi.org/10.1093/bib/bby007>



treated 1	treated 2
treated 3	treated 4



control 1	control 2
control 3	control 4



control 3	control 2
treated 1	treated 4



control 1	treated 3
treated 2	control 4

After sequencing (pre-processing)

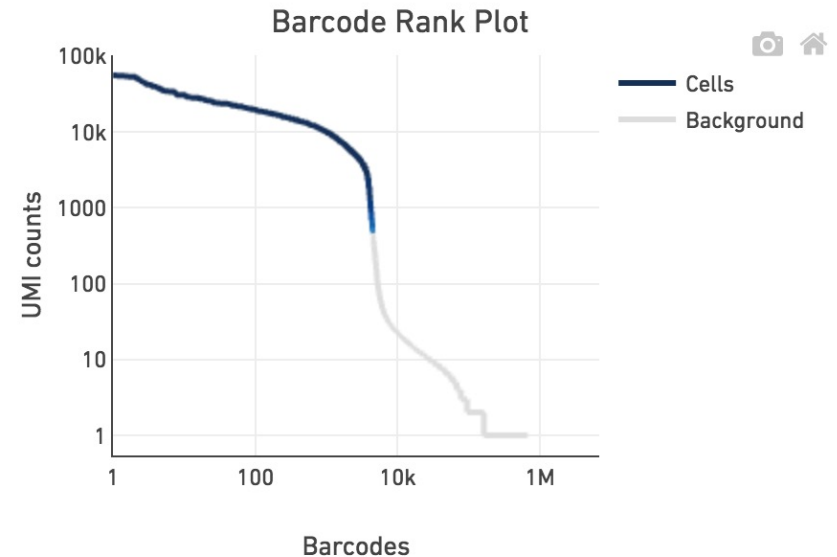
1. Demultiplexing
2. Alignment
3. Quantification: # UMI/gene
4. Cell calling

For 10x (human/mouse/rat)
all with cellranger

Cell calling

Dropouts: low #UMI/cell

Cells ?



genes ->

cells ->

	ATAC-1	CCG-1	GCGA-1
RPL22	5	13	3
PARK7	0	9	3
ENO1	1	35	0
PLA2G2A	0	0	0
CAMK2N1	0	6	0
CDC42	0	8	1
C1QA	0	0	25
C1QC	0	0	25
C1QB	0	0	29
ID3	0	35	0
RPL11	18	29	16
CLIC4	0	4	0

Further cell filtering

- Find doublets (e.g. with DoubletFinder)
- % UMI in mitochondrial genes:
 - Points to dying/stressed cells
 - Filter by threshold (e.g. 20%)
- Number of detected genes
 - Can point to dropouts/doublets

Normalization & scaling

- **Normalization** (per cell): remove technical effects (i.e. library size)
- **Scaling** (per gene): standardize range, mean and variance
- Both are mainly for the purpose of clustering and annotation