Introduction to single cell RNAseq

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TRANSCRIPTOMICS
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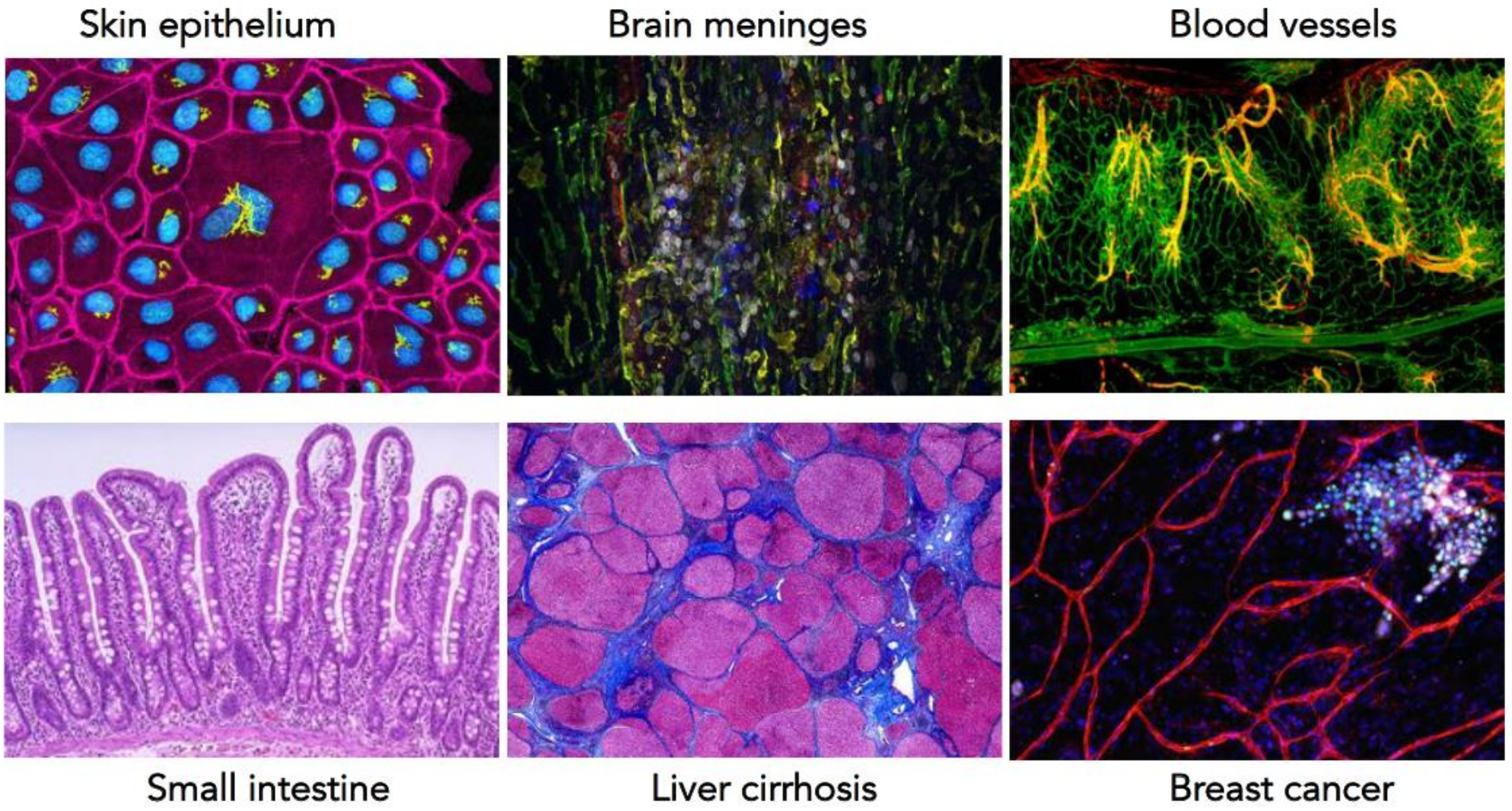
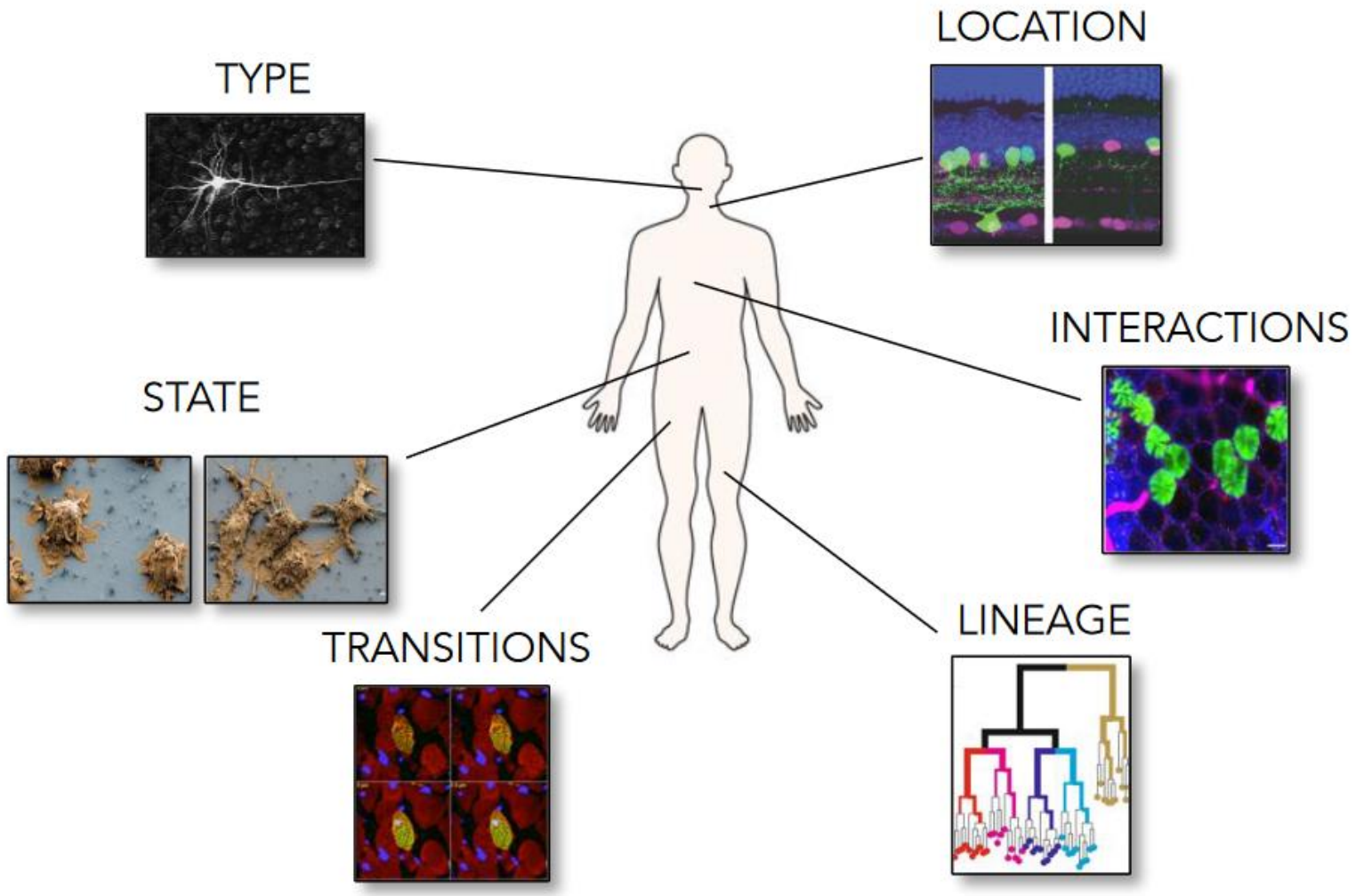
An incredible diversity of cells across human tissues



http://www.cell.com/pictureshow/skin
https://library.med.utah.edu/WebPath/webpath.html

A cell's identity and fate are shaped by many features



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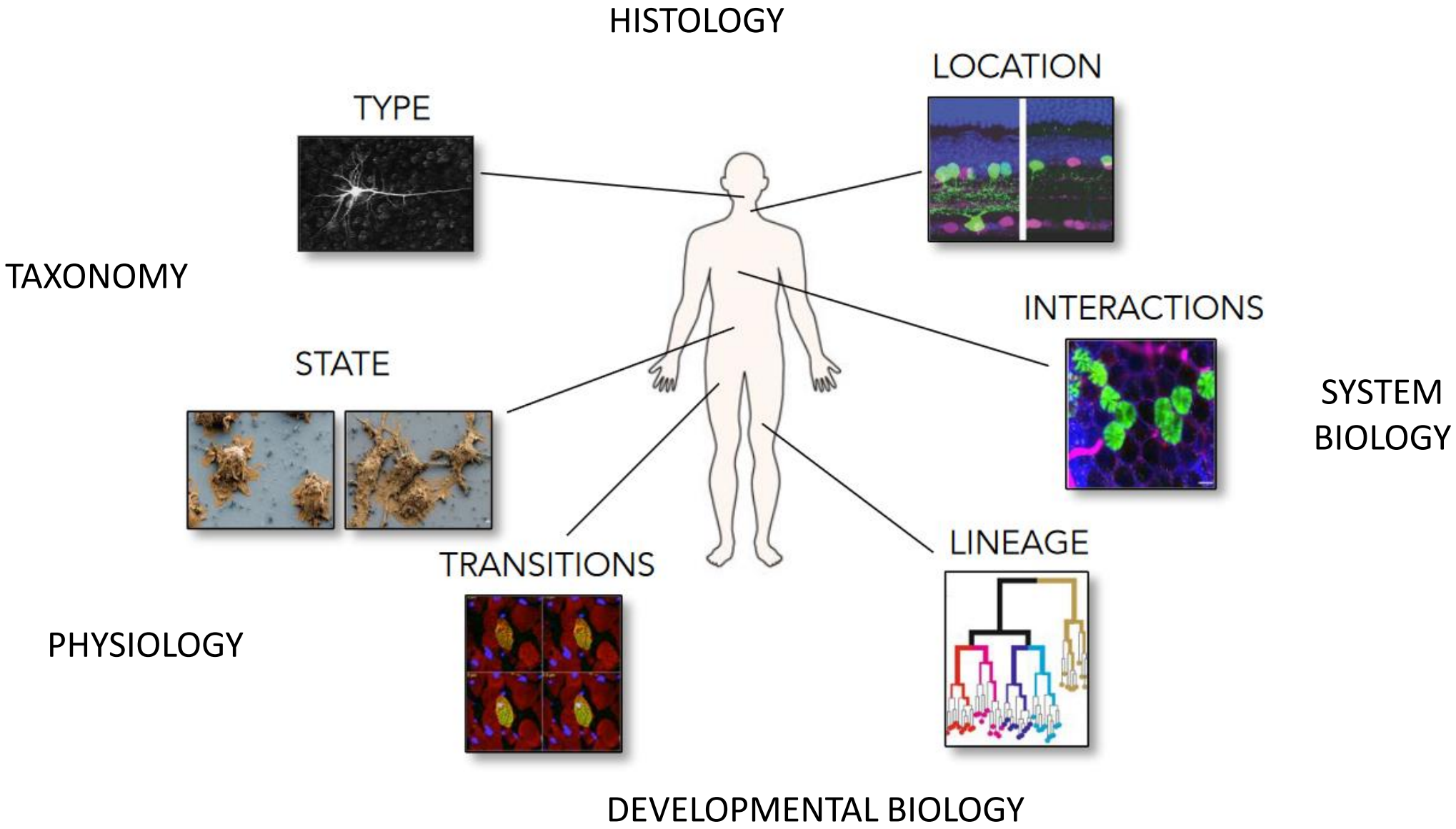

Single-cell RNA sequencing has grown exponentially

Svensson *et al.*, *Nature Protocols*, 2018

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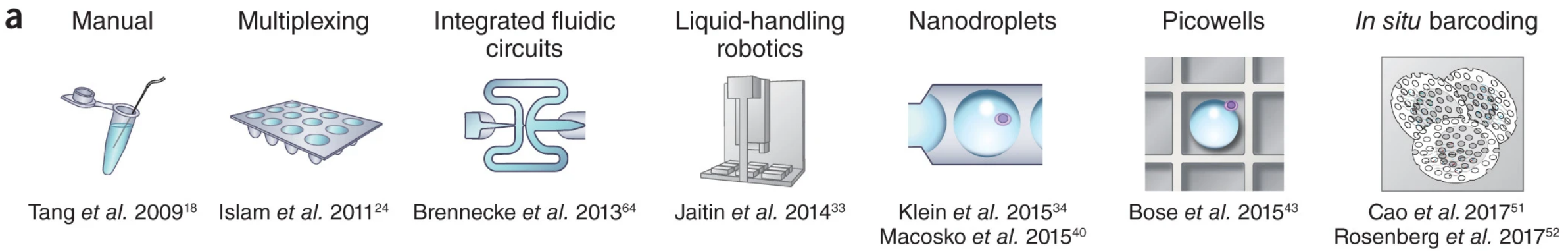
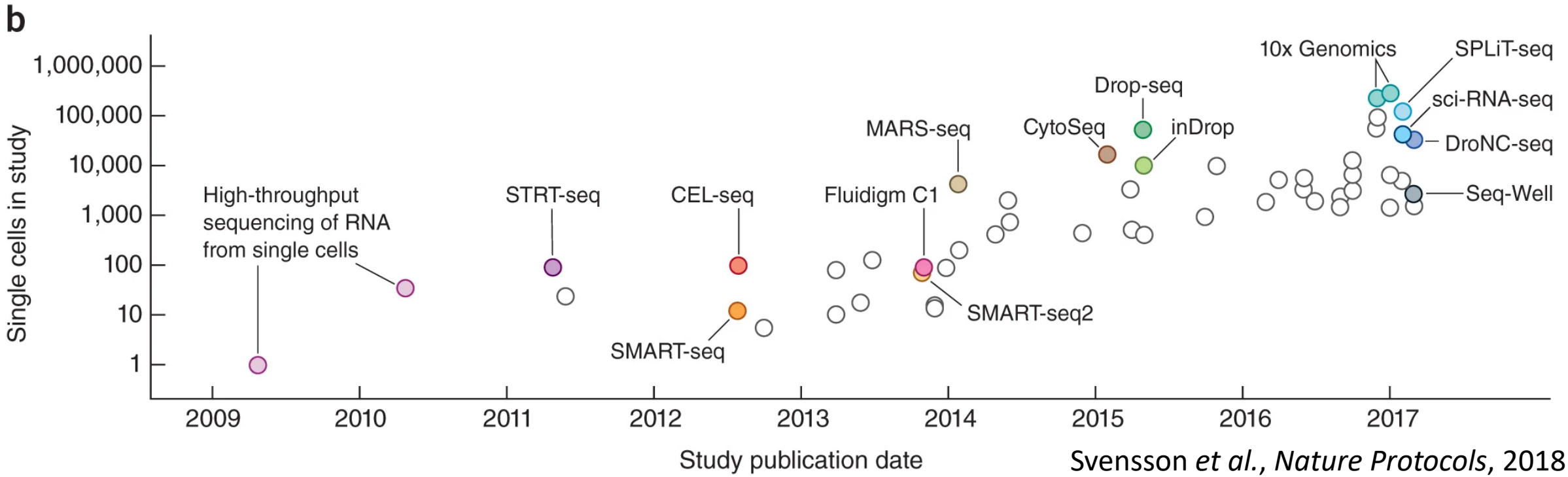
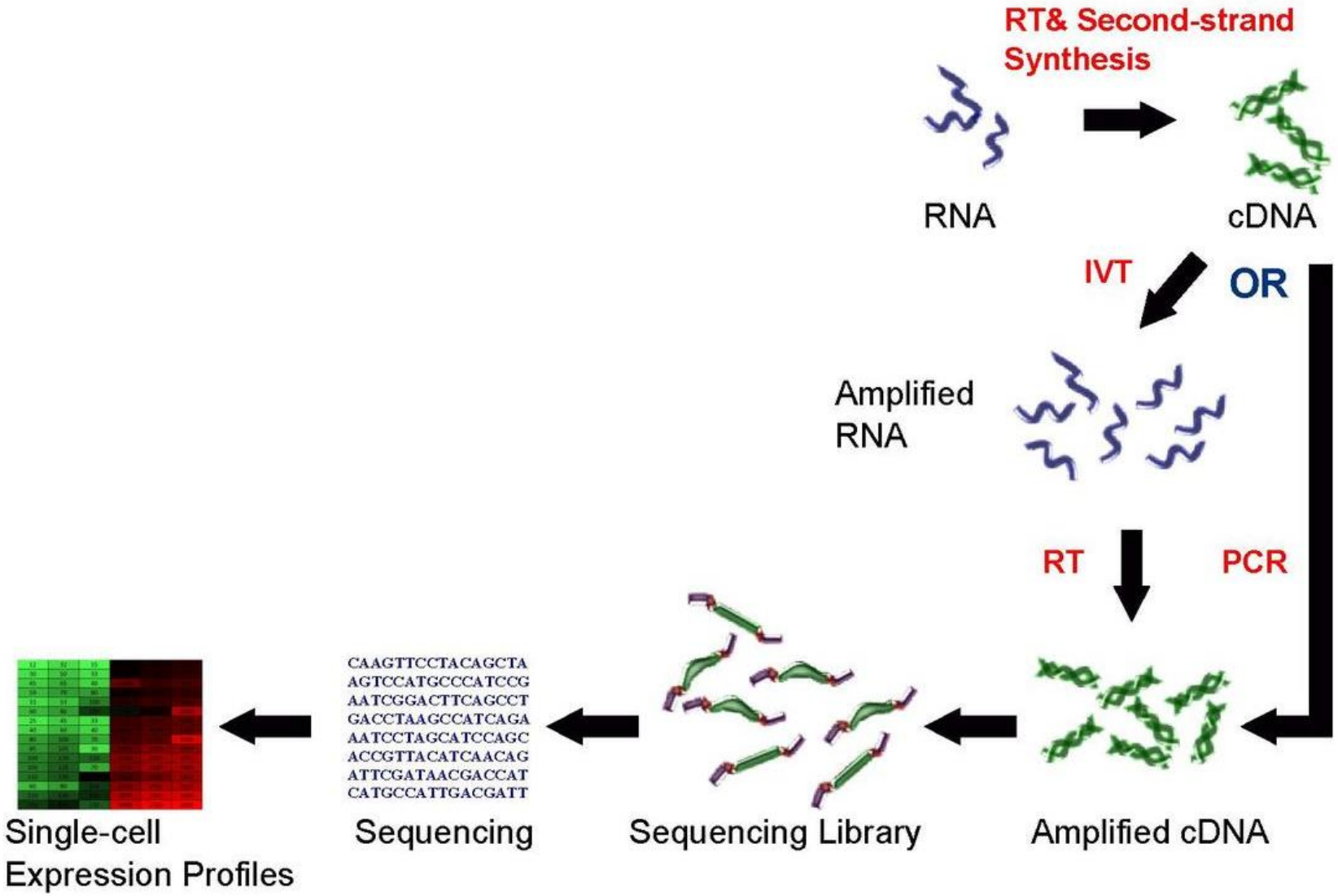




Svensson *et al.*, *Nature Protocols*, 2018

Experimental design: single cell RNA-Seq



https://en.wikipedia.org/wiki/Single_cell_sequencing

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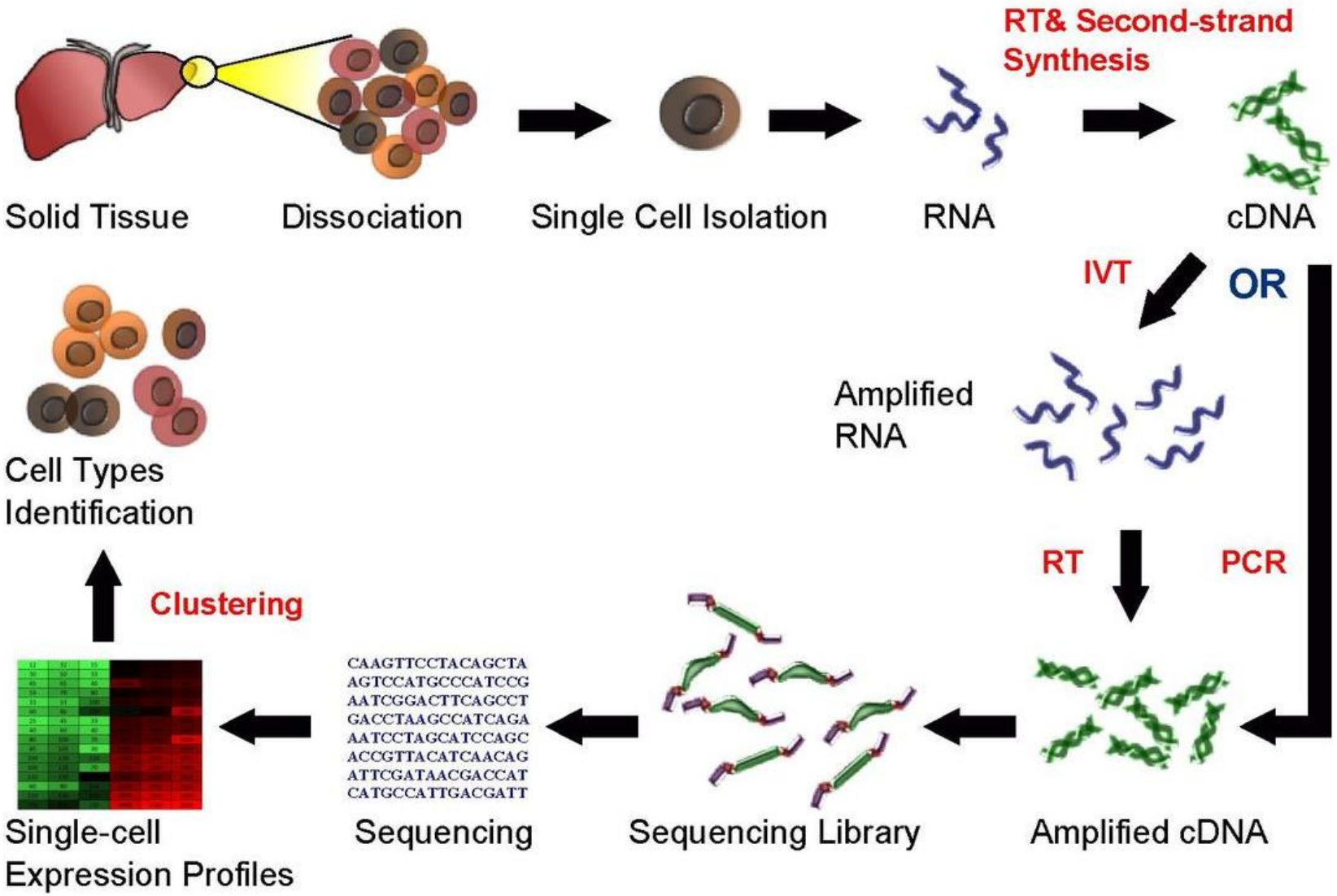

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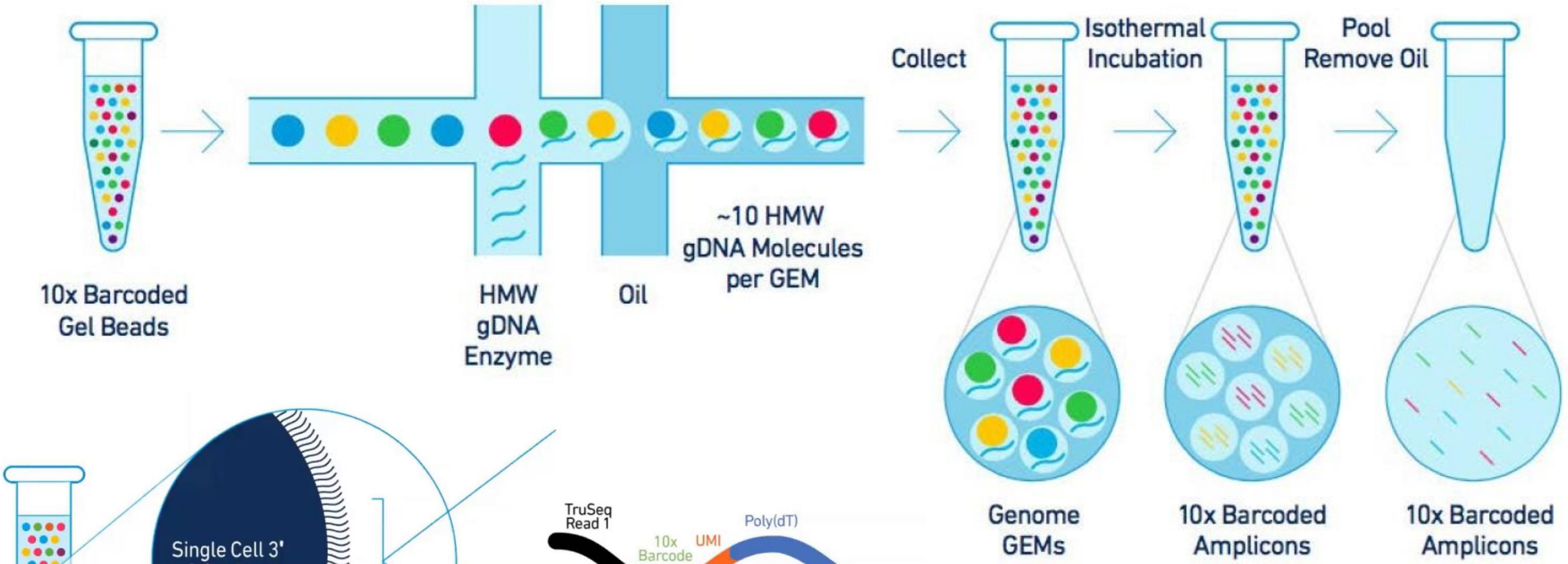
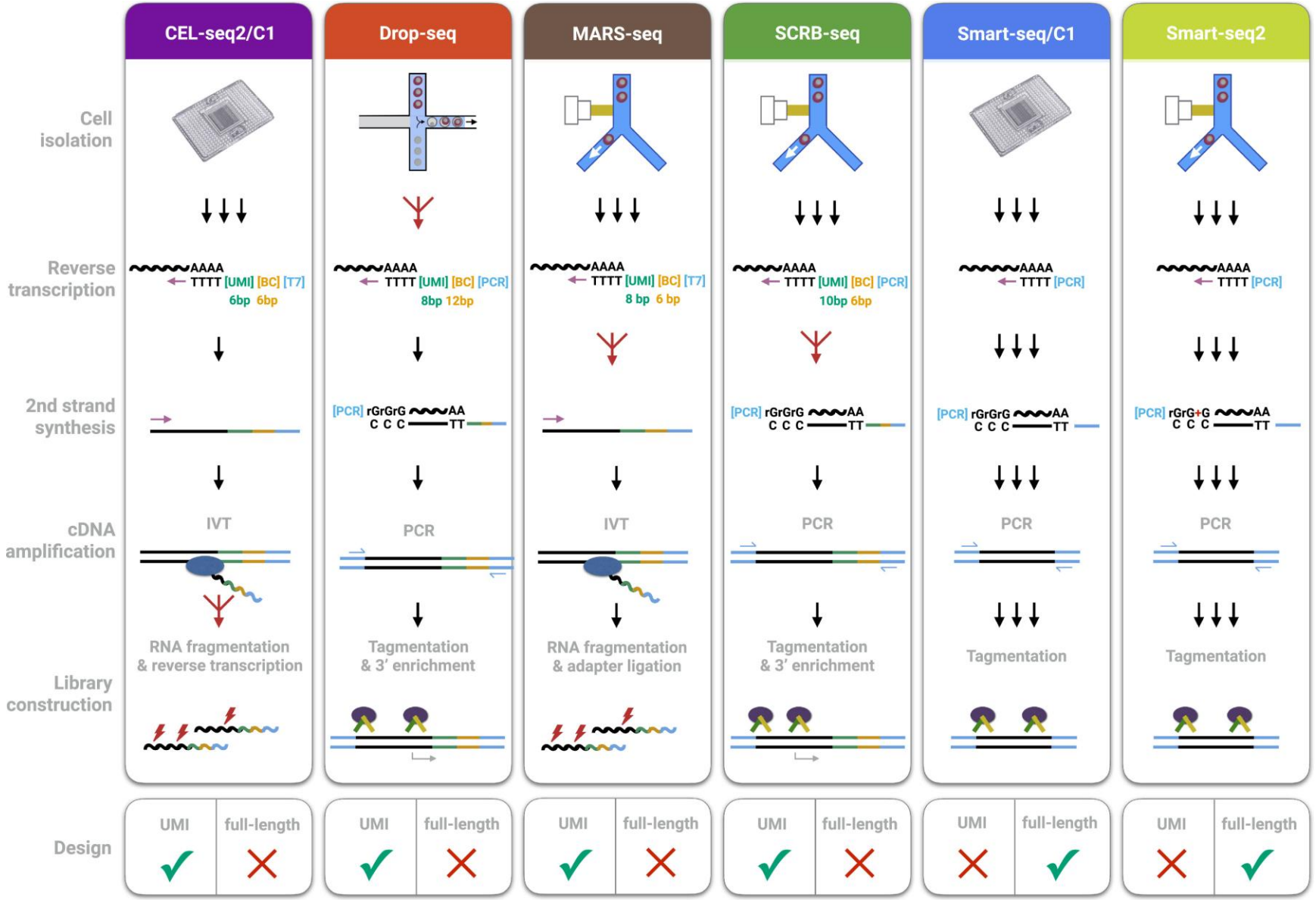
Single cell transcriptomics using 10x Chromium system



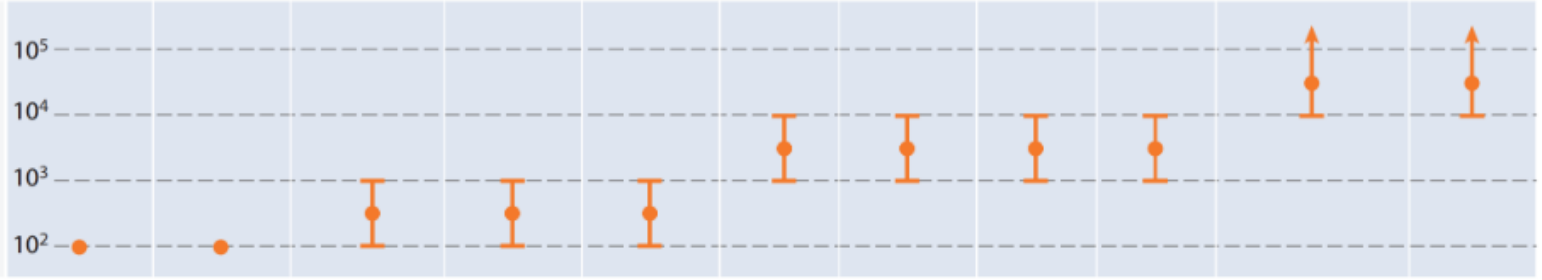
There are many single-cell RNA sequencing methods



Ziegenhain *et al.*, *Mol Cell*, 2017

There are many single-cell RNA sequencing methods

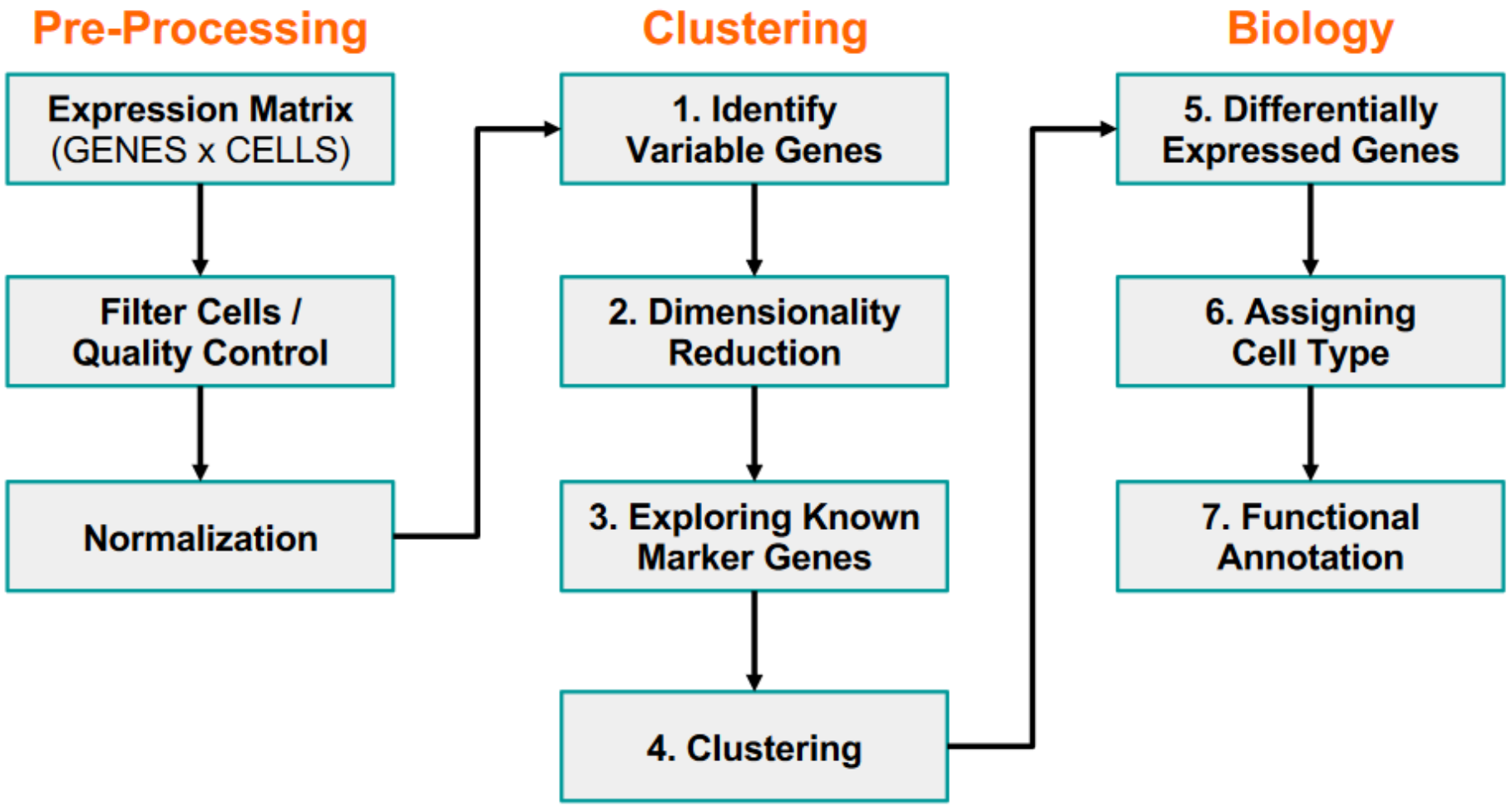
	SMART-seq2	CEL-seq2	STRT-seq	Quartz-seq2	MARS-seq	Drop-seq	inDrop	Chromium	Seq-Well	sci-RNA-seq	SPLiT-seq
Single-cell isolation	FACS, microfluidics	FACS, microfluidics	FACS, microfluidics, nanowells	FACS	FACS	Droplet	Droplet	Droplet	Nanowells	Not needed	Not needed
Second strand synthesis	TSO	RNase H and DNA pol I	TSO	PolyA tailing and primer ligation	RNase H and DNA pol I	TSO	RNase H and DNA pol I	TSO	TSO	RNase H and DNA pol I	TSO
Full-length cDNA synthesis?	Yes	No	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes
Barcode addition	Library PCR with barcoded primers	Barcoded RT primers	Barcoded TSOs	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers and library PCR with barcoded primers	Ligation of barcoded RT primers
Pooling before library?	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Library amplification	PCR	In vitro transcription	PCR	PCR	In vitro transcription	PCR	In vitro transcription	PCR	PCR	PCR	PCR
Gene coverage	Full-length	3′	5′	3′	3′	3′	3′	3′	3′	3′	3′
Number of cells per assay											



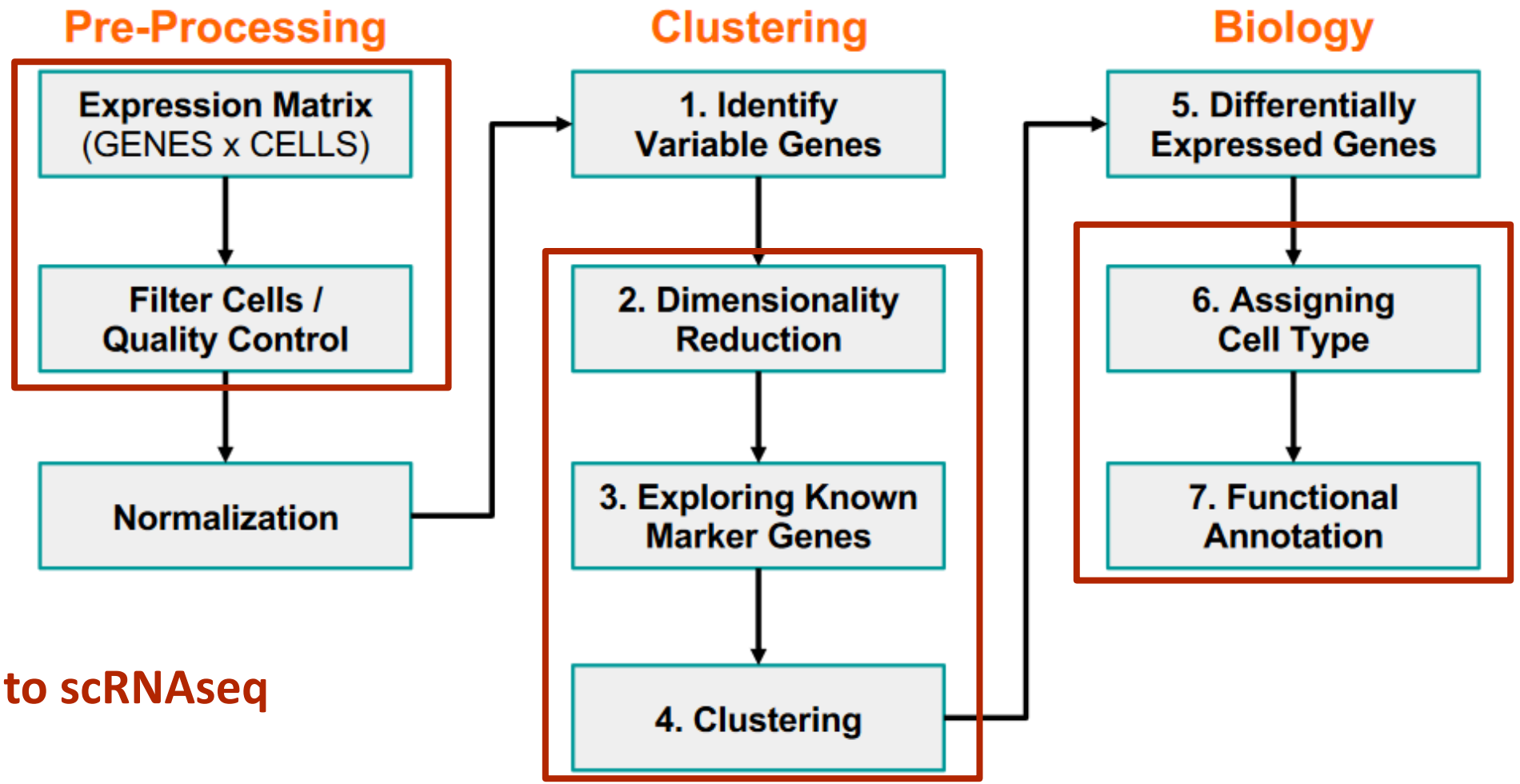
Each protocol has **advantages** and **limitations**. What one ends up using is often dictated by multiple features - the **biological context, cost, objective** etc.

Chen X. et *al.* (2018) *Annual Review of Biomedical Data Science*

ScRNAseq pipeline

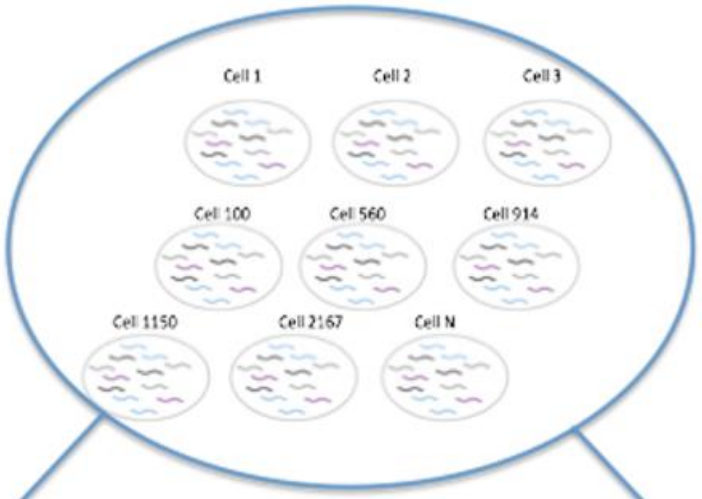


ScRNAseq pipeline



Specific to scRNAseq

Single-cell gene expression distributions are very different from bulk gene expression distributions



Gene Expression Matrix

	Exp
Gene 1	100
Gene 2	3.5
Gene 3	10
...	
Gene K	95
...	
Gene 25K	0.4

	Cell 1	Cell 2	Cell 3	....	Cell 5K
Gene 1	3	0	1		2
Gene 2	0	2	0		1
Gene 3	1	0	3		5
...					
Gene K	14	7	1		0
...					
Gene 25K	0	13	1		0

Population Average

Bulk

Cellular resolution

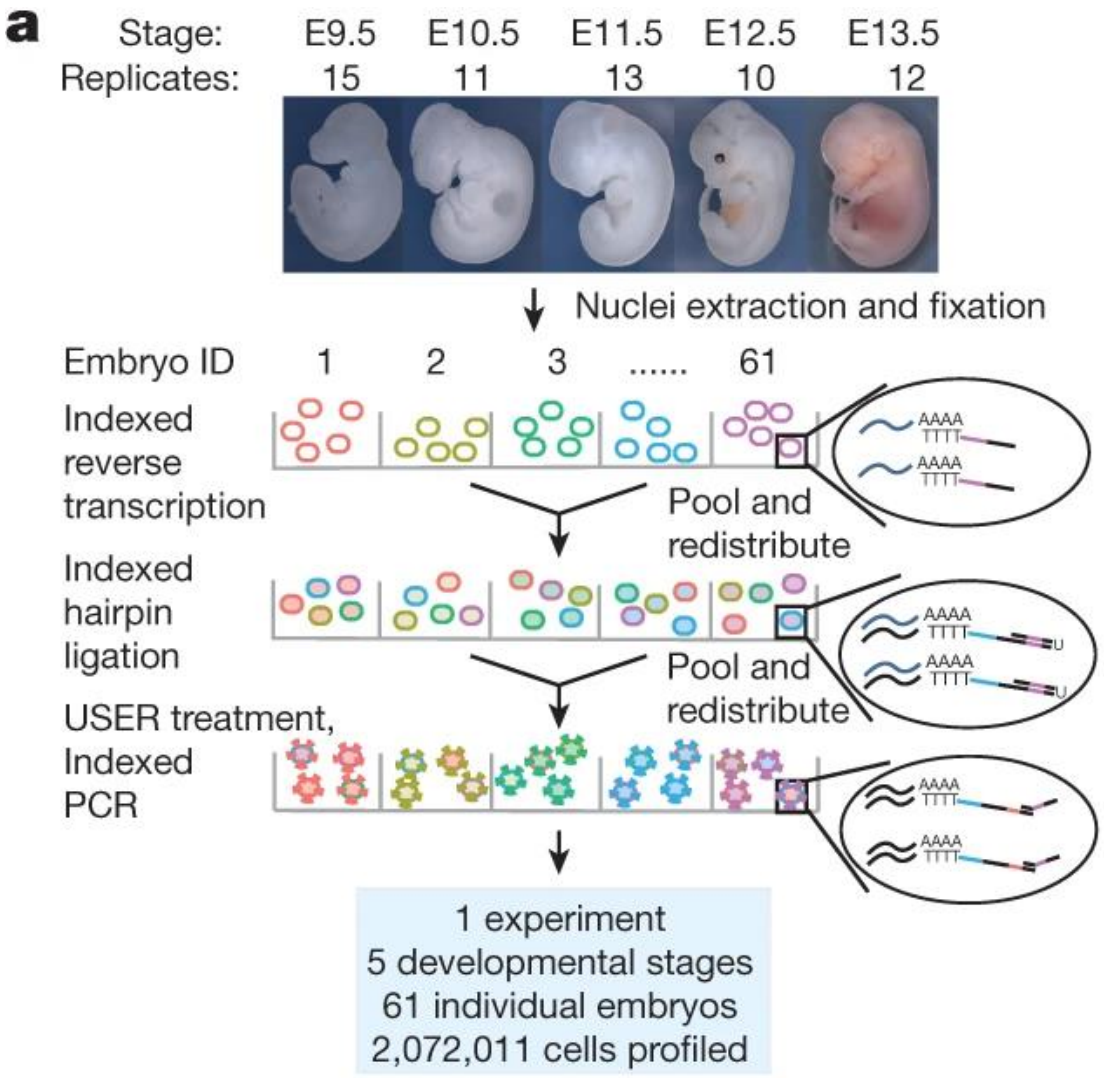
Single cell

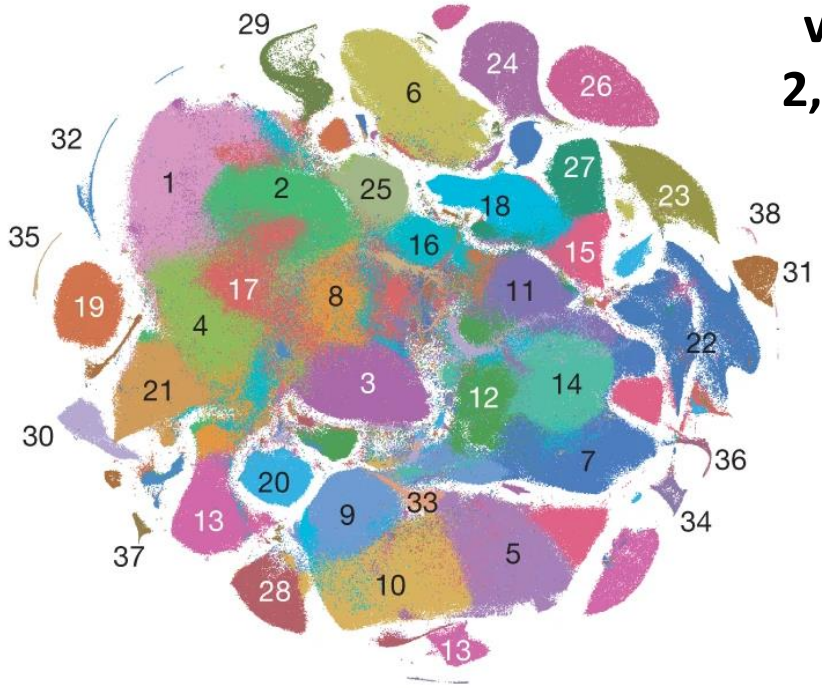
Slide courtesy of Karthik Shekhar

Limitations

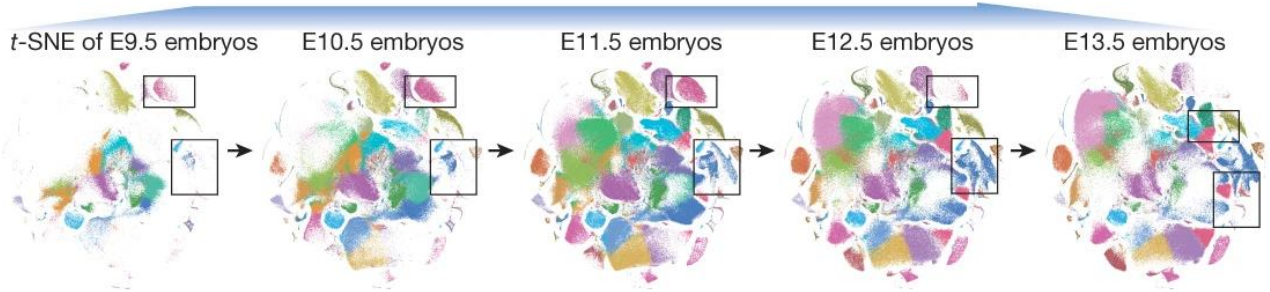
- High power computing facilities
- High data storage
- High cost of the experiments
- Dropouts, doblets and noisy data
- Lowly expressed genes might be undetected
- High batch effect in the replicates

Mouse organogenesis studied by single-cell RNA sequencing





Clustering and visualization of 2,026,641 mouse embryo cells



Cao, J., *at al., Nature* 2019

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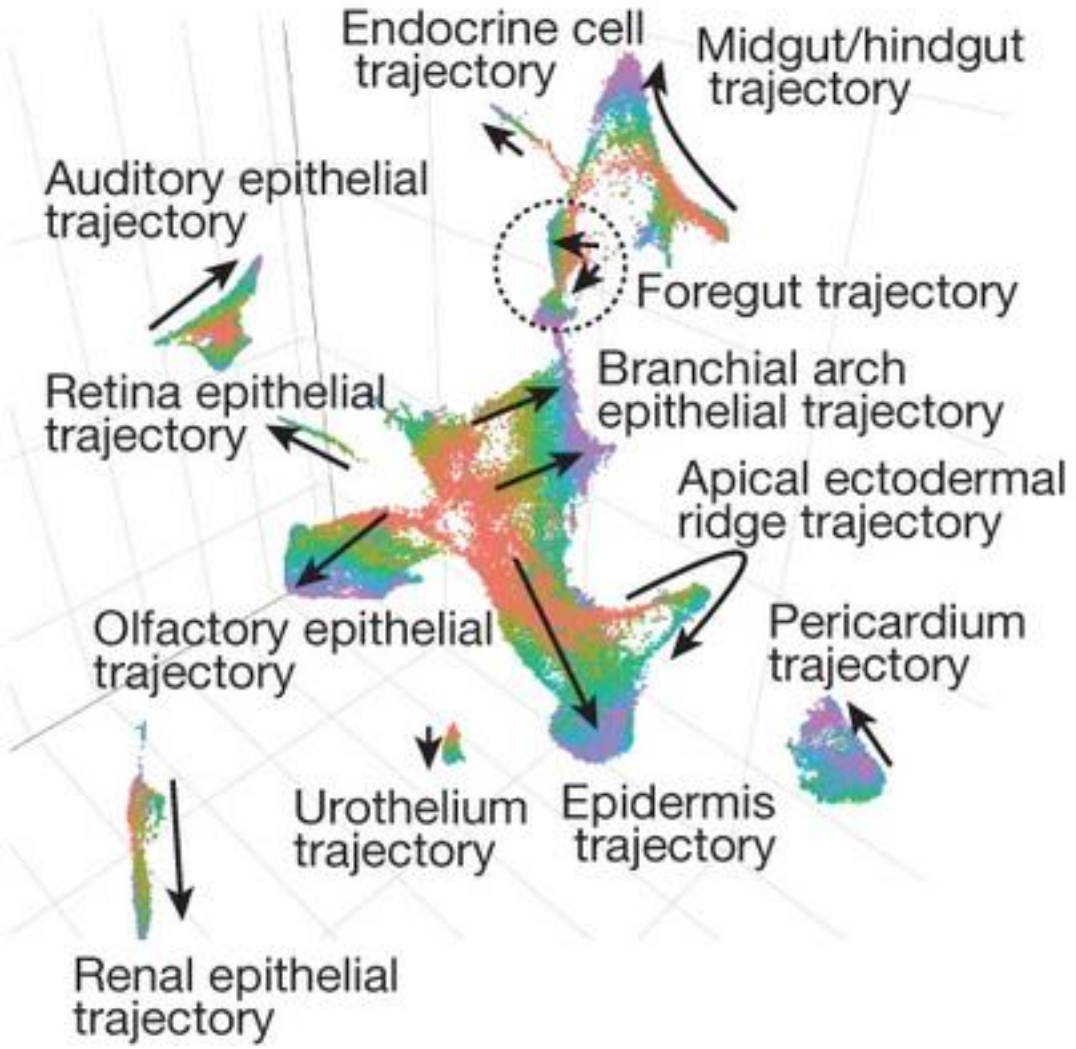

Cao, J., *at al., Nature* 2019

https://www.youtube.com/watch?v=cWG2CkkDcrM