

Single-cell Genomics

- Analyzing the DNA or RNA of single cells

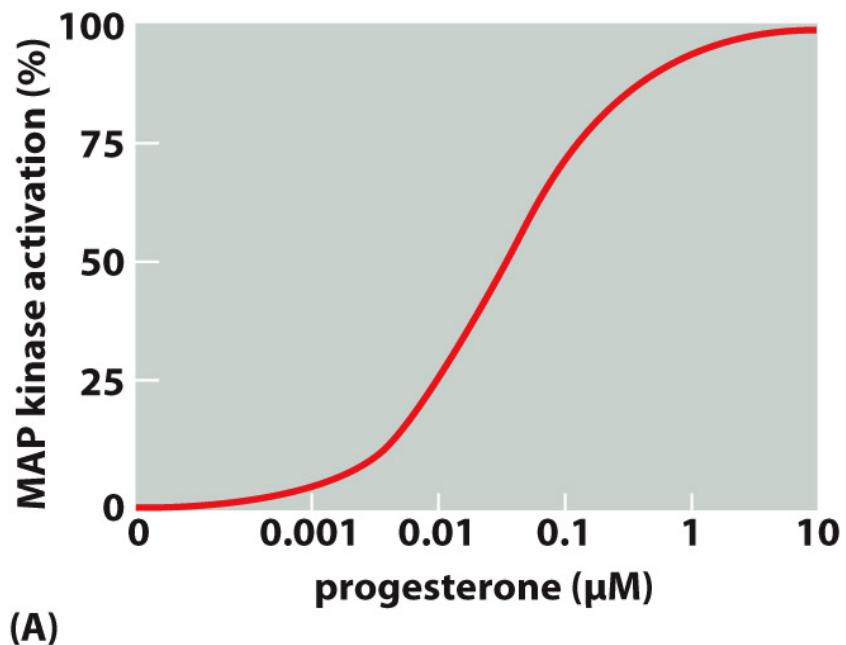
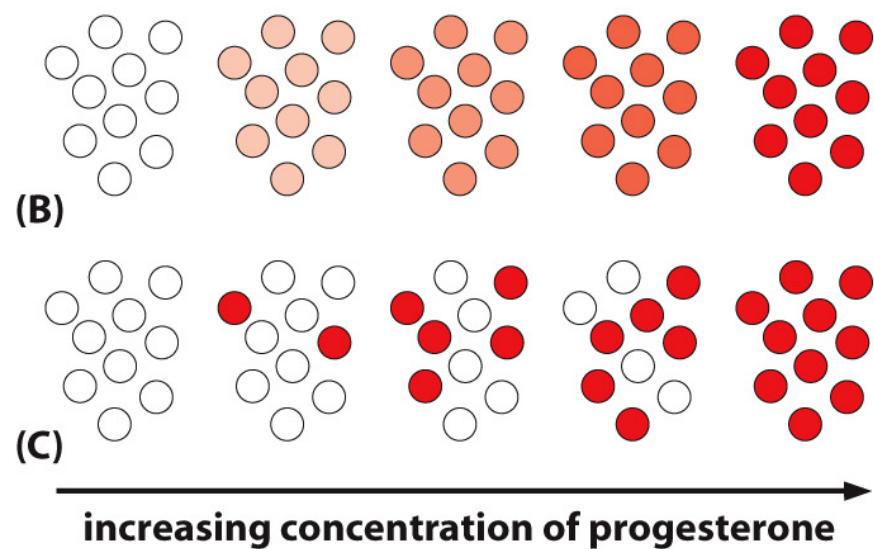


Figure 15-19 Molecular Biology of the Cell 6e (© Garland Science 2015)



Cancer cells are constantly evolving, so tumors are very heterogeneous

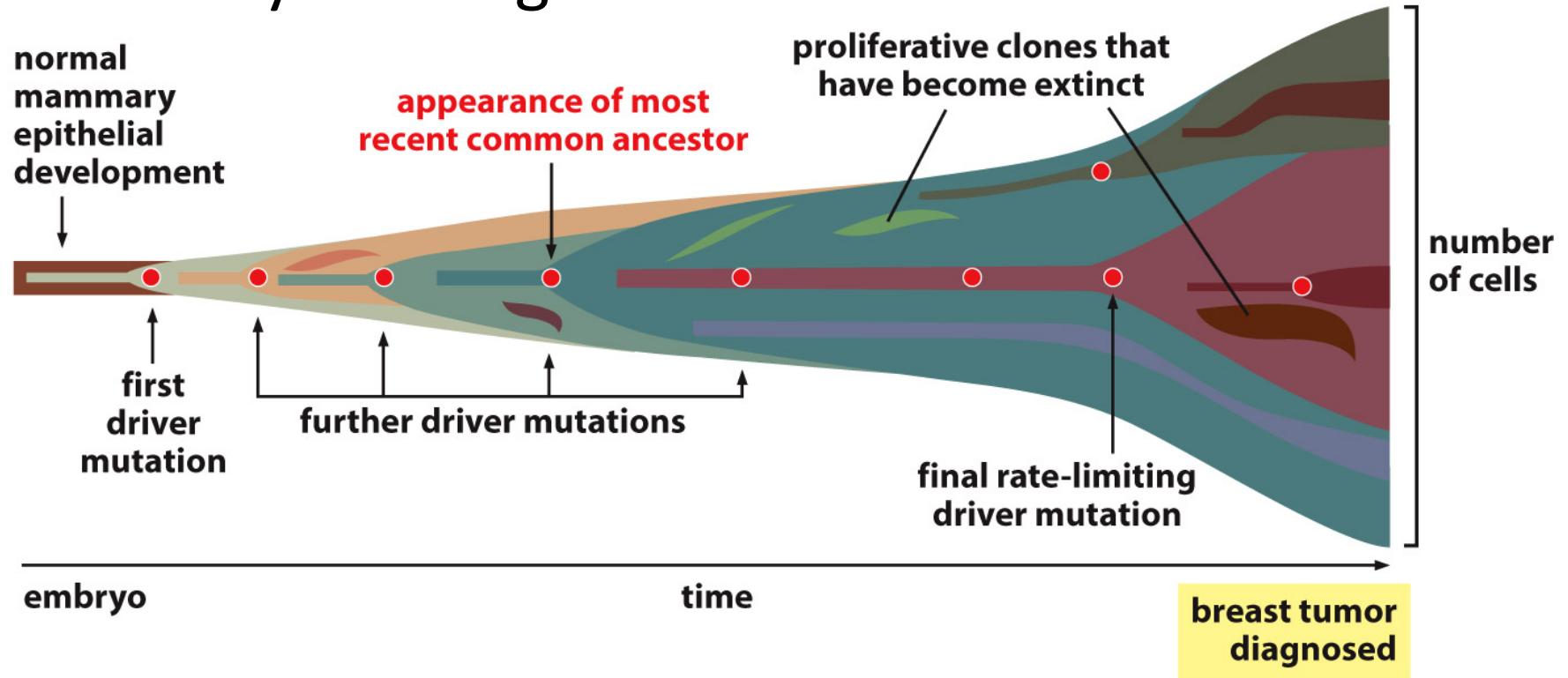
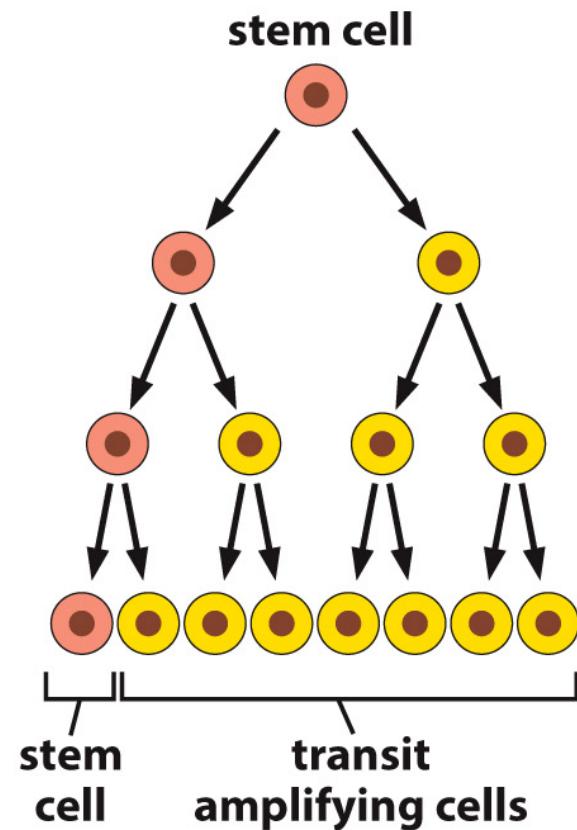
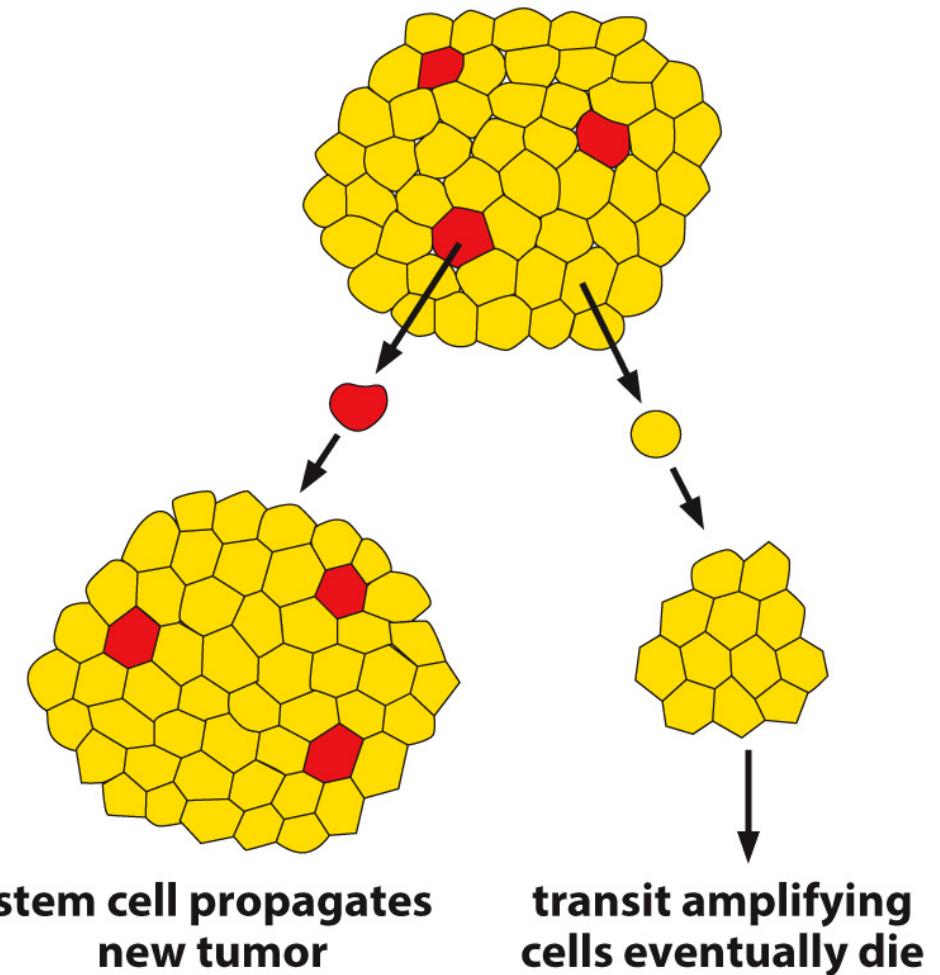


Figure 20-30c Molecular Biology of the Cell 6e (© Garland Science 2015)



(A)

mixed cell population in tumor



(B)

Figure 20-32 Molecular Biology of the Cell 6e (© Garland Science 2015)

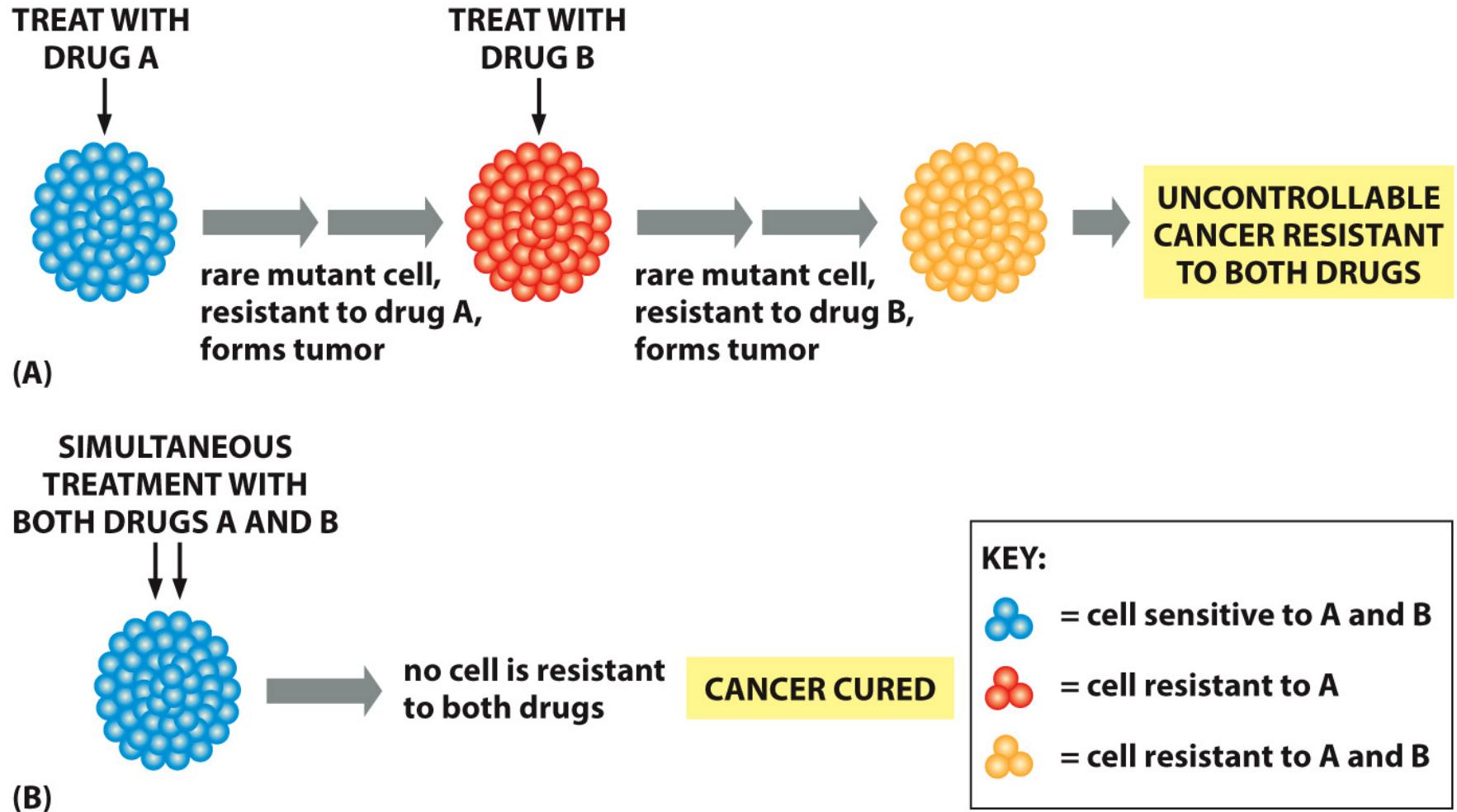
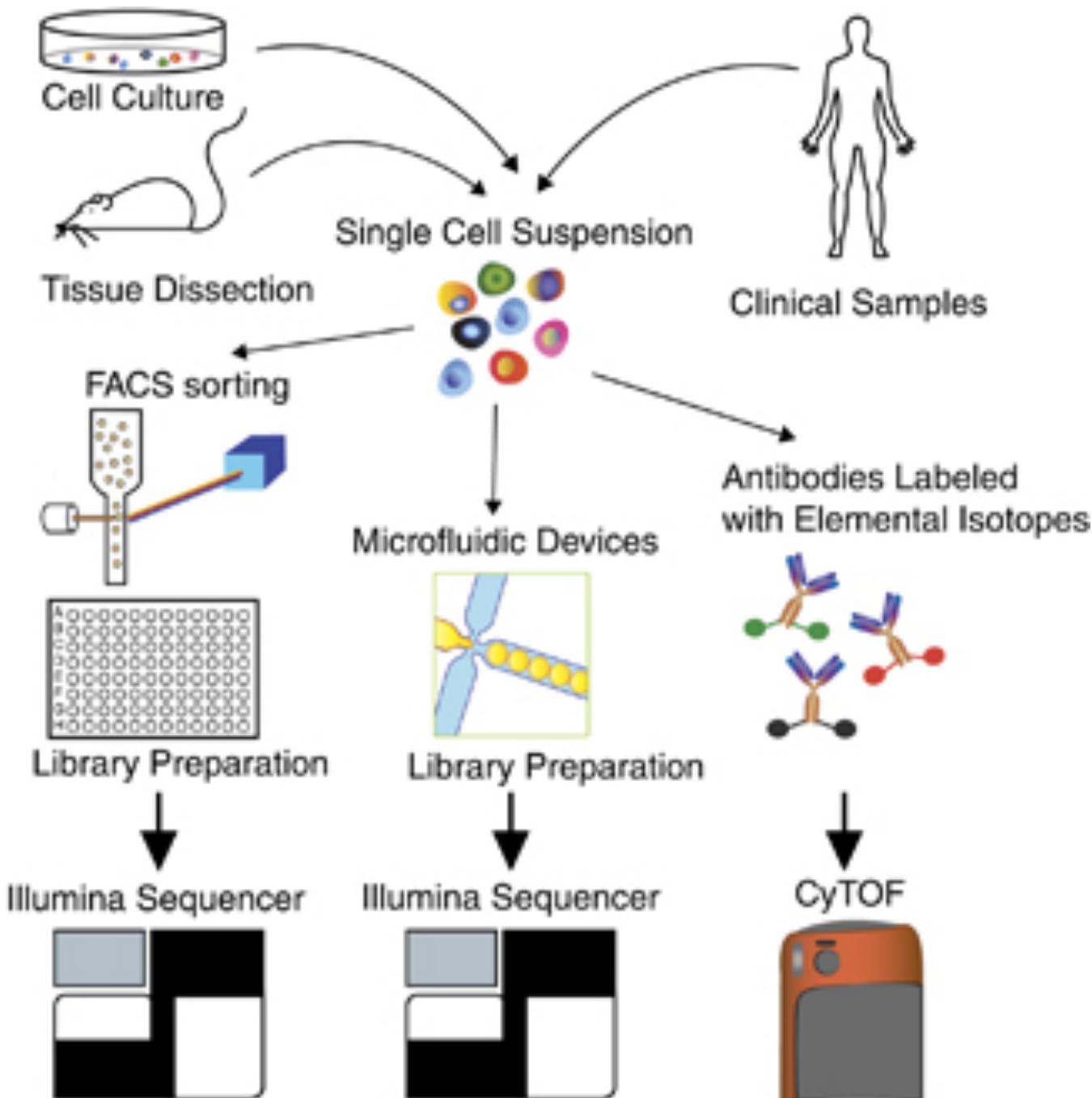


Figure 20-46 Molecular Biology of the Cell 6e (© Garland Science 2015)

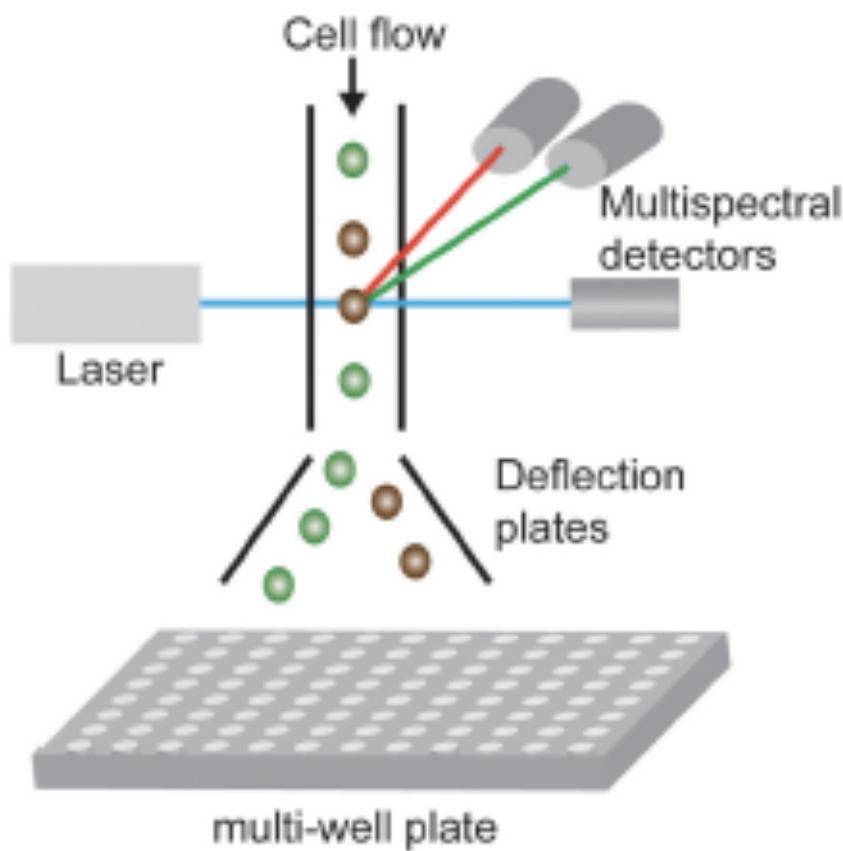
What are two challenges faced by single-cell genomics that are different than genomics in a population of cells?

What are two challenges faced by single-cell genomics that are different than genomics in a population of cells?

1. Need to isolate individual cells and extract nucleic acids
2. Generating enough signal from infinitesimally small amounts of starting material
 - Usually through amplification
3. Lack of complete data
 - Even coverage is hard to achieve

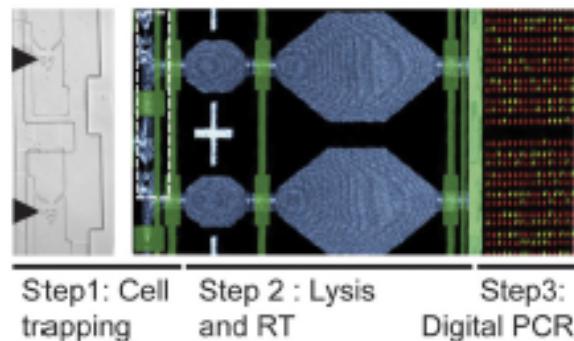


A Fluorescence-activated cell sorting

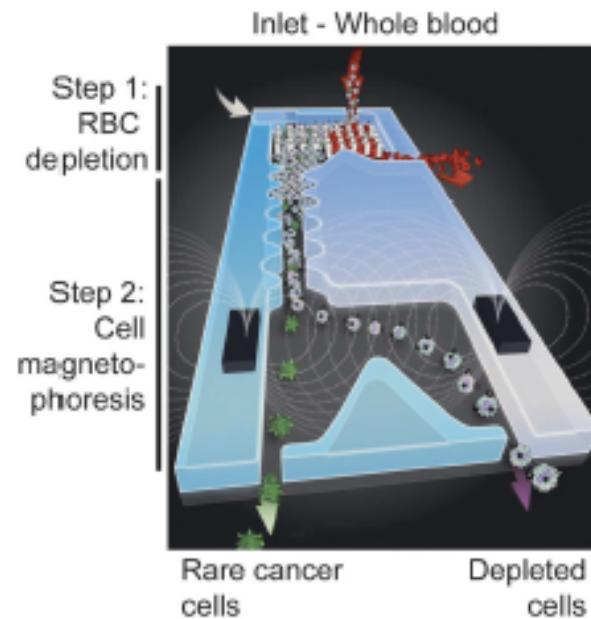


C Microfluidic-based cell handling

(i) Single-cell digital PCR device



(ii) Rare cell sorting

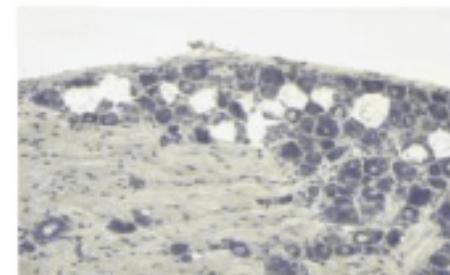


D Laser-capture microdissection

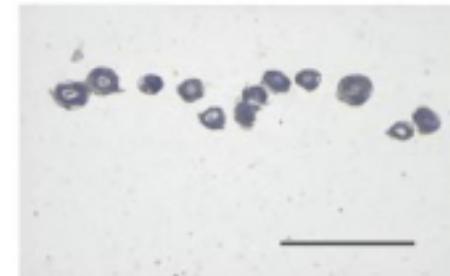
(i) Cell selection



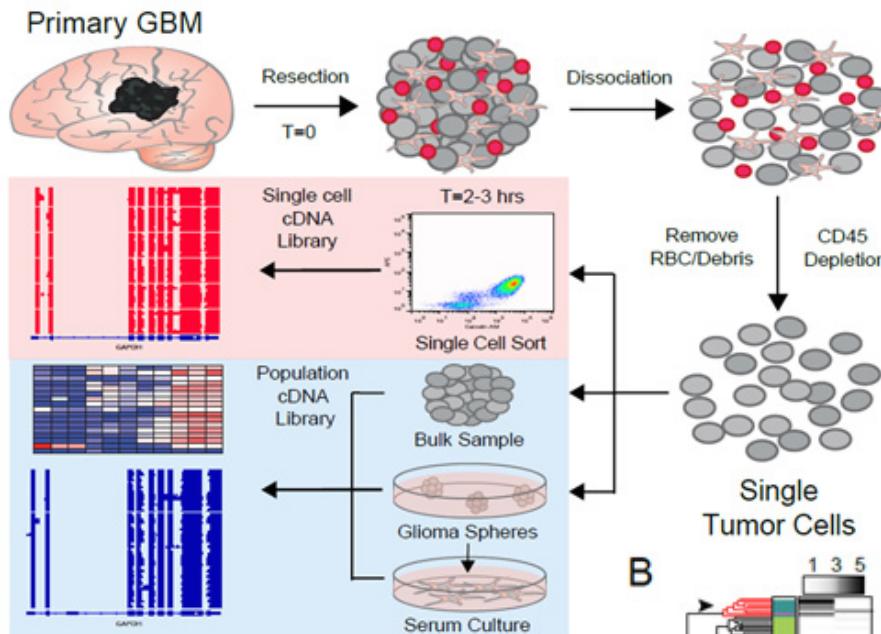
(ii) Laser sectionning



(iii) Cell transfer on a membrane



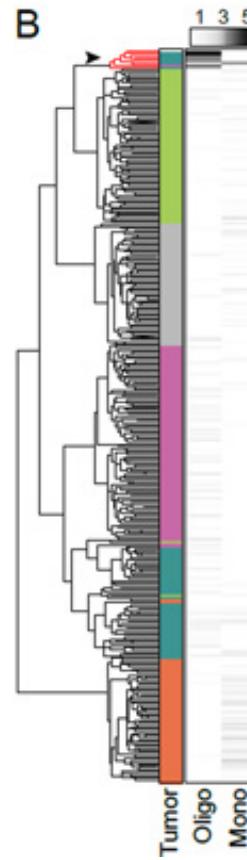
A



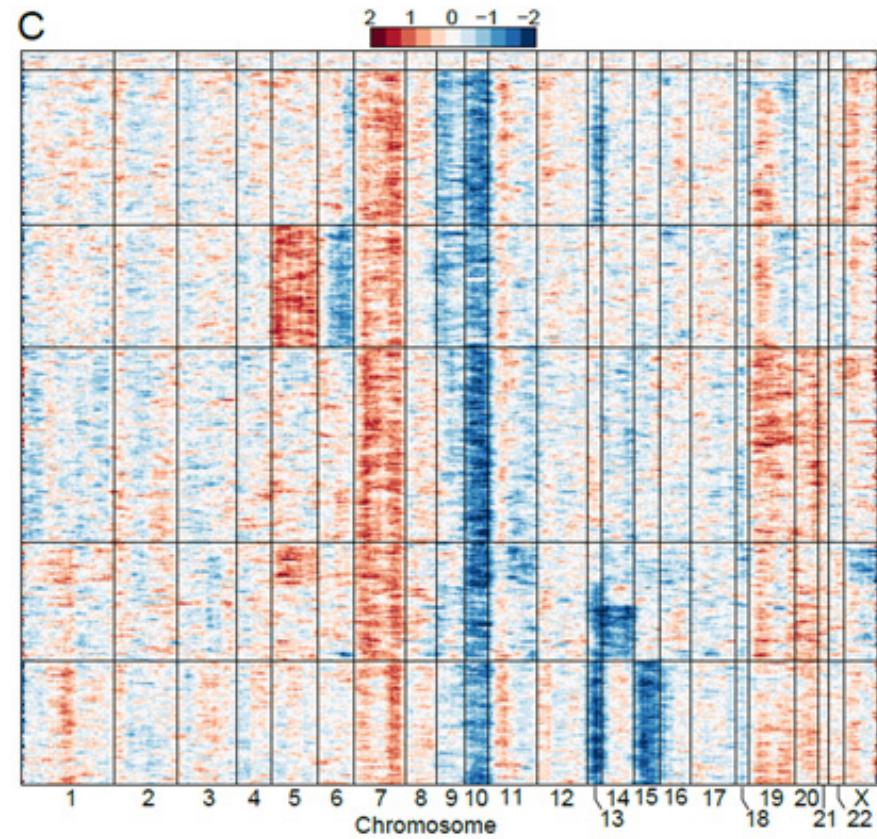
Sort cells in tumor
Extract RNA
Make cDNA library
Sequence

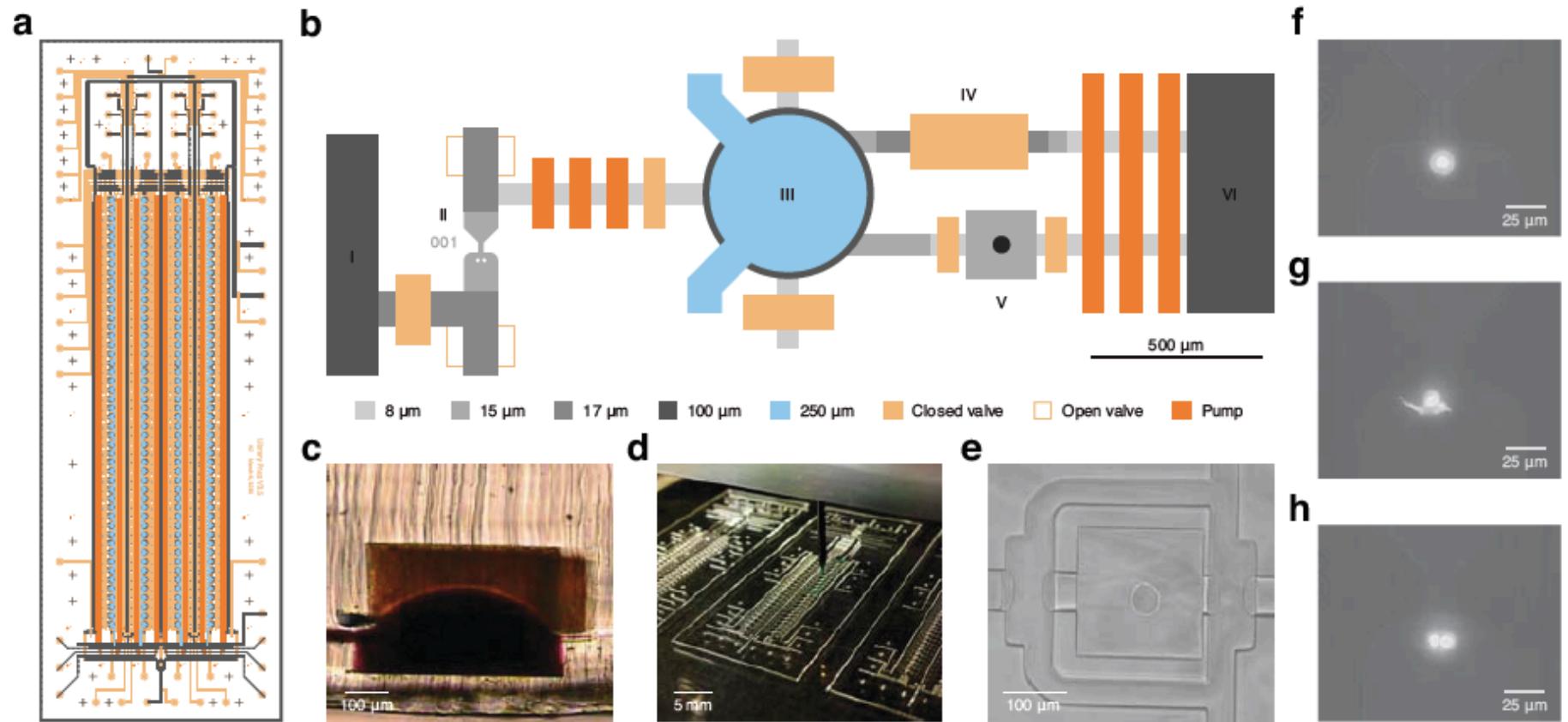
Infer genetic changes
from RNAs

- Tumors contain non-cancerous cells
- 5 different types of cancer cells (broadly) in this individual tumor



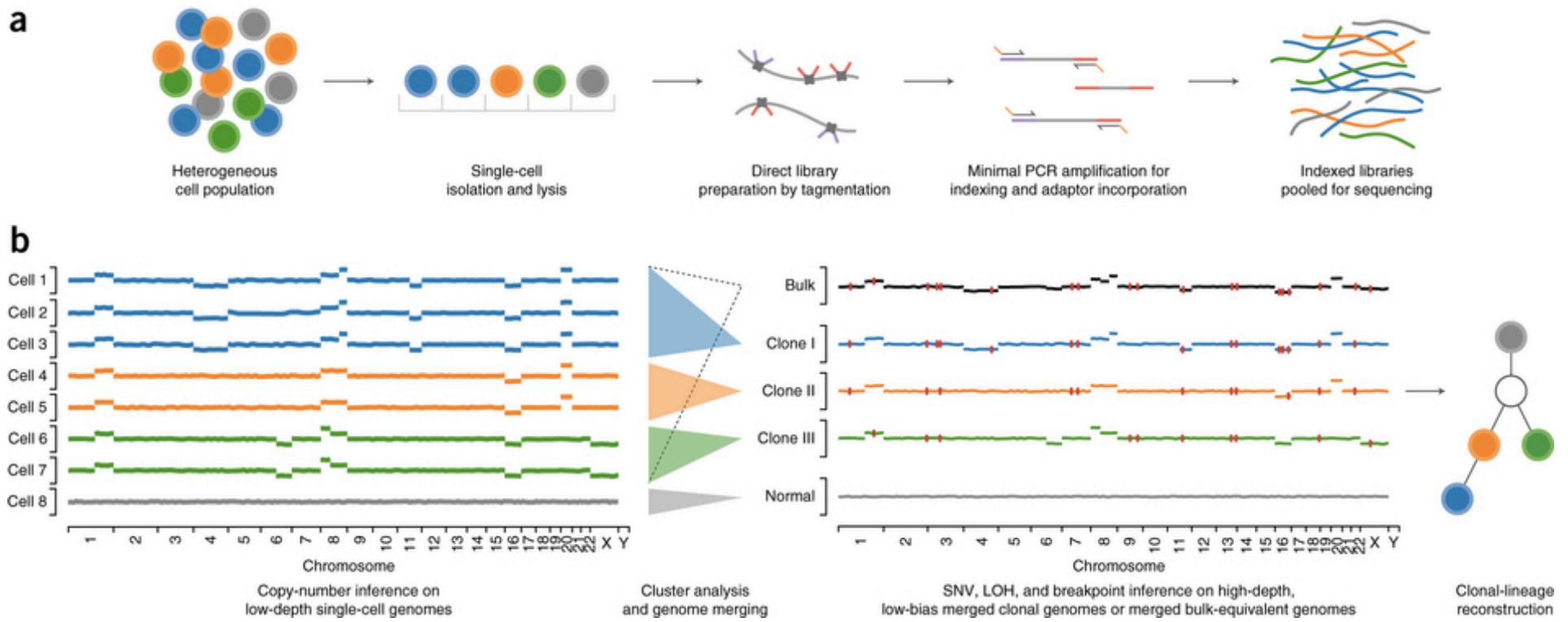
B





Zahn, H., et al; *Nature Methods* **14**, 167–173 (2017) doi:10.1038/nmeth.4140

(b) Expanded view of one cell processing unit, featuring: (I) the cell lysis inlet, (II) a cell trap, (III) an inflatable reaction chamber, (IV) the reagent inlet, (V) an index-spotting chamber, (VI) the reagent supply channel.

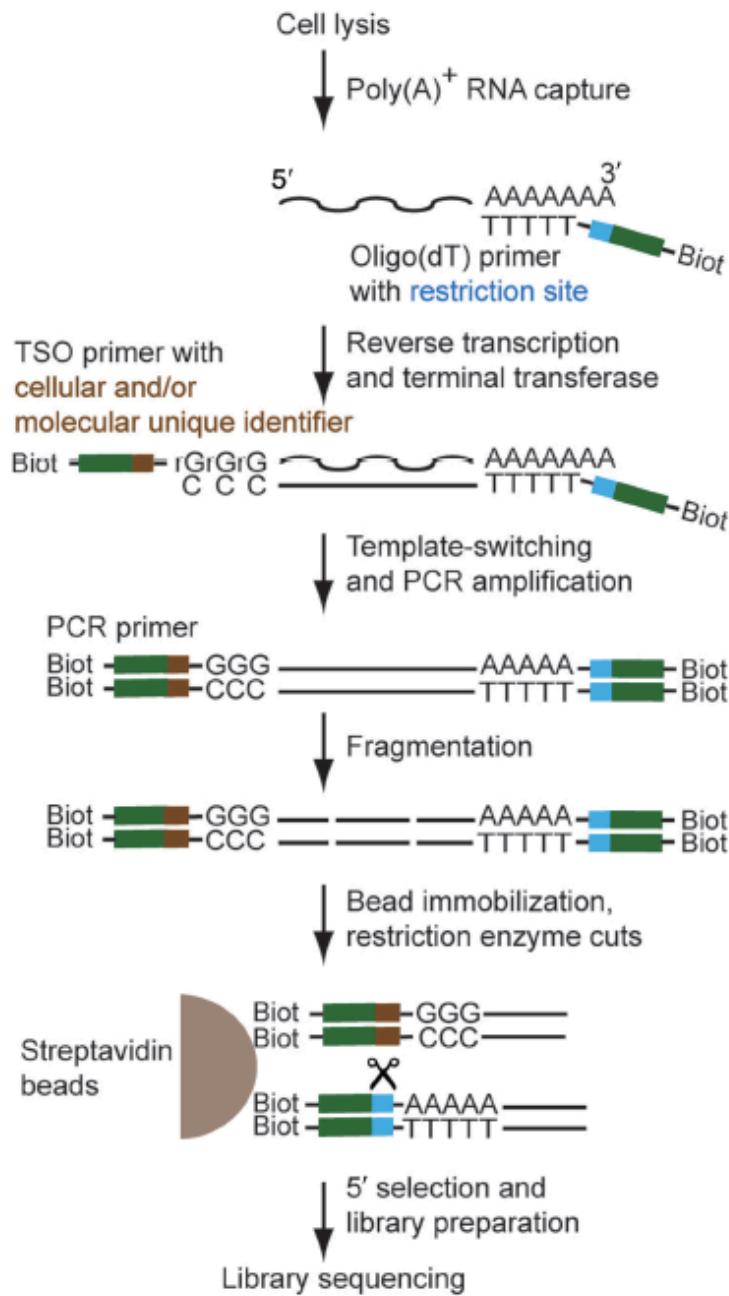


Laser Microdissection

- <https://youtu.be/kXGja36hnmw>

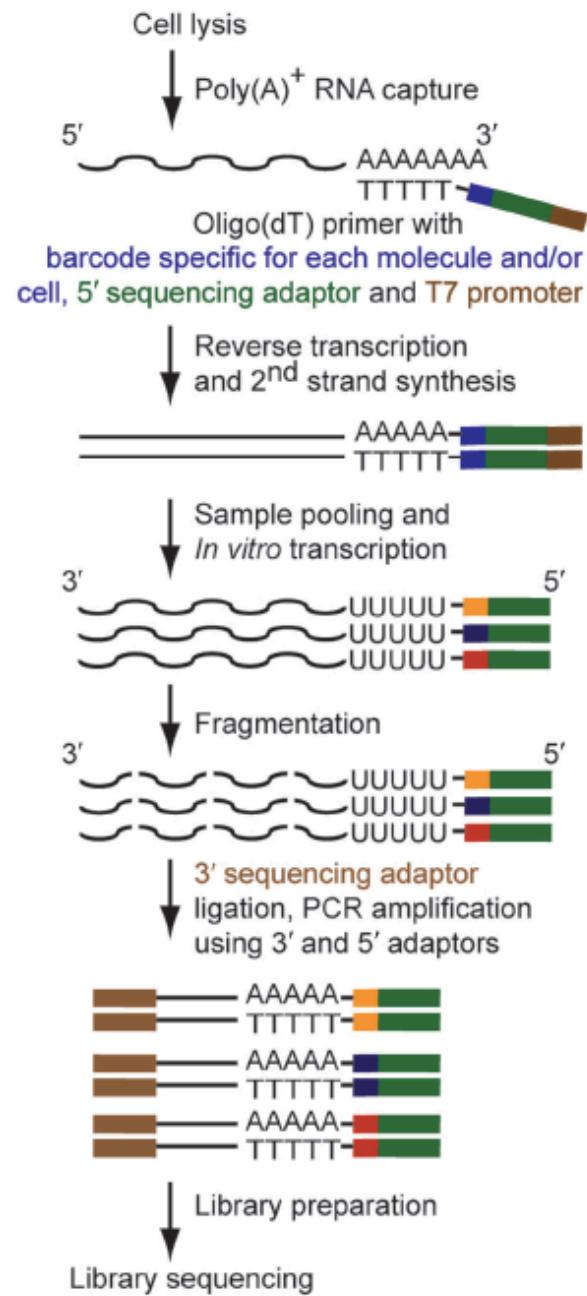
A

5' selection



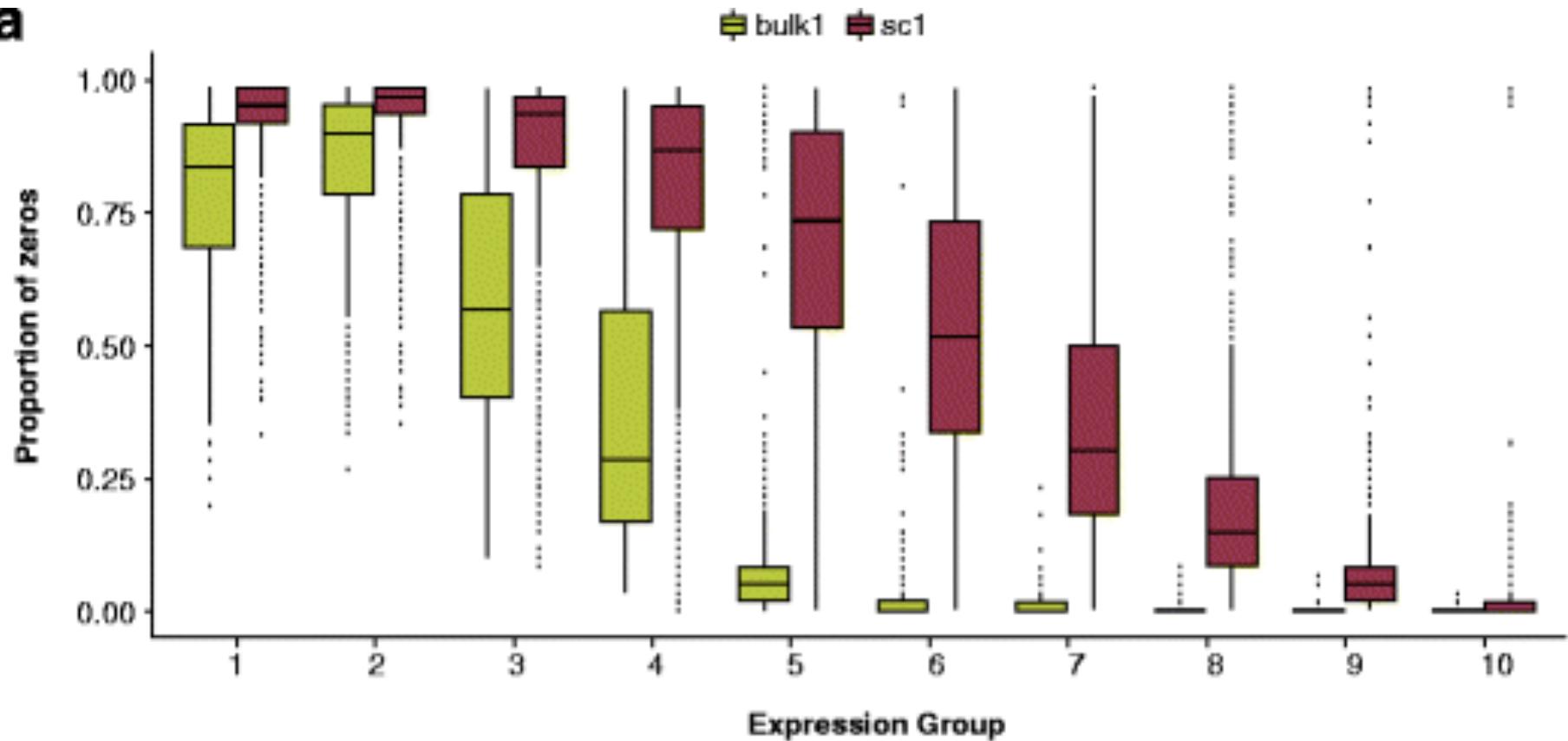
B

3' selection



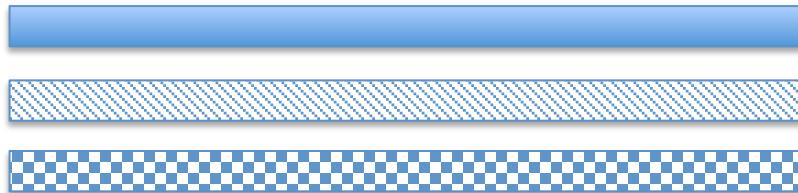
Challenges in quantifying in single-cells

What challenges do you think there might be in analyzing the data of scRNA-seq data?

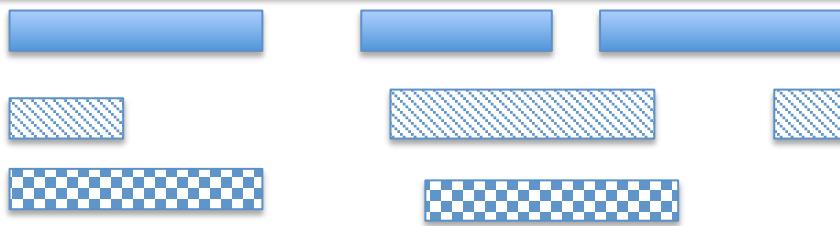
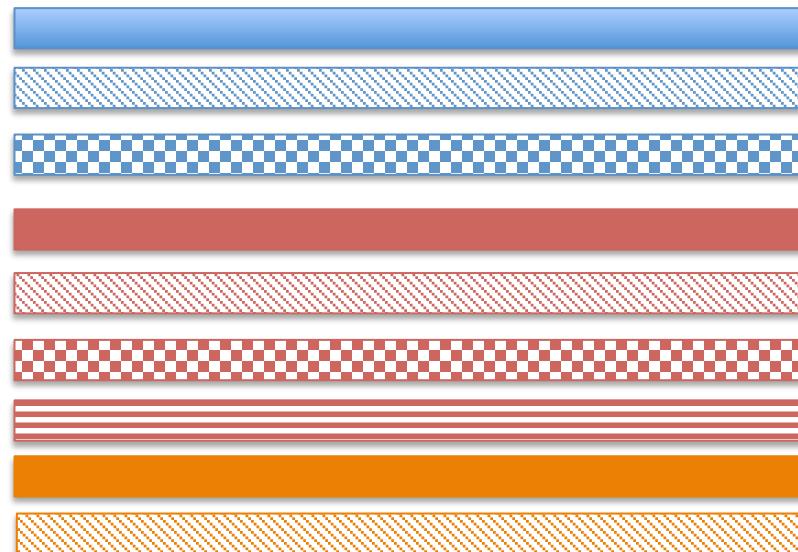
a

Rhonda Bacher & Christina Kendziorski; *Genome Biology* 2016 **17**:63 DOI: 10.1186/s13059-016-0927-y

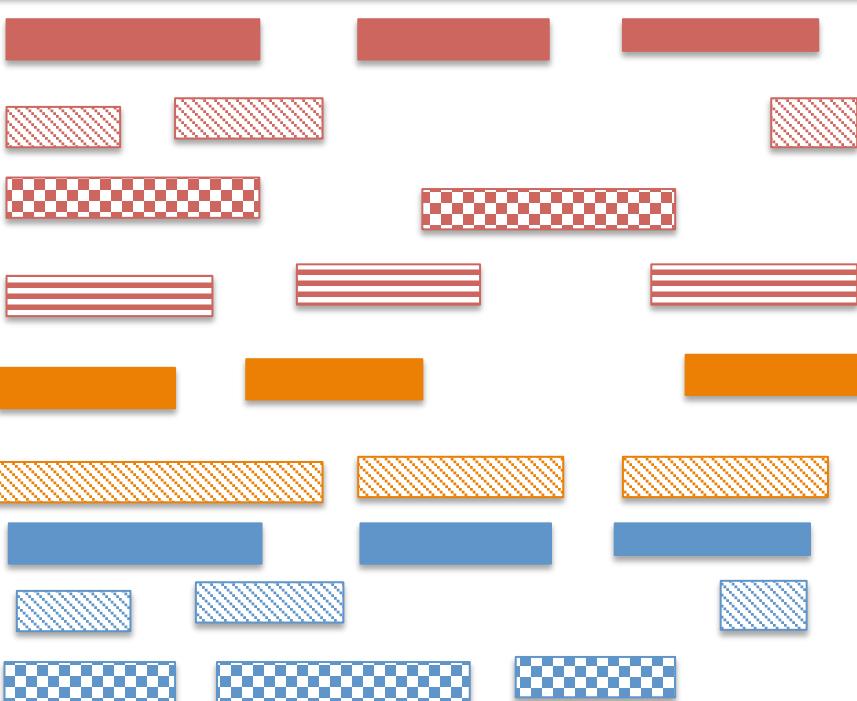
Many more zeros in the data



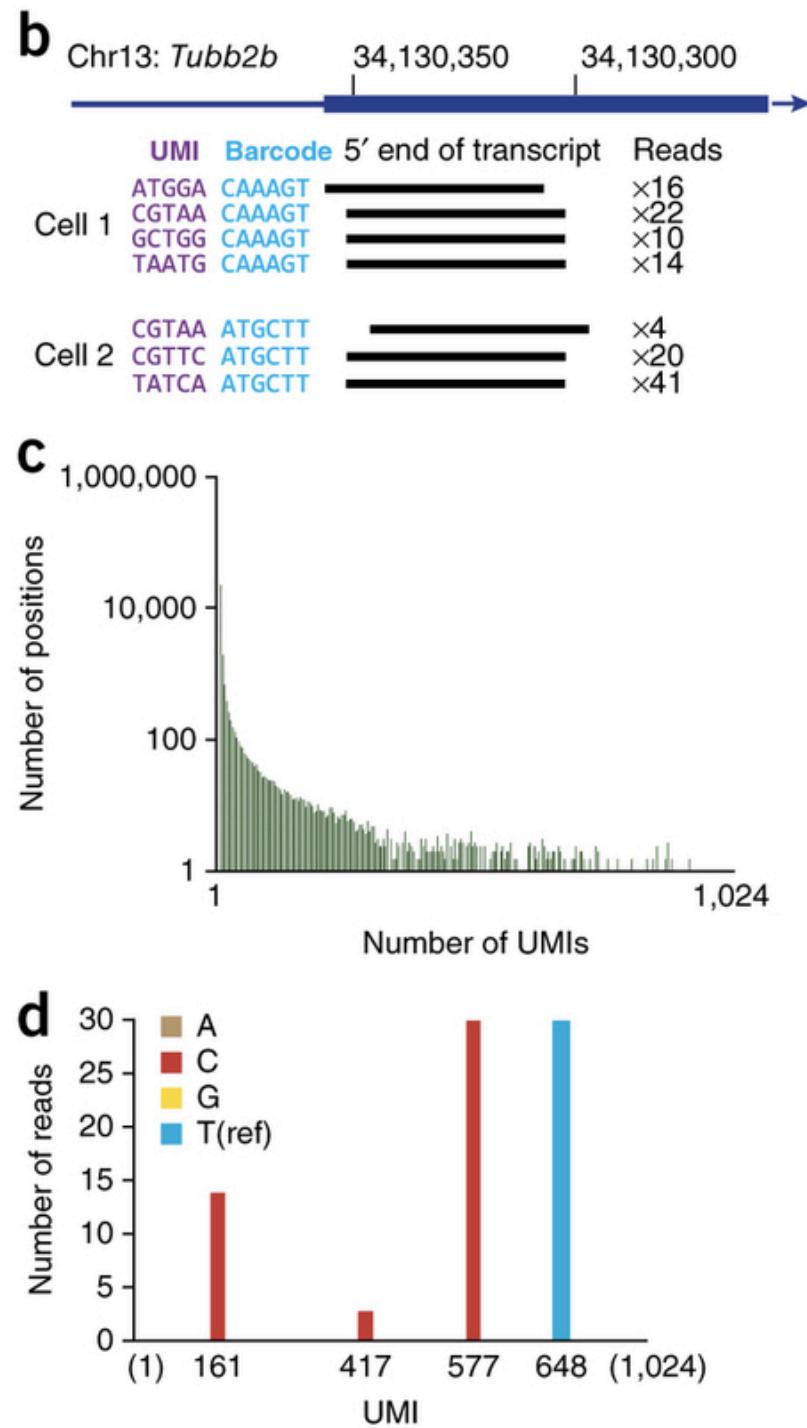
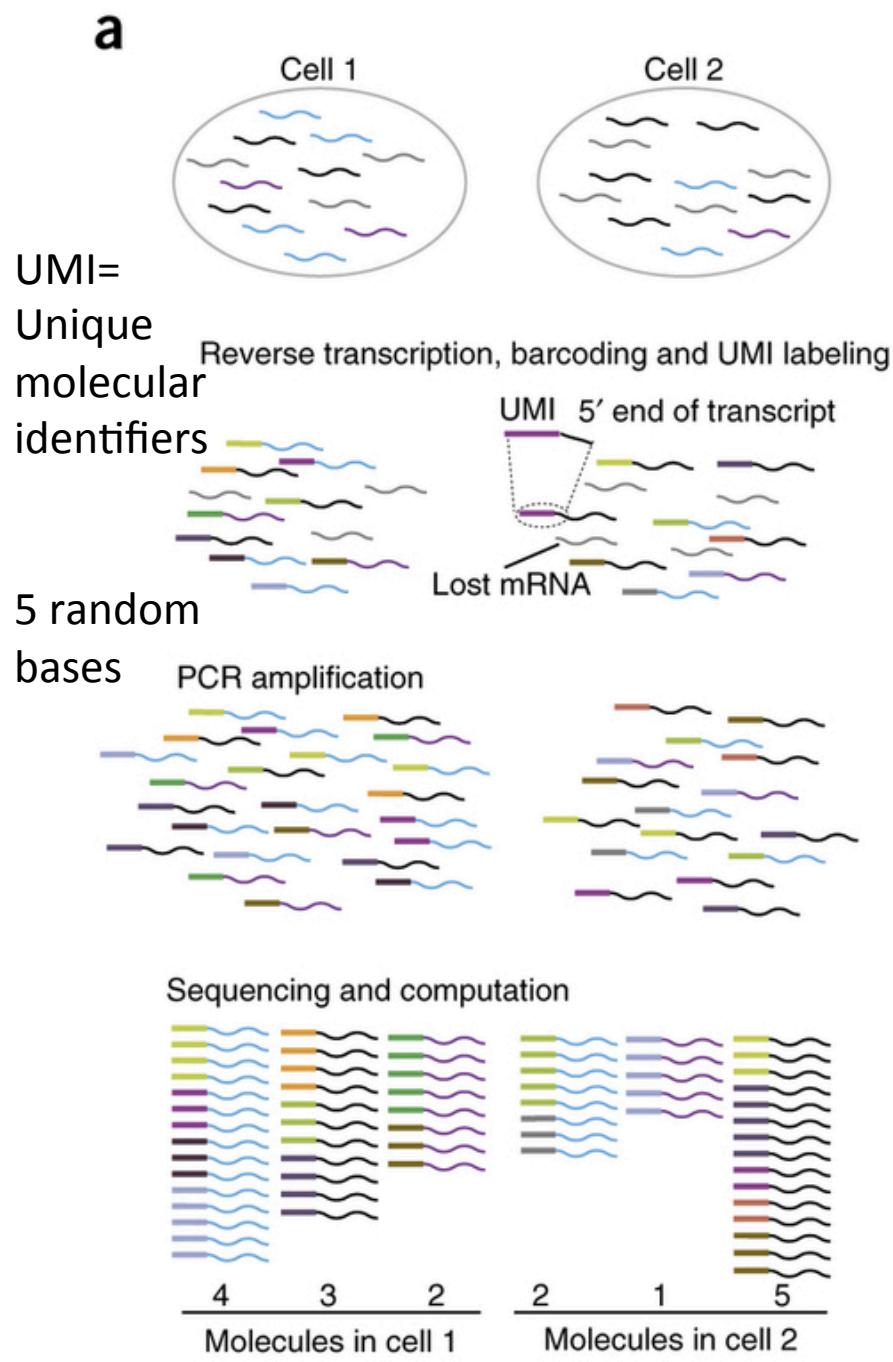
More likely to end up with regions that are not in covered in the library - RPKM is no longer as useful



Need to be able to work with data that is incomplete
Uneven (stochastic) amplification of fragments may lead to errors in quantification



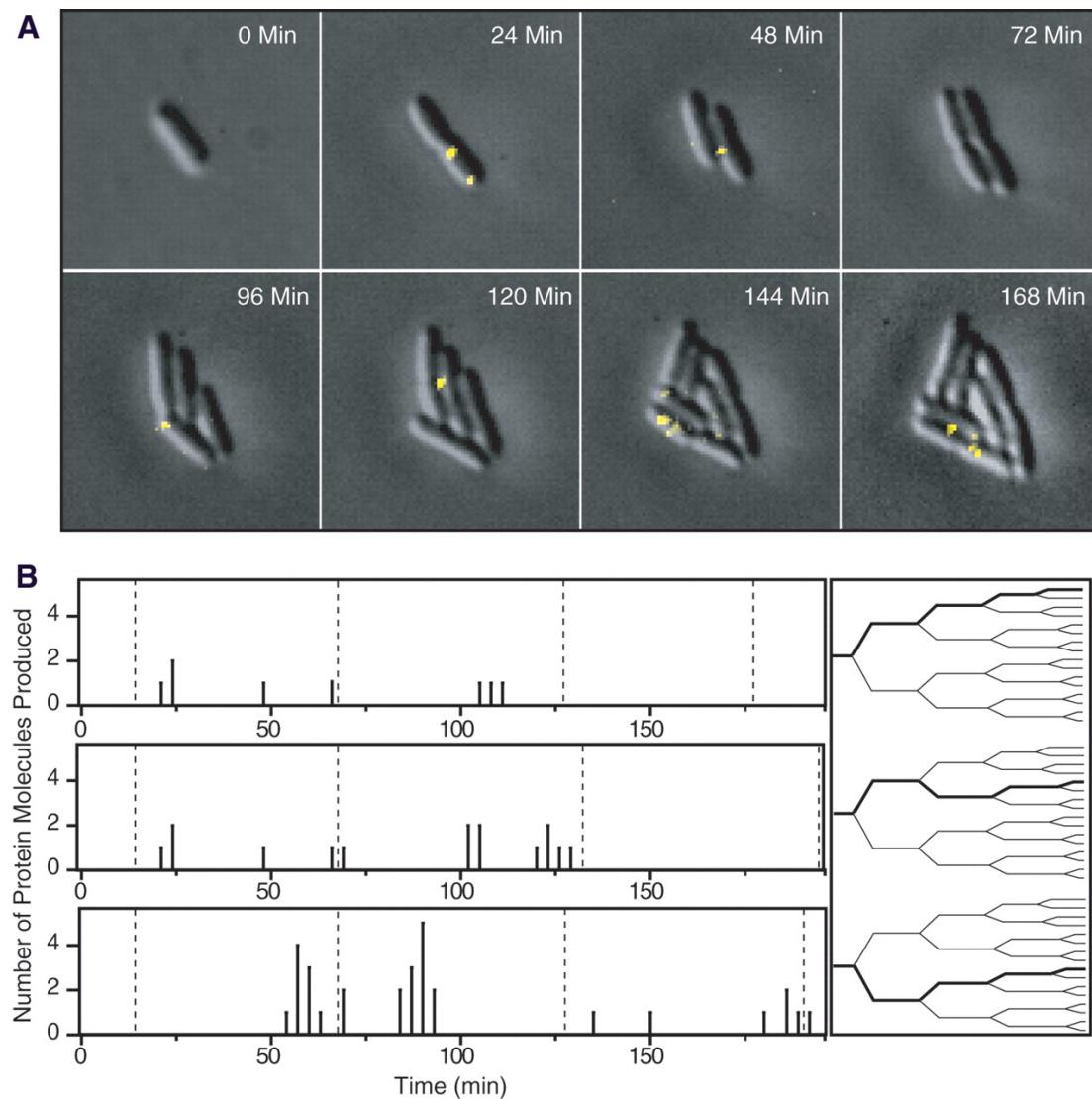
How might we better quantify RNAs in
single-cell experiments?



Things we've learned from scRNA-Seq

- Even cells that are genetically the same have different RNA expression profiles.

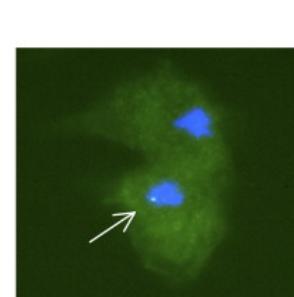
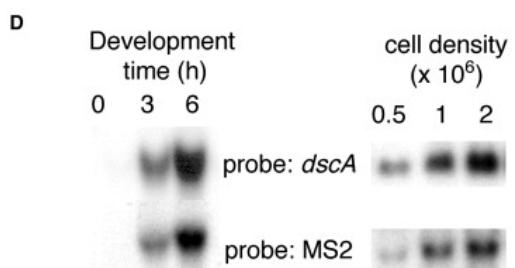
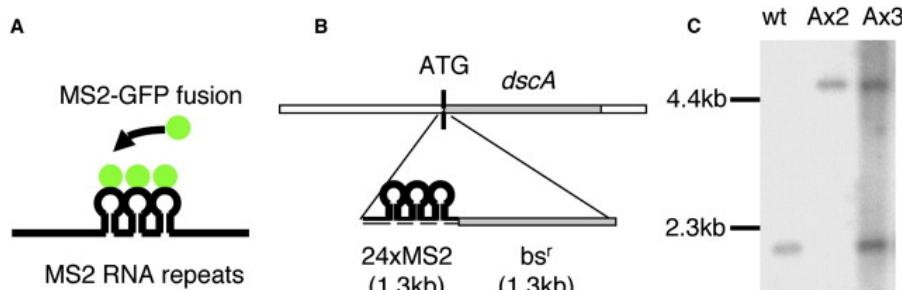
Fig. 3. Real-time monitoring of the expression of Tsr-Venus under the control of repressed lac promoter.



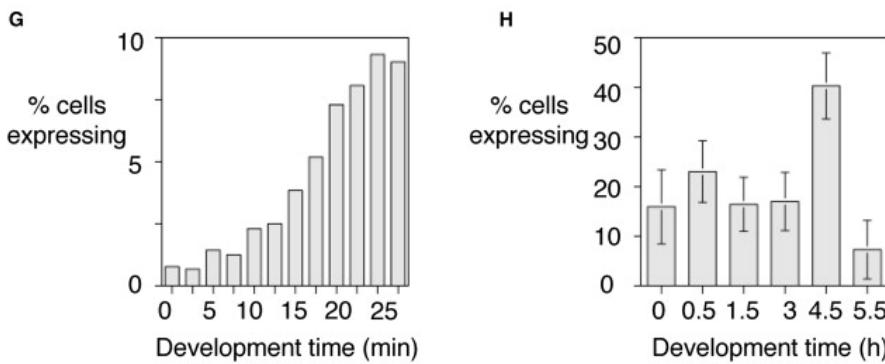
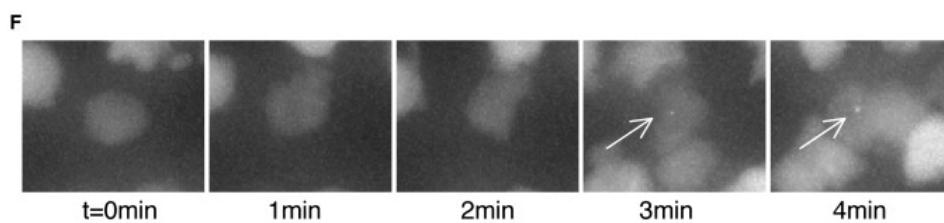
Repressor binding is stochastic

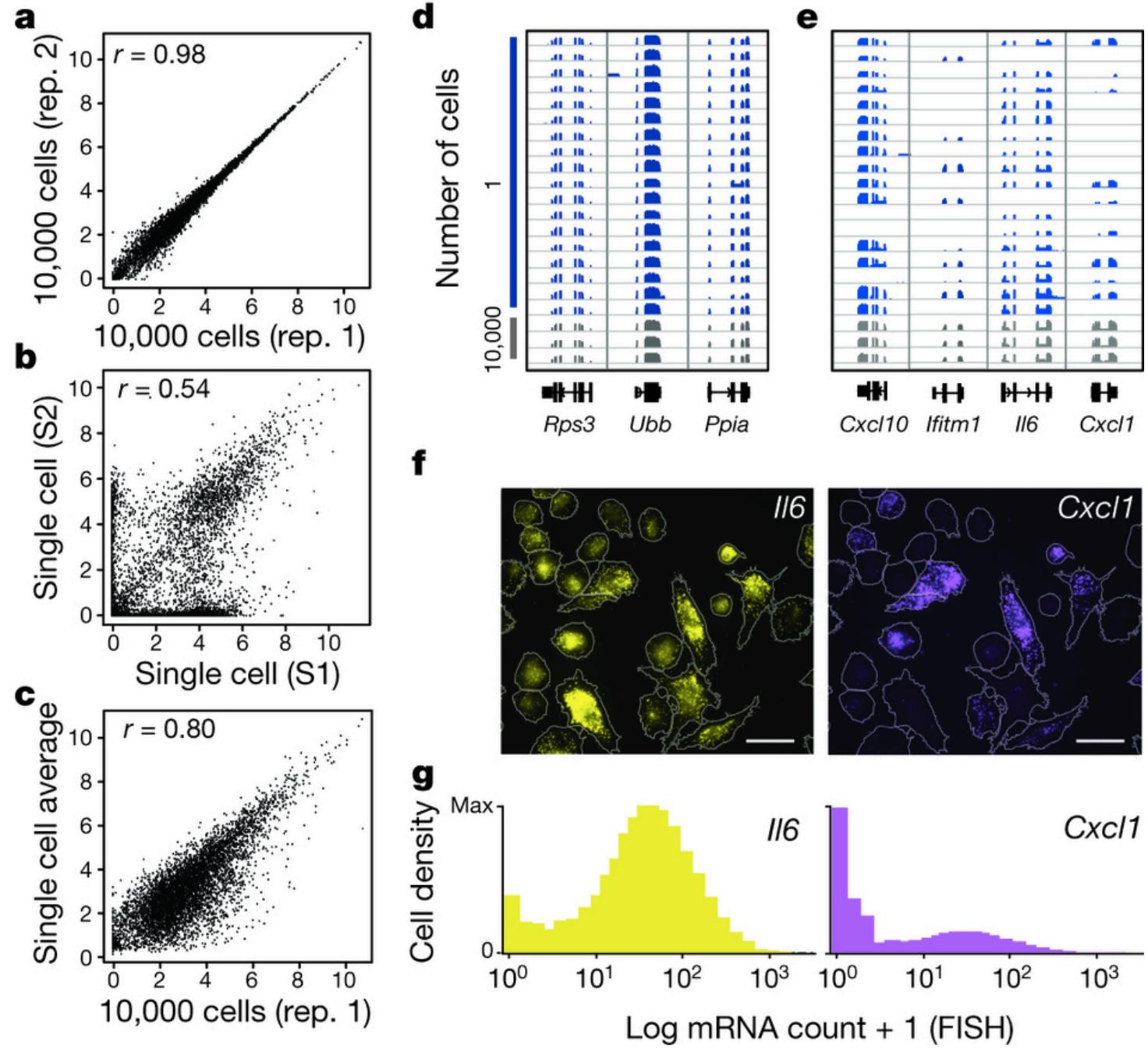
Occasionally it falls off, allowing RNAP to transcribe an mRNA





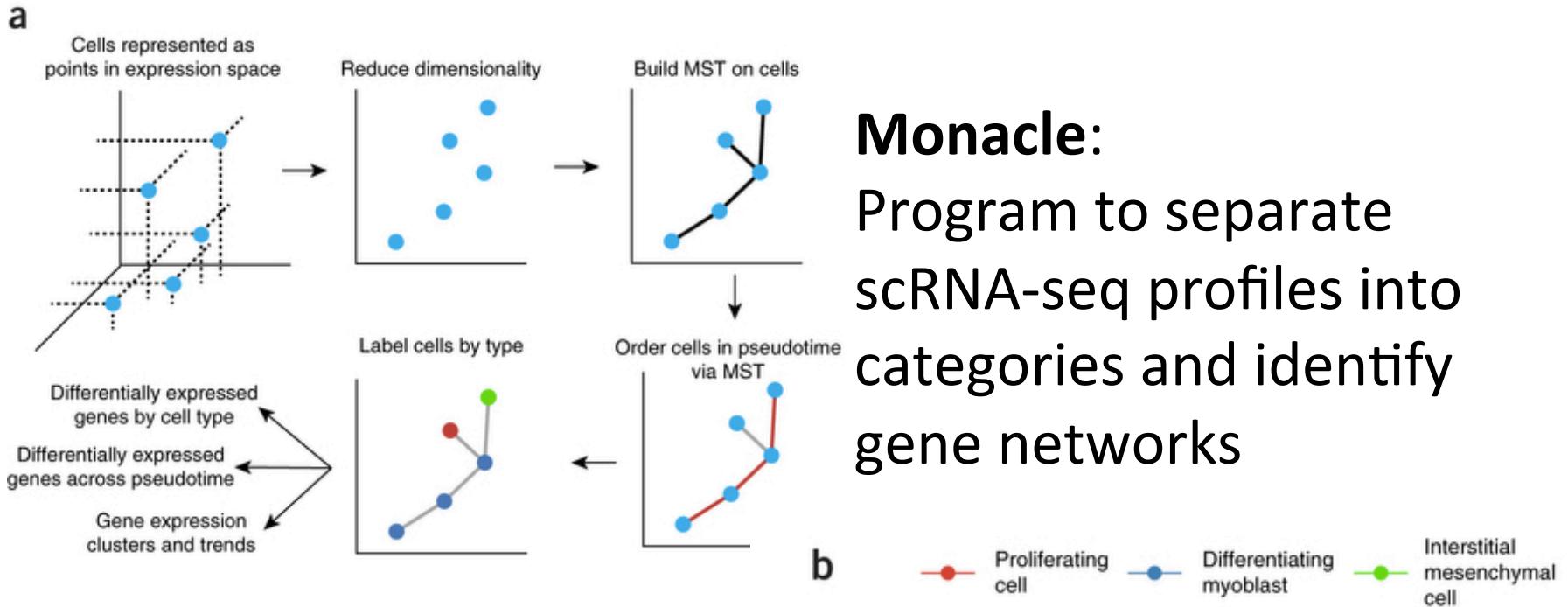
Stochastic, bursting gene expression in eukaryotic cells





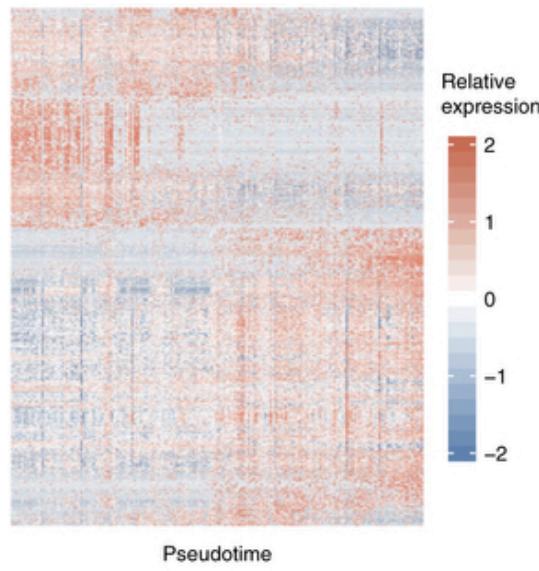
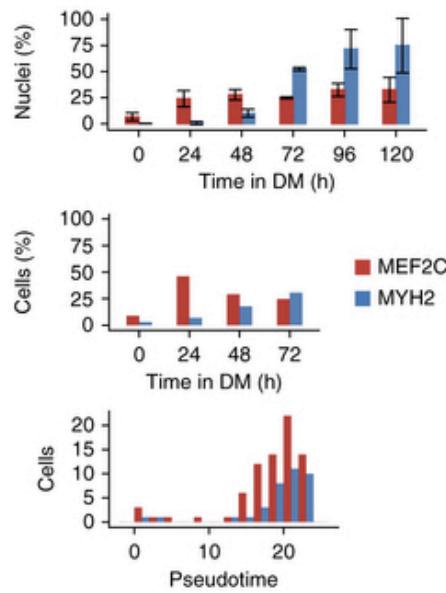
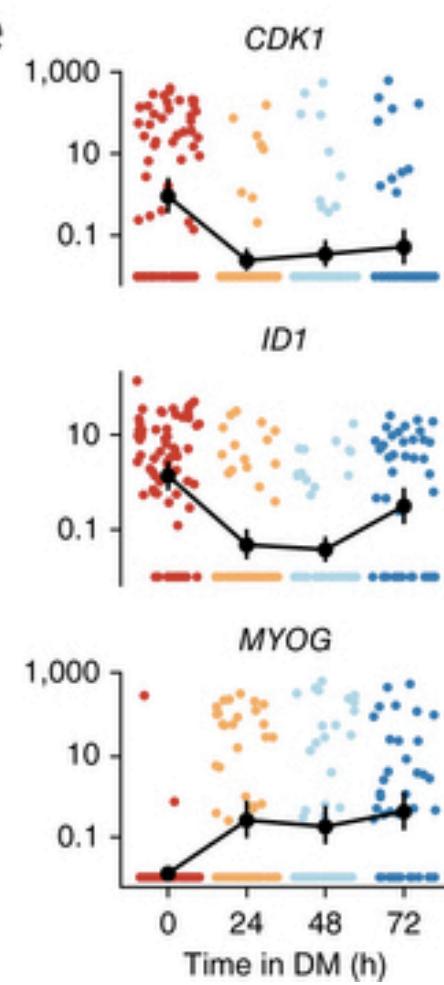
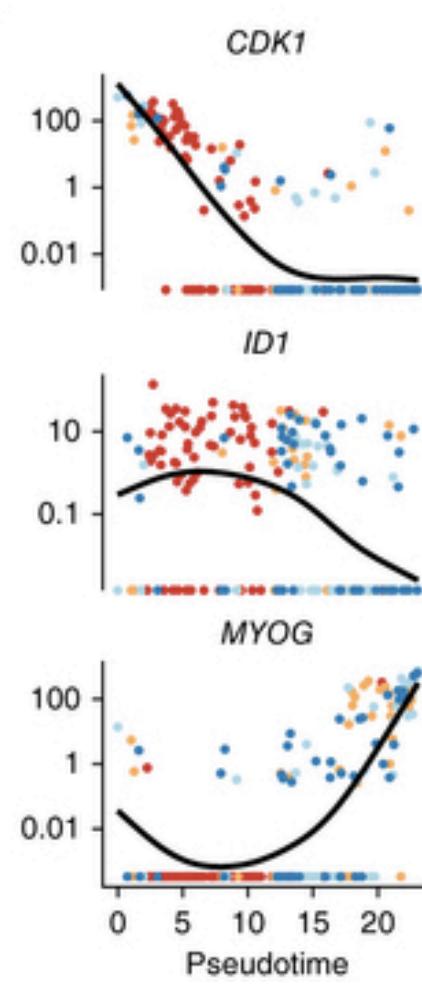
Things we've learned from scRNA-Seq

- Even cells that are genetically the same have different RNA expression profiles.
- Better understanding of genetic pathways
 - Less noise in pathway data



Monocle:

Program to separate scRNA-seq profiles into categories and identify gene networks

c**d****e****f**

What is a question that you think might be best answered using single-cell genomics? Why do you need single-cell measurements?

Questions?