

Single-cell Sequencing Platforms

UCD scRNA-seq workshop

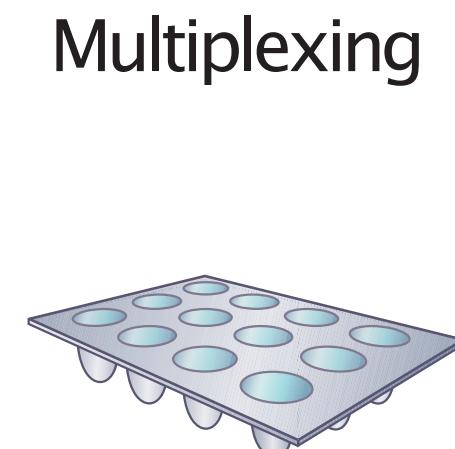
Eric Chow, UCSF Center for Advanced Technology

July 2, 2019

CAT intro

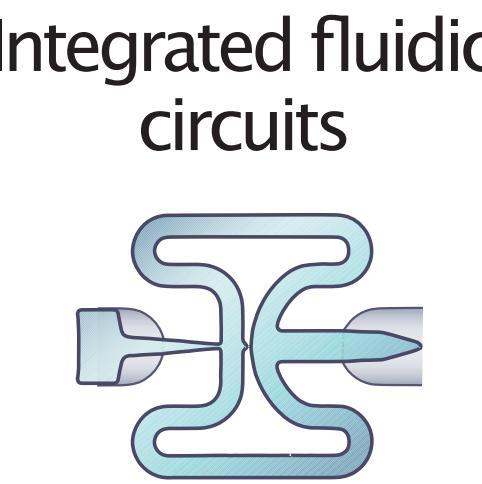
- UCSF Center for Advanced Technology
- Offers NovaSeq and HiSeq 4000 services
- A lot of equipment users operate on their own - purchase your own reagents.
 - MiSeq, iSeq, 10X, CelSee, BioAnalyzer, Qubit, Covaris, Pippin, Biomek FX, Labcyte Echo.
- cat.ucsf.edu

scRNA-seq output has increased significantly



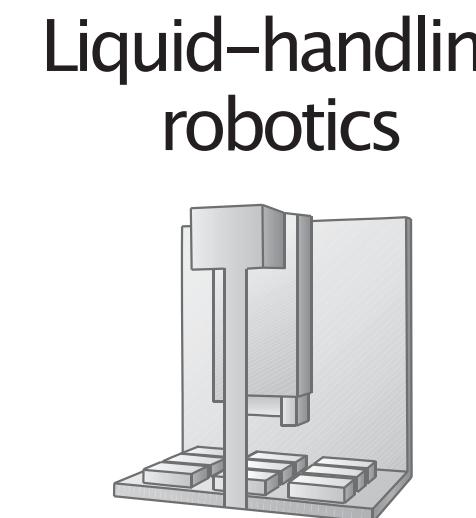
Multiplexing

Islam et al. 2011



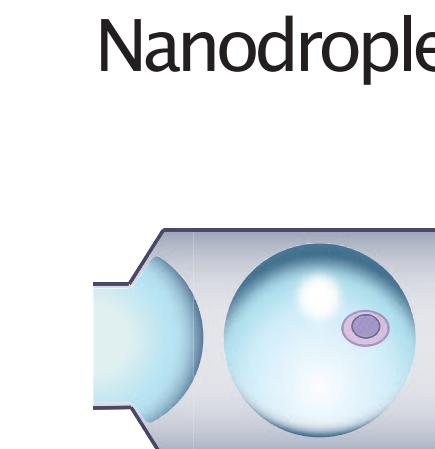
Integrated fluidic circuits

Brennecke et al. 2013



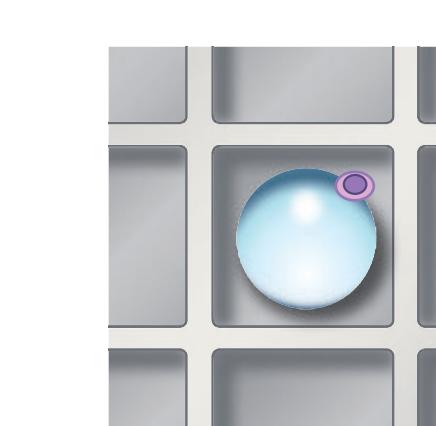
Liquid-handling robotics

Jaitin et al. 2014



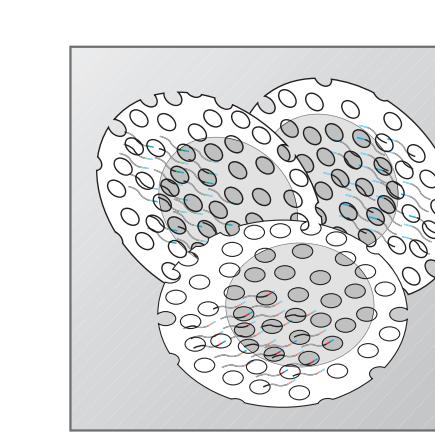
Nanodroplets

Klein et al. 2015
Macosko et al. 2015



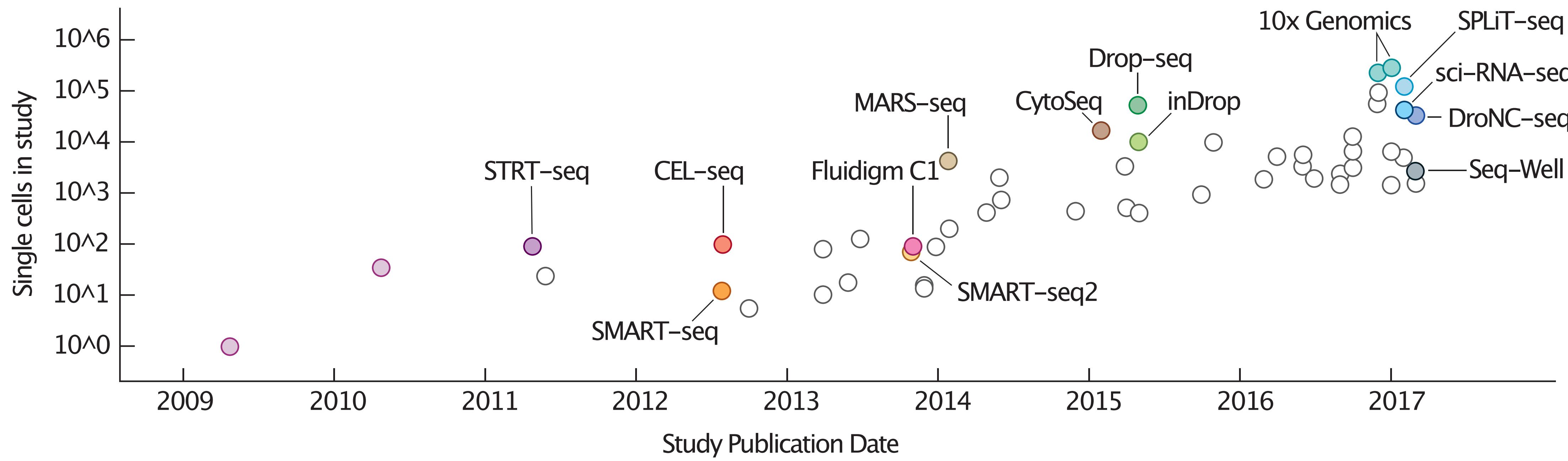
Picowells

Bose et al. 2015



In situ barcoding

Cao et al. 2017
Rosenberg et al. 2017



Methods covered today

- Plate based Smart-seq
- DropSeq
- SCl-seq
- 10X Genomics
- BioRad Illumina ddSEQ
- BD Precise/Resolve
- Wafergen/Takara ICell8
- Scienion/Cellenion

Methods not covered

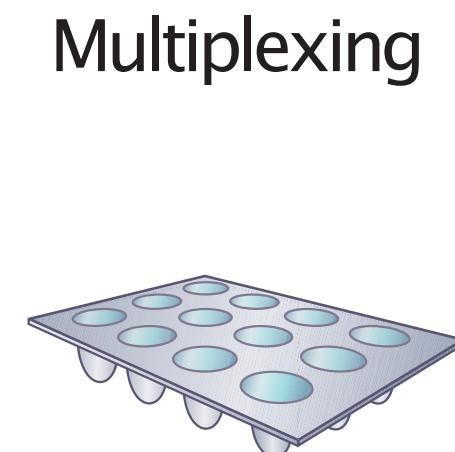
- CelSee
- SeqWell/Honeycomb Bio
- CEL-seq
- SPLIT-seq
- inDrops
- Many others
- Useful resource: https://teichlab.github.io/scg_lib_structs/
- NGS technology talk on iBiology Techniques

NGS intro talk online



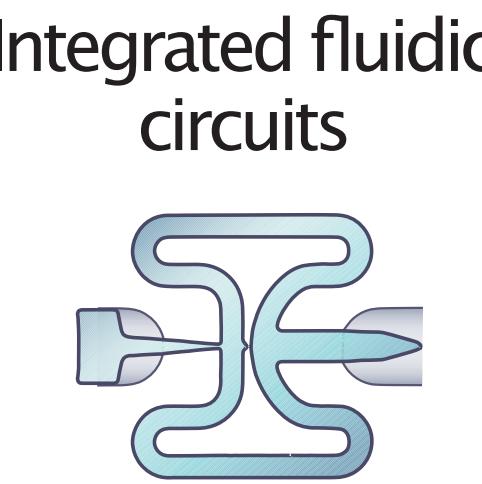
www.ibiology.org

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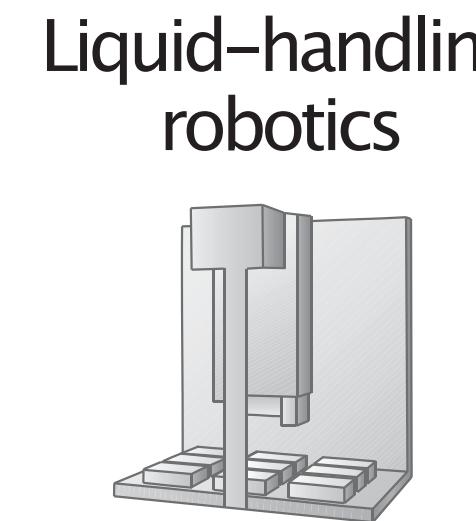
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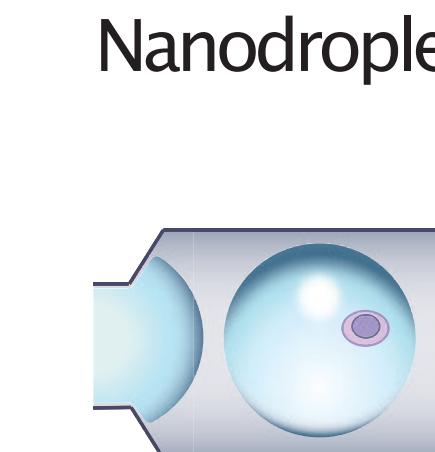
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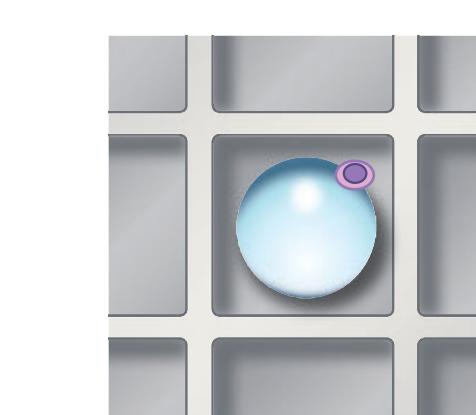
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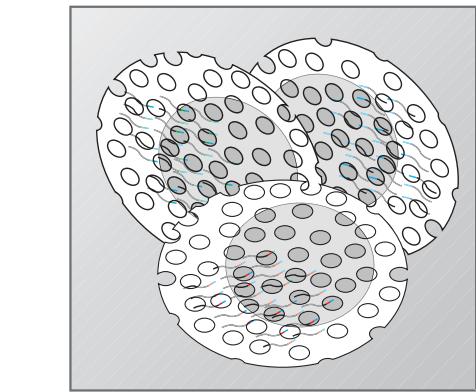
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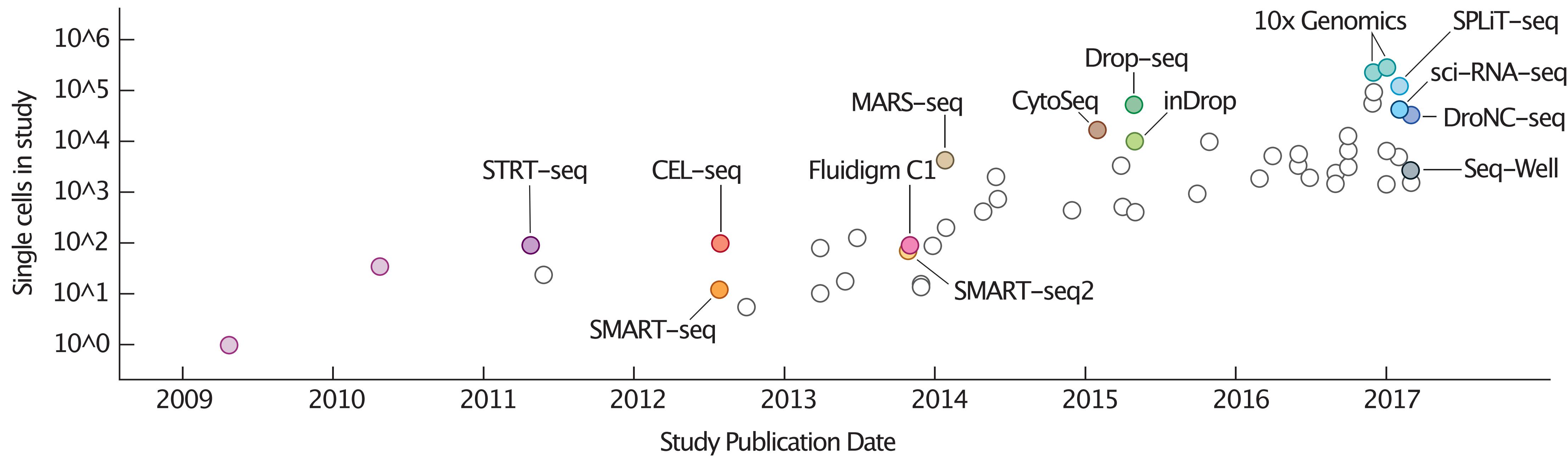


Plate-based SMART-seq

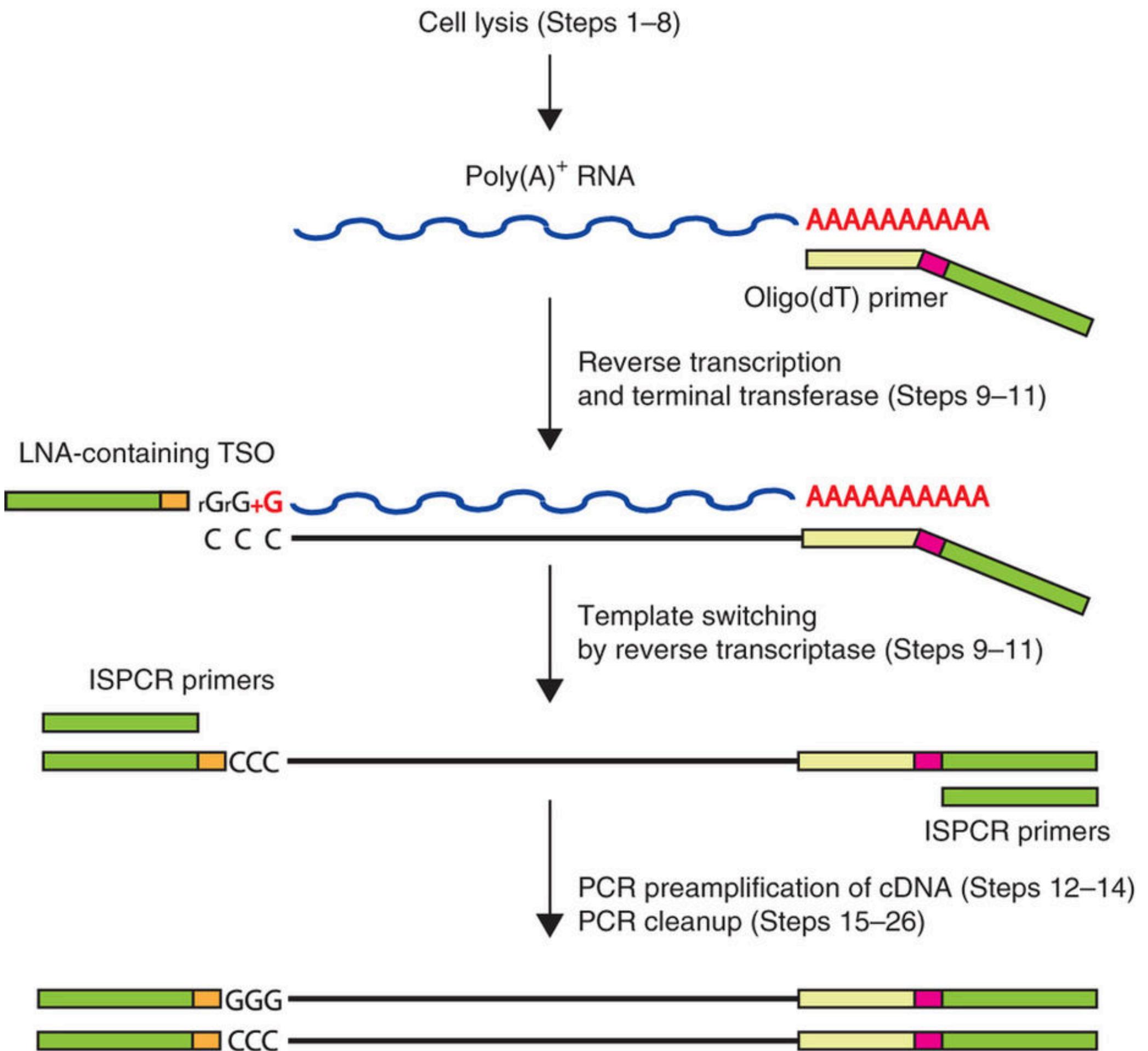
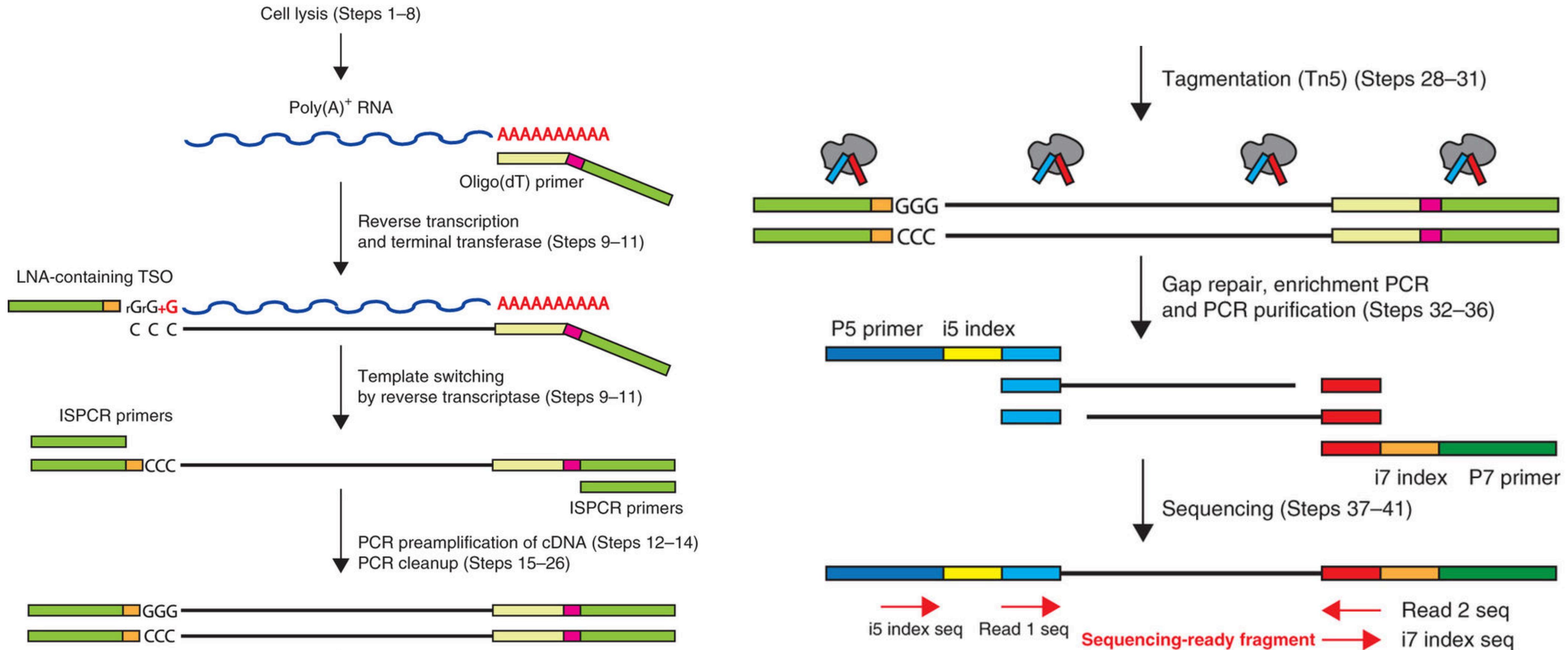
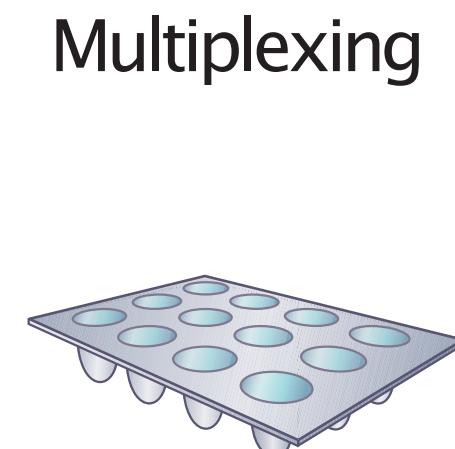


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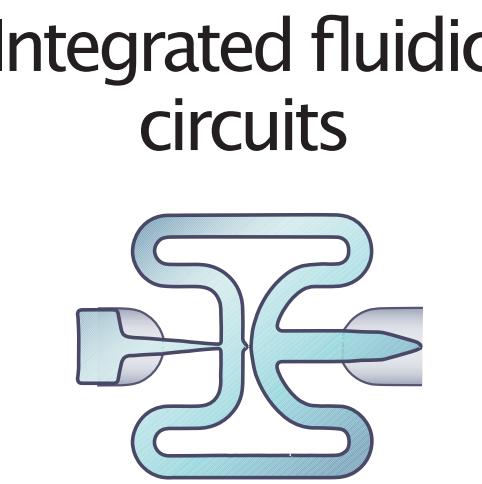


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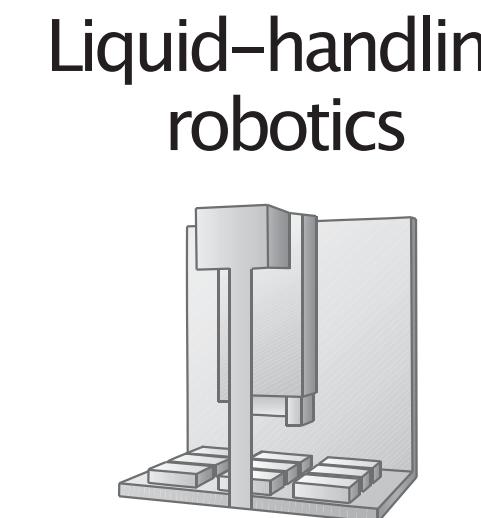
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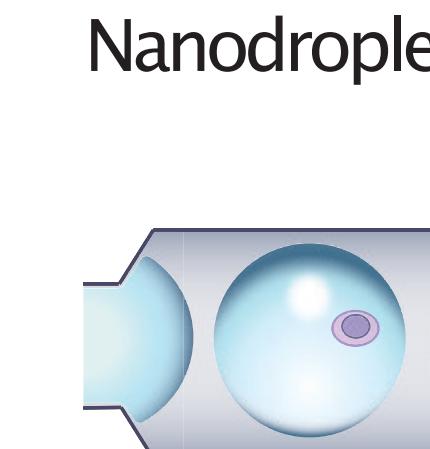
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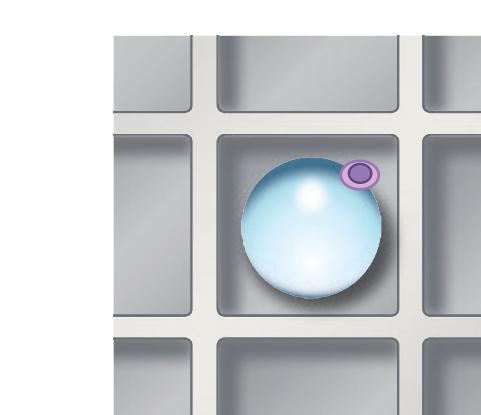
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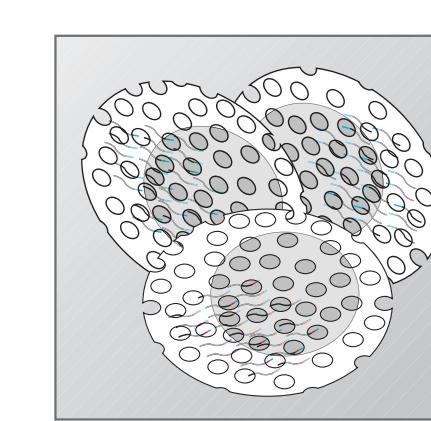
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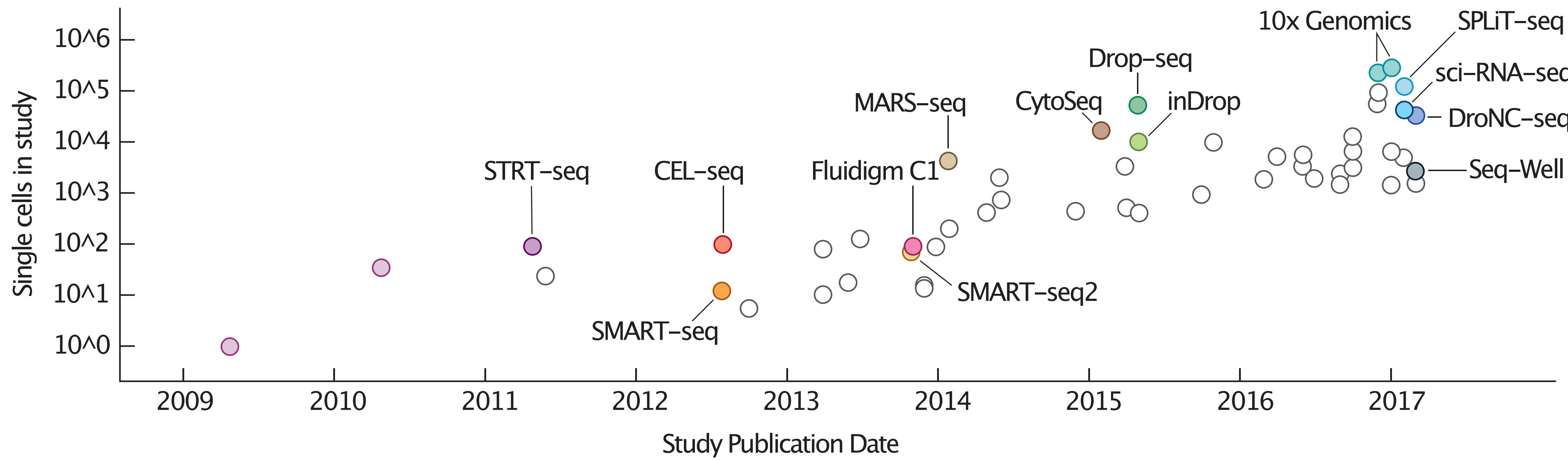
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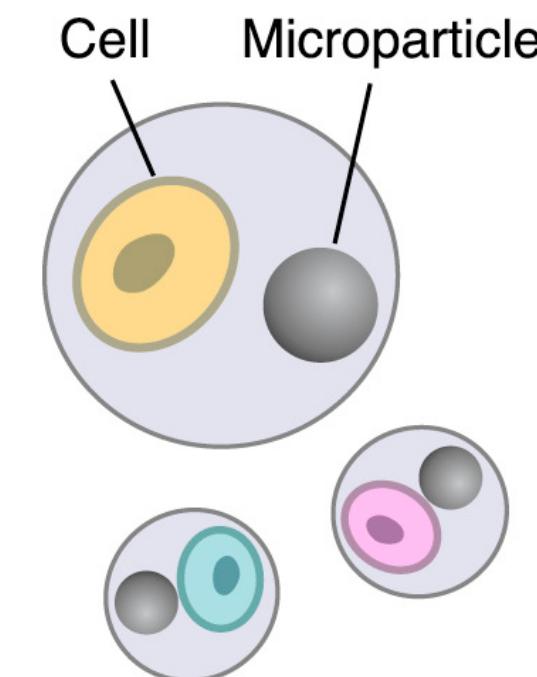
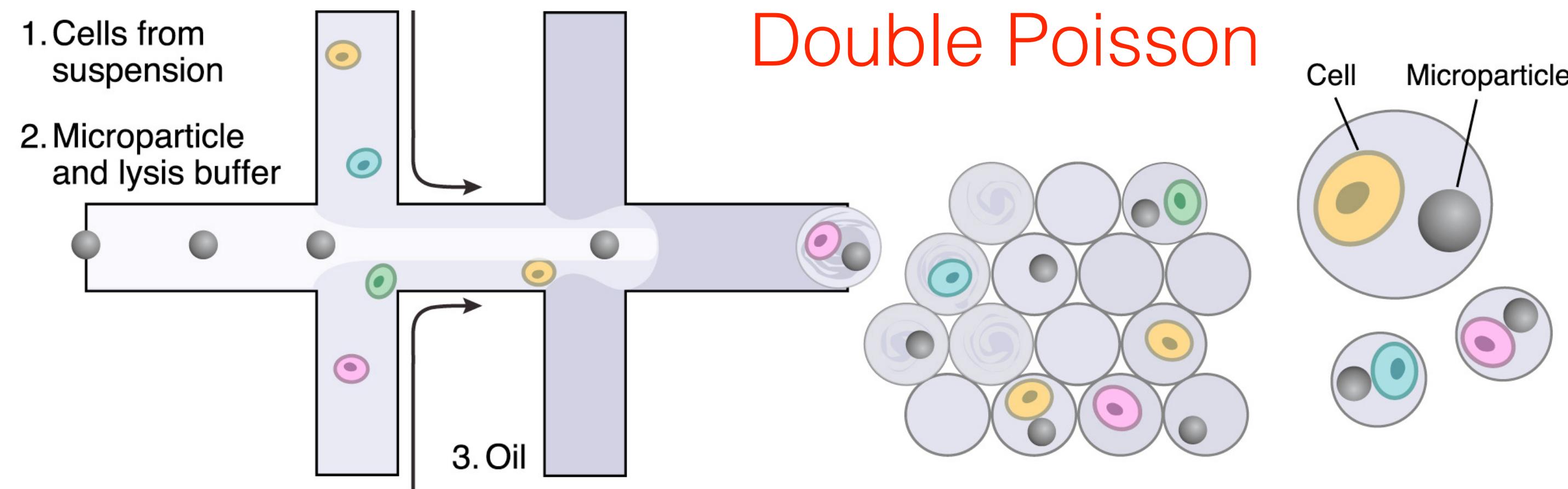
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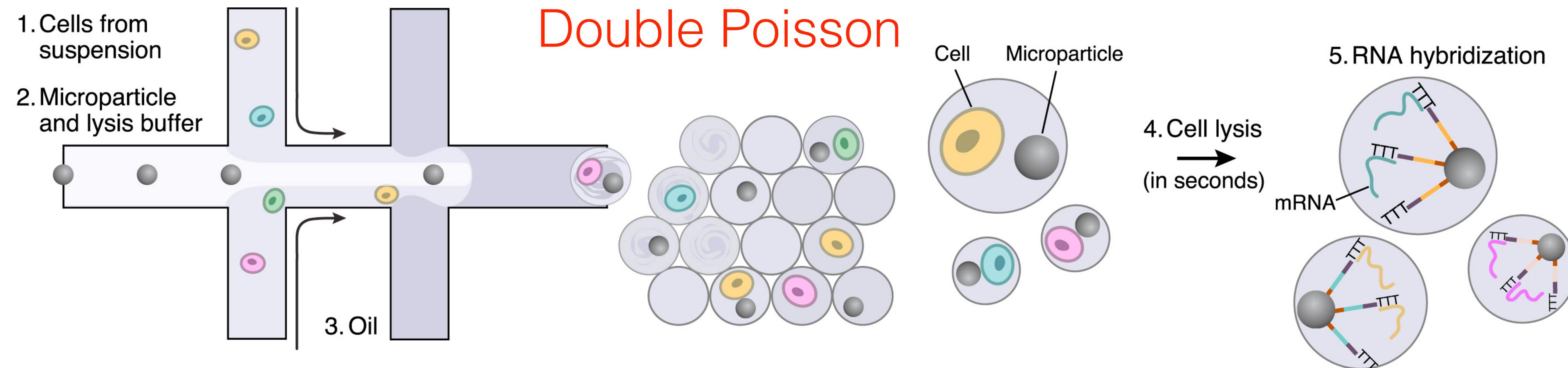


Svensson et al 2018

DropSeq

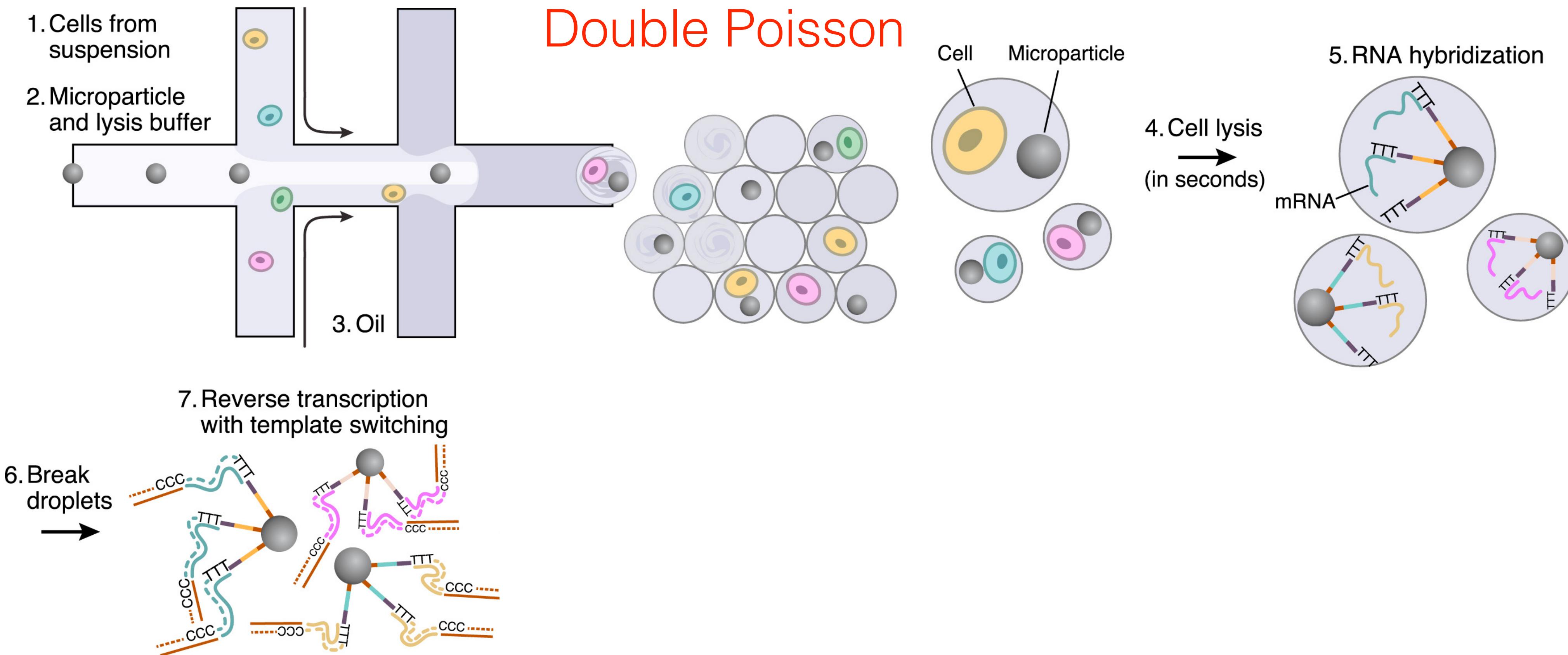


DropSeq



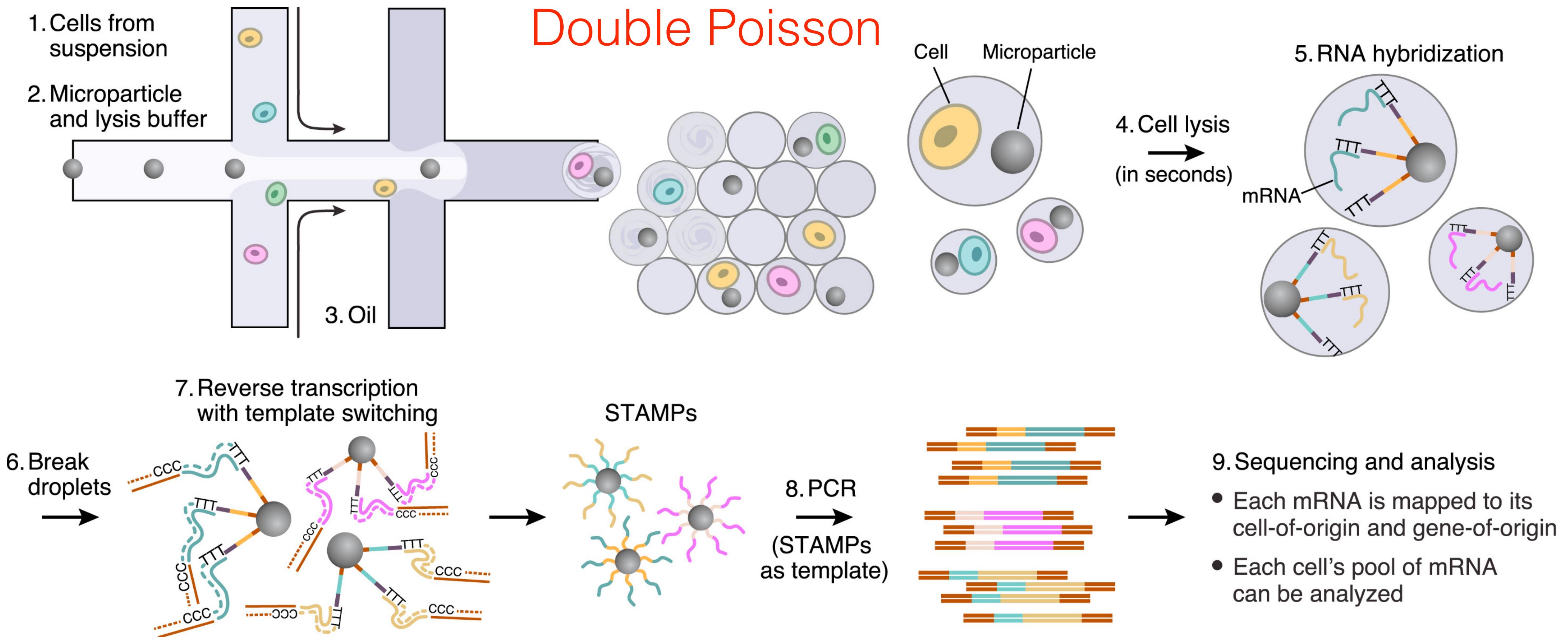
Macosko et al 2015 Cell

DropSeq



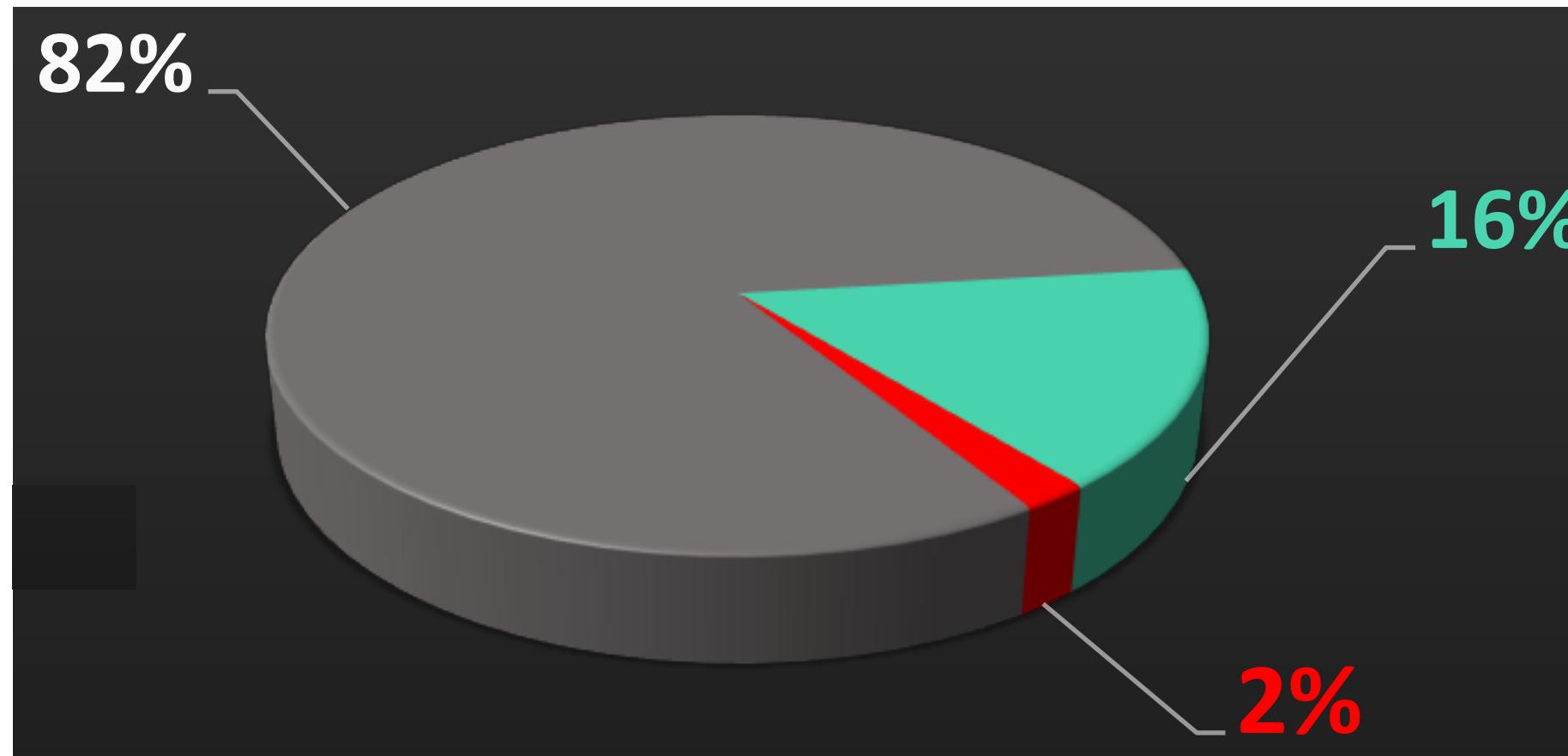
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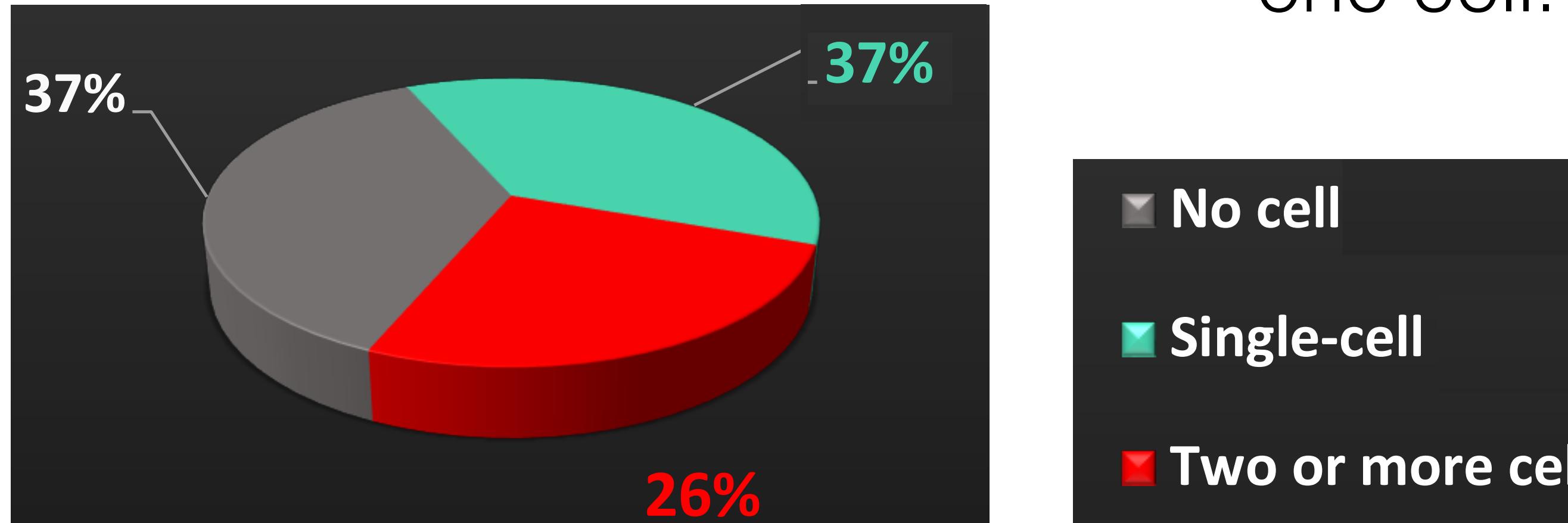


Macosko et al 2015 Cell

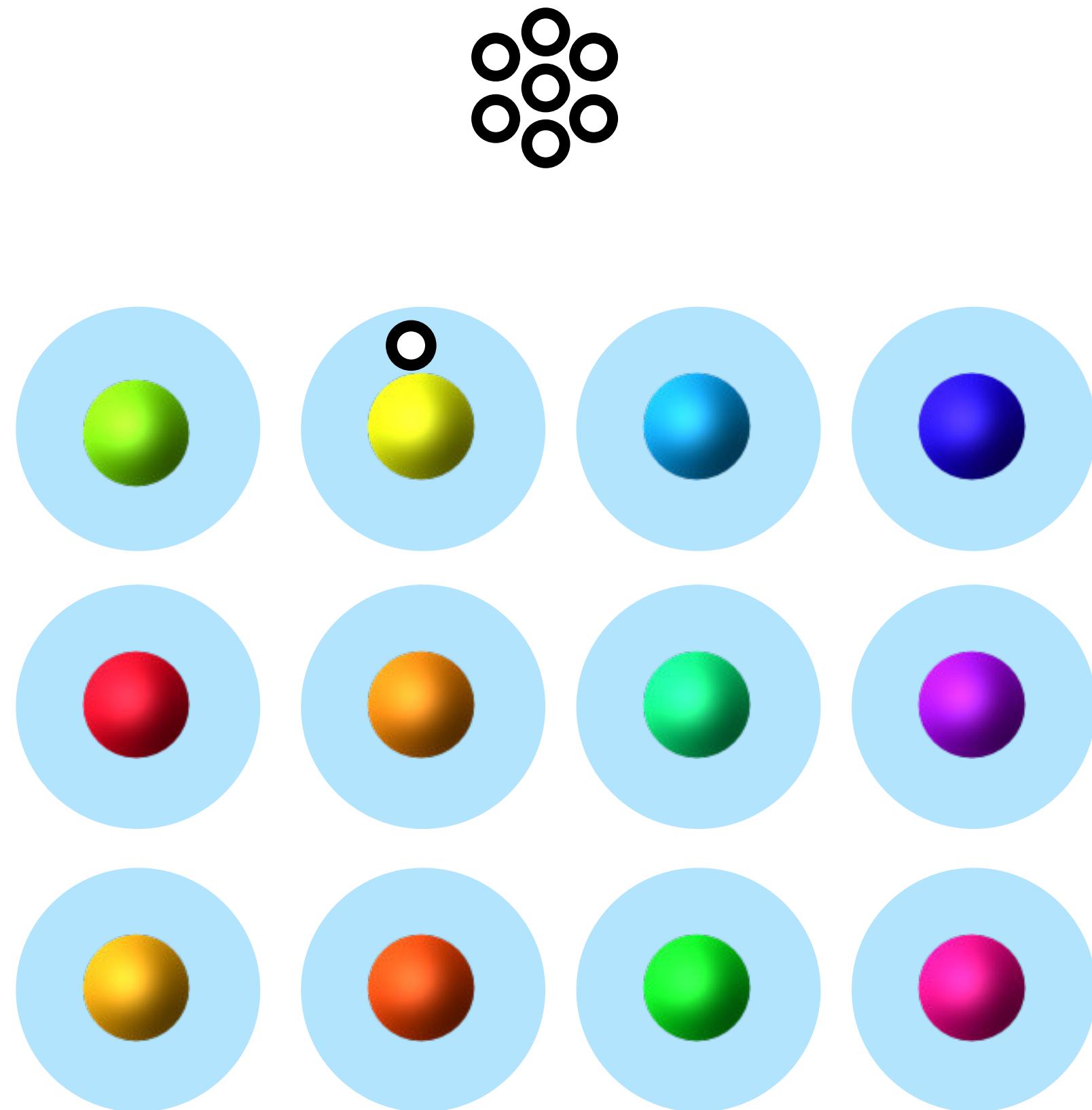
Poisson distribution



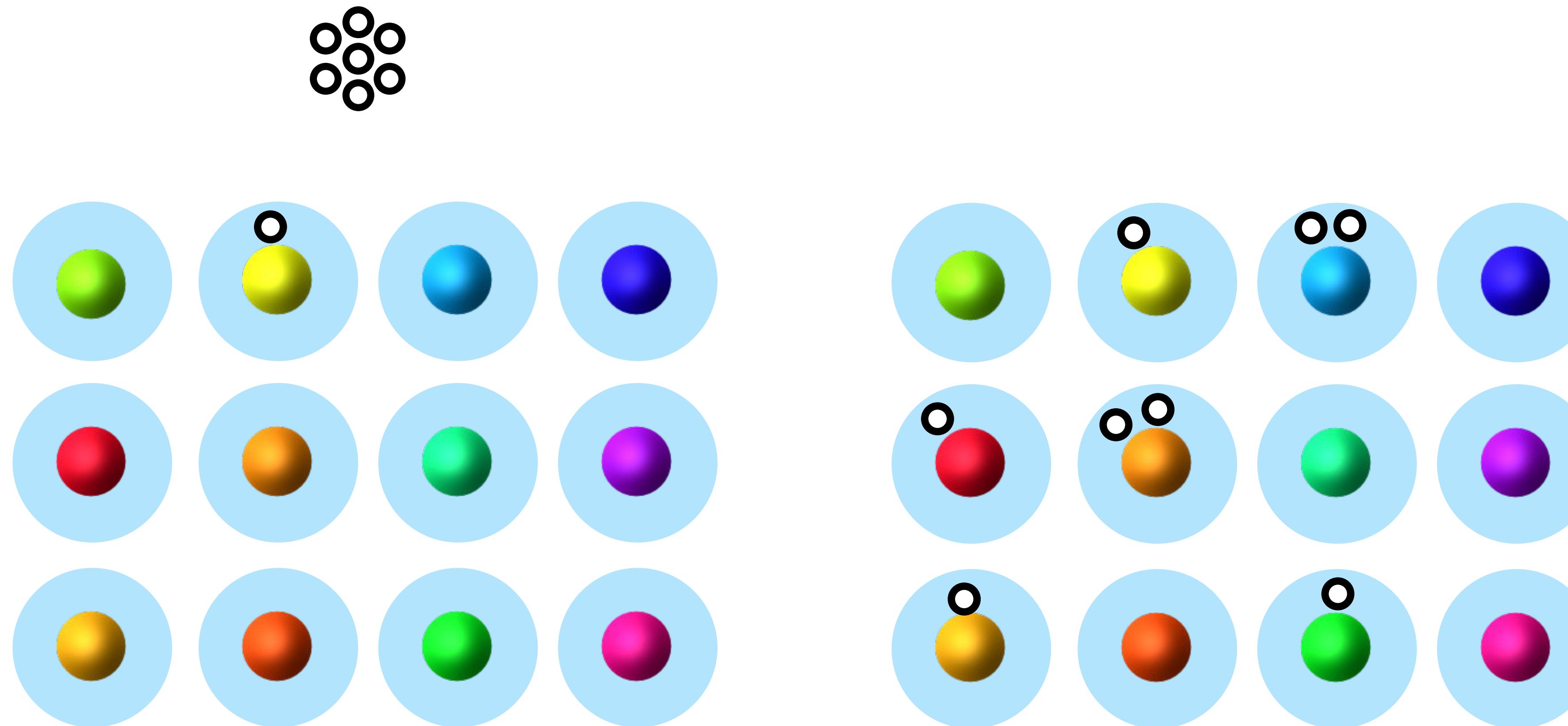
- Only a fraction of droplets contain a bead
- Only a fraction of droplets contain a cell
- Very few droplets will have one bead AND one cell.



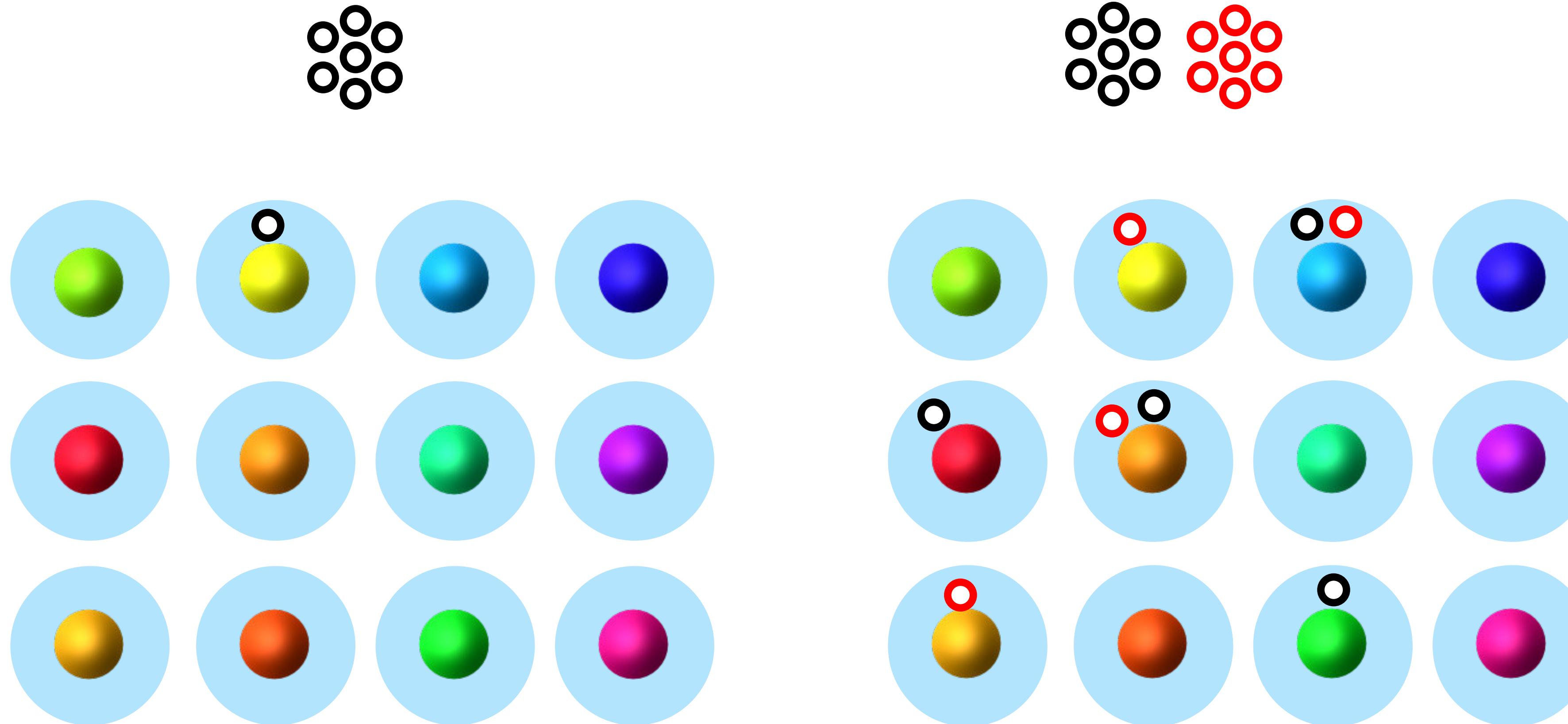
Multiplets limit loading concentration



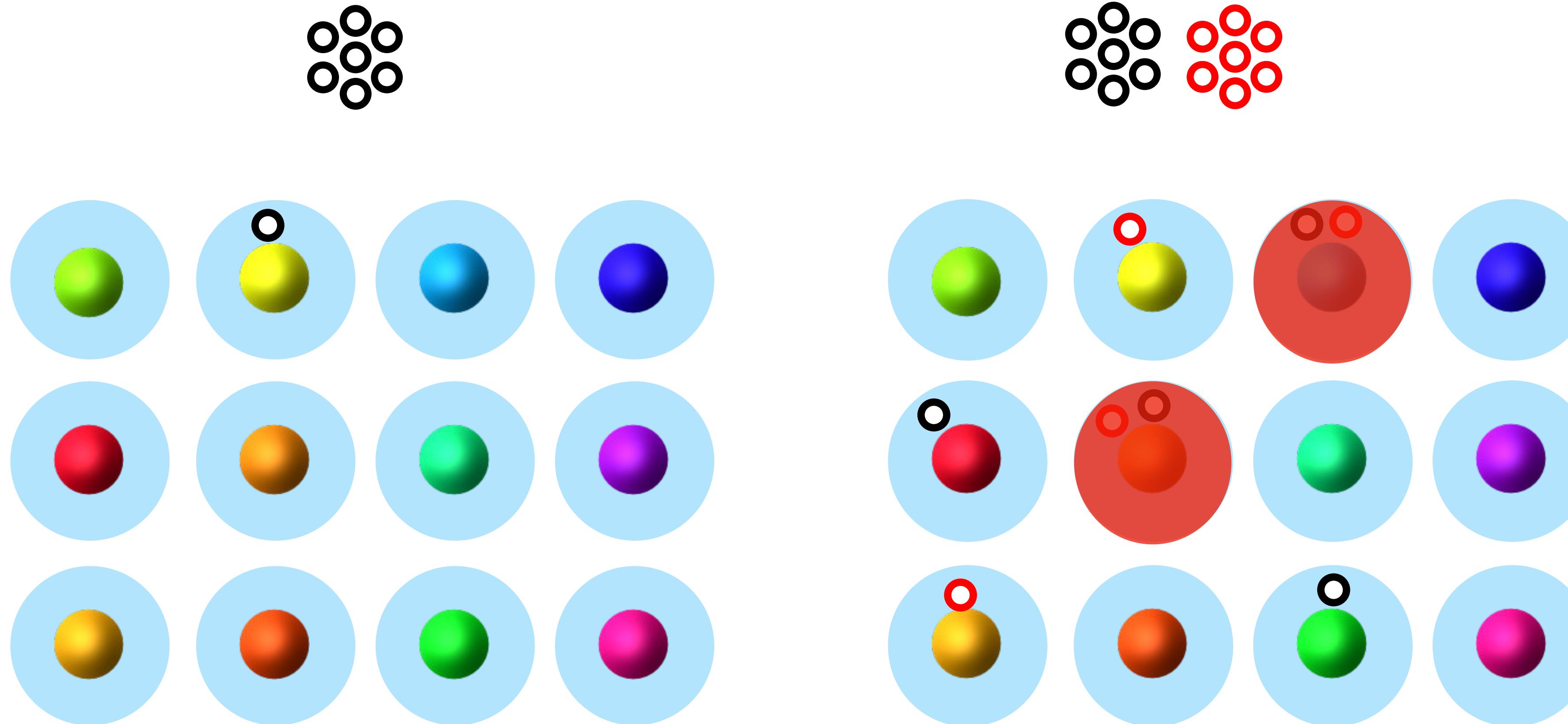
Multiplets limit loading concentration



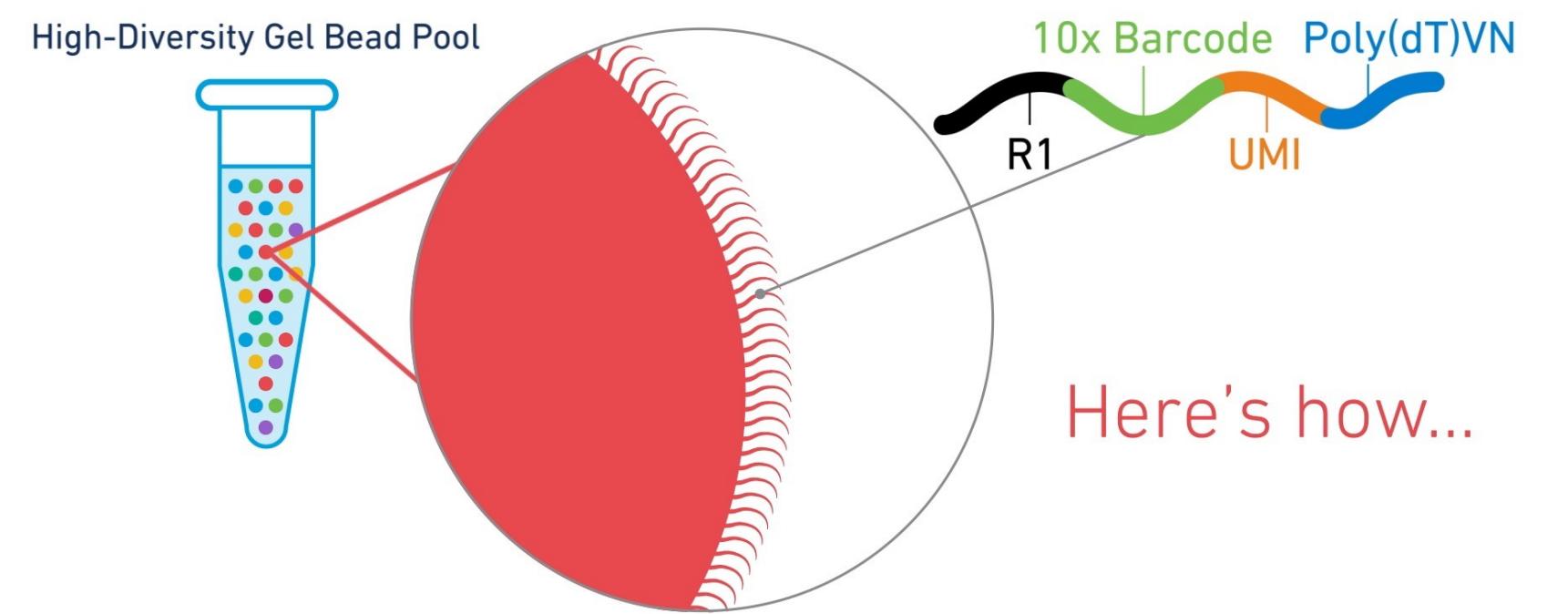
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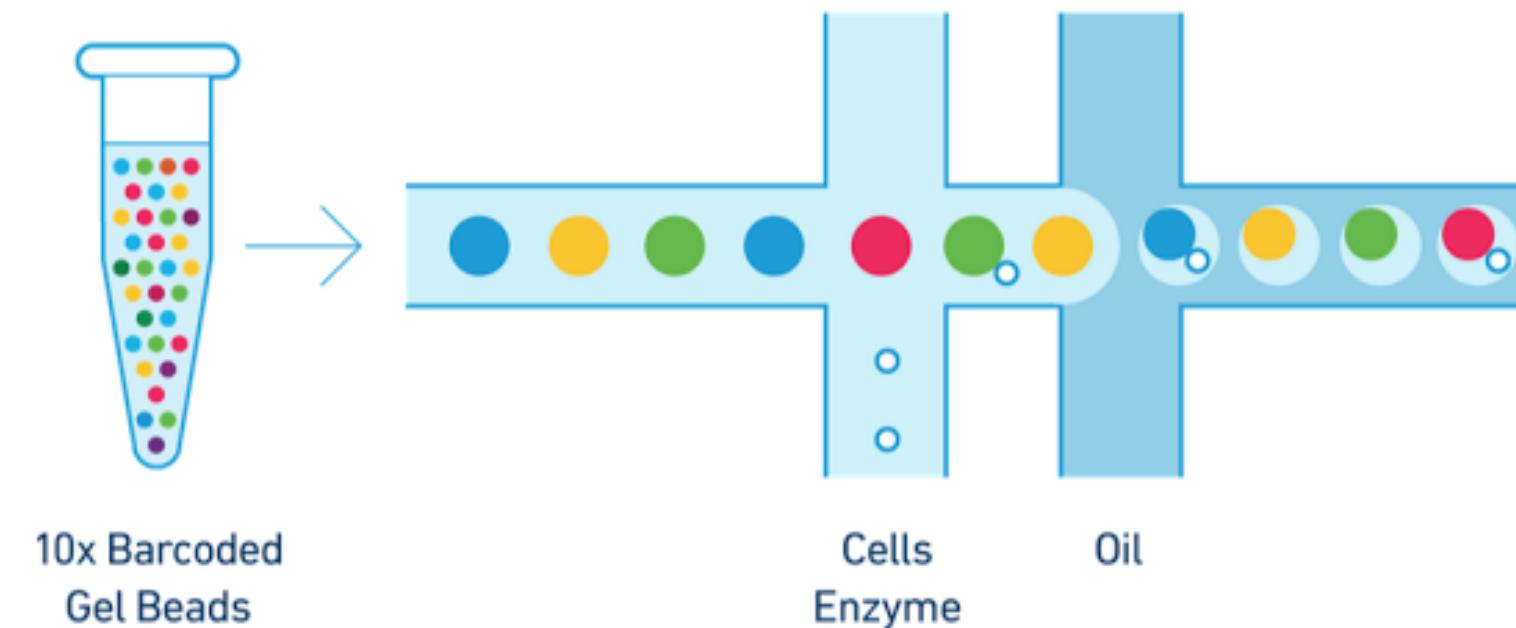
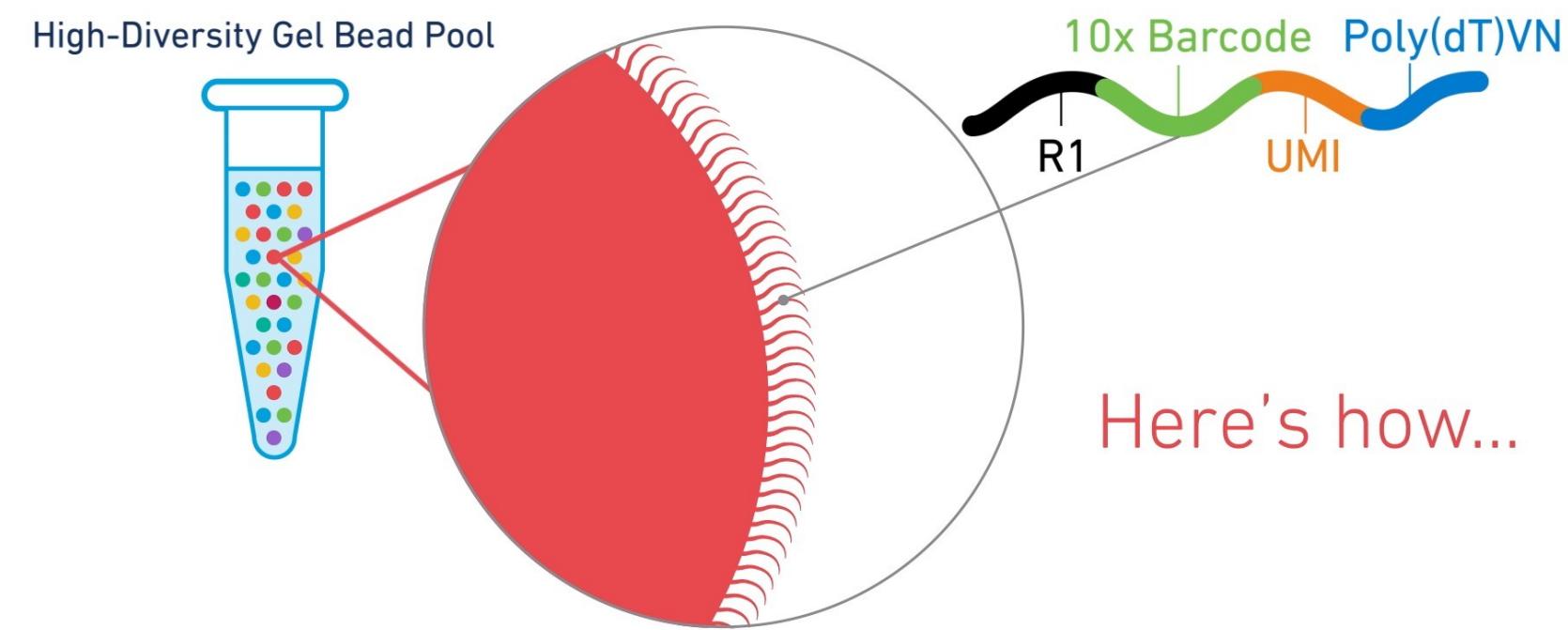


10X Genomics

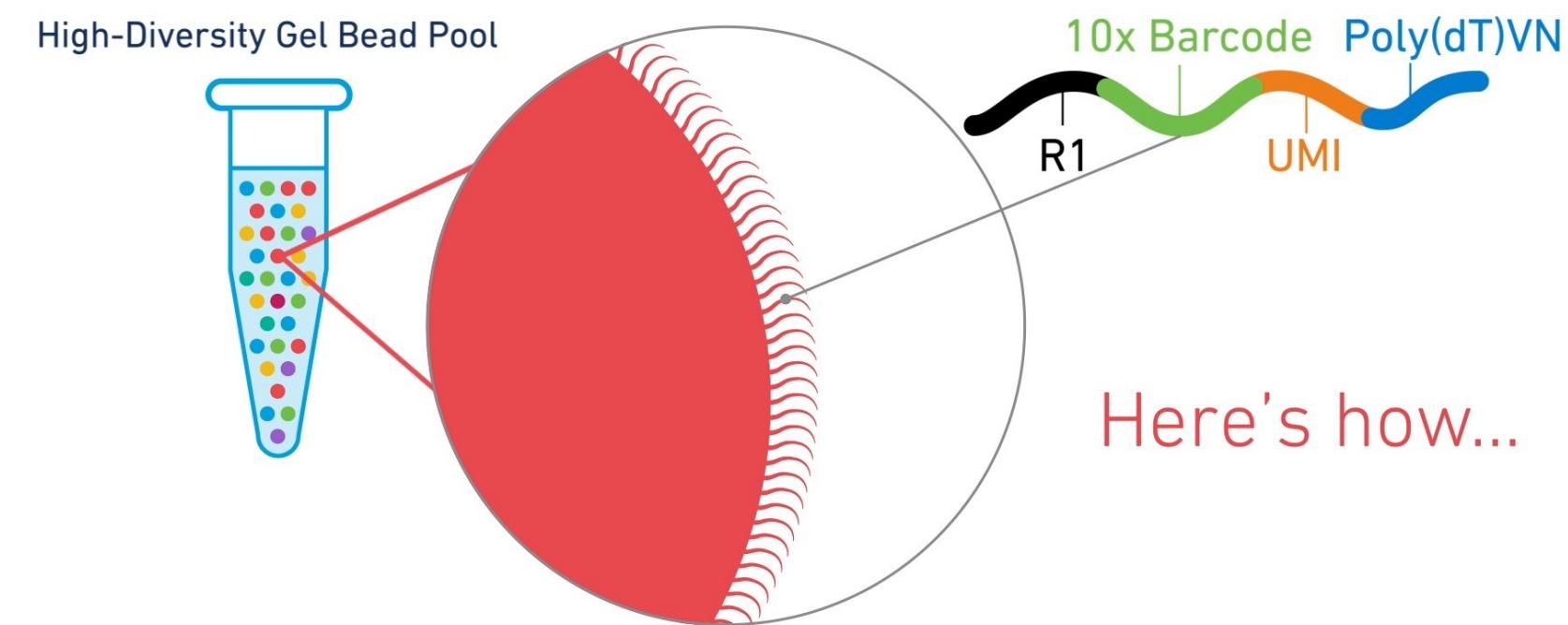


Here's how...

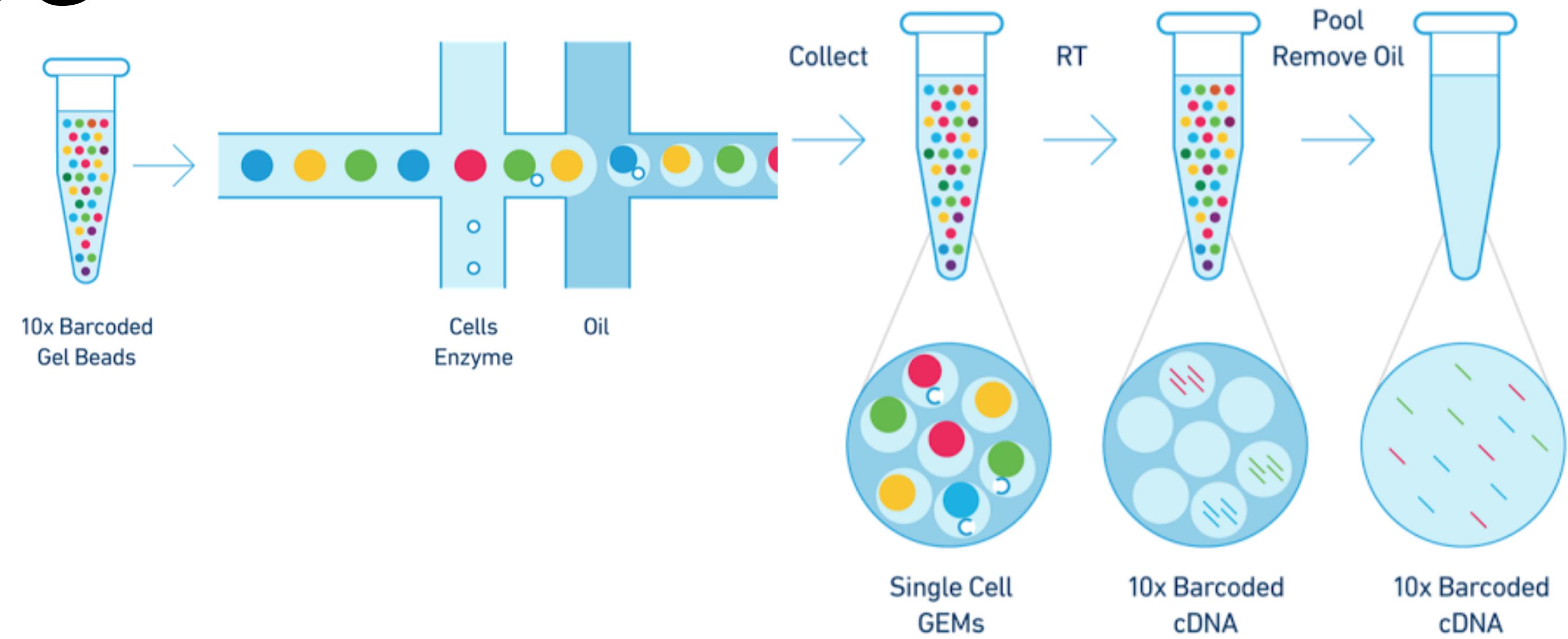
10X Genomics



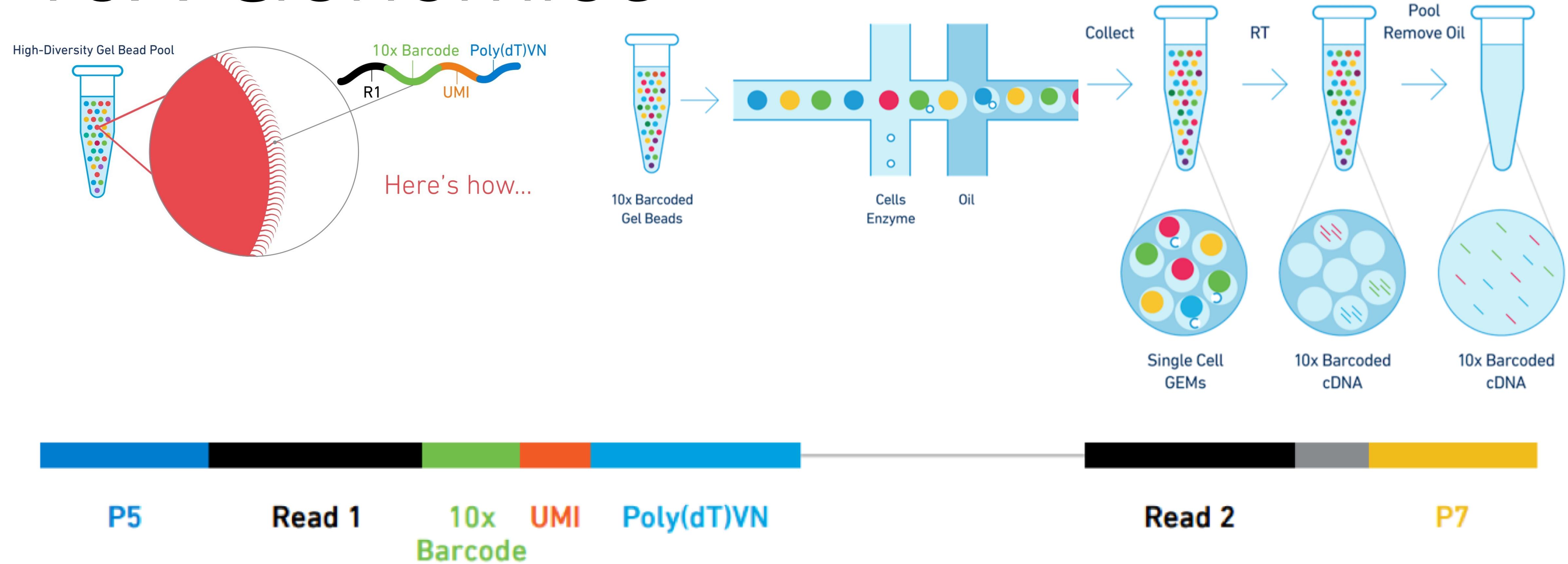
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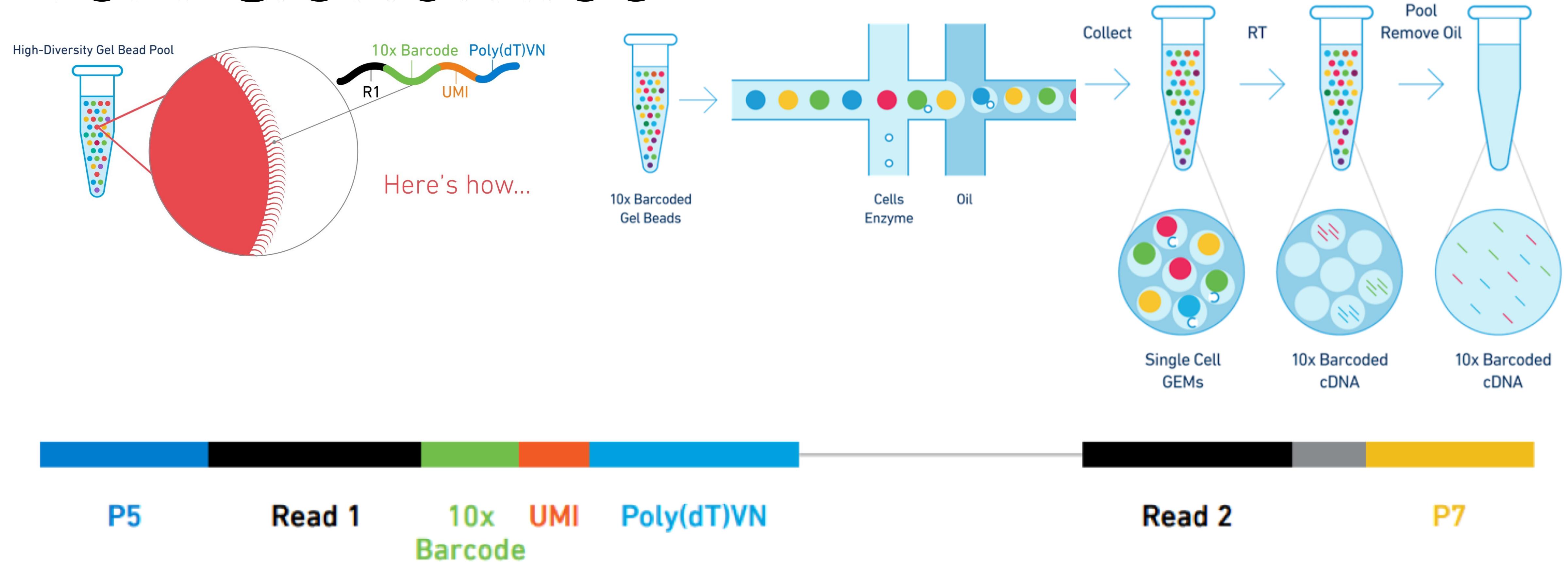
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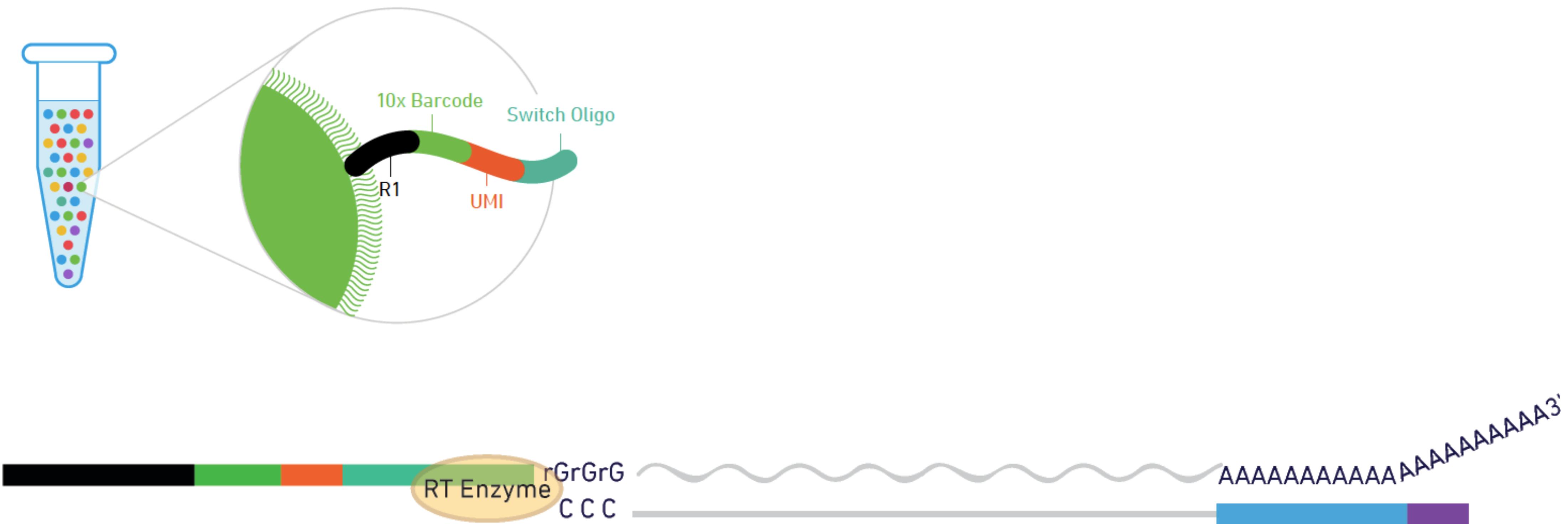


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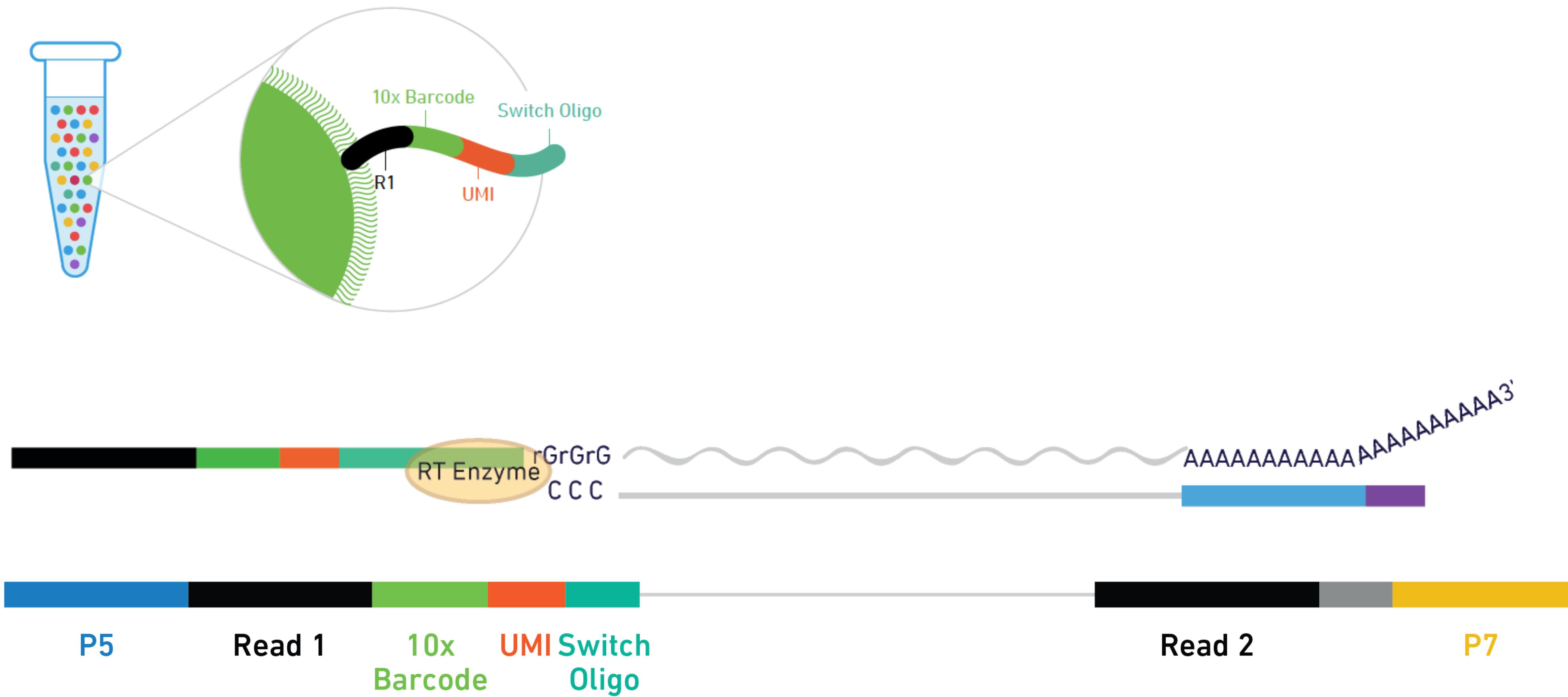


System is single poisson

10X Genomics 5' sequencing

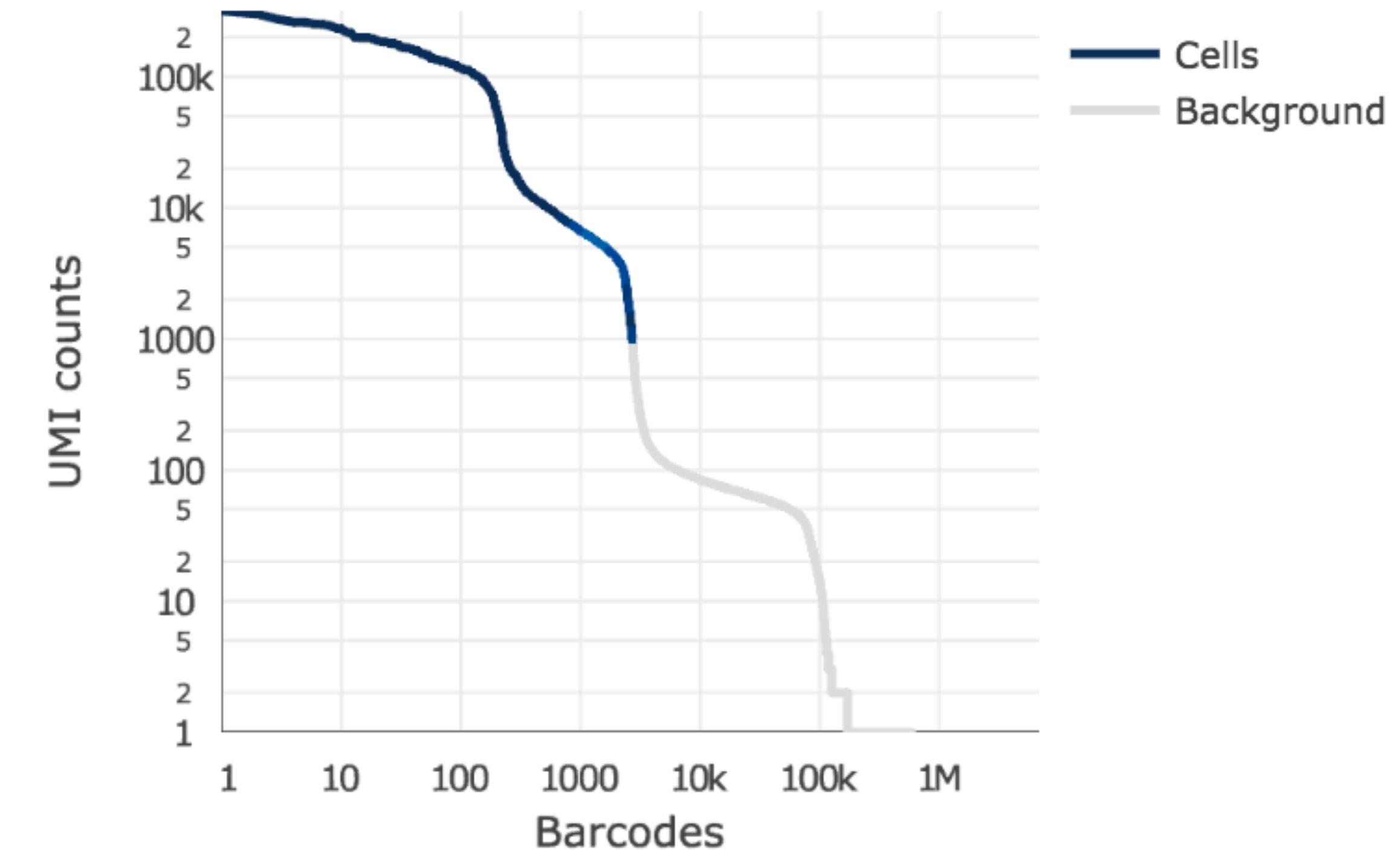
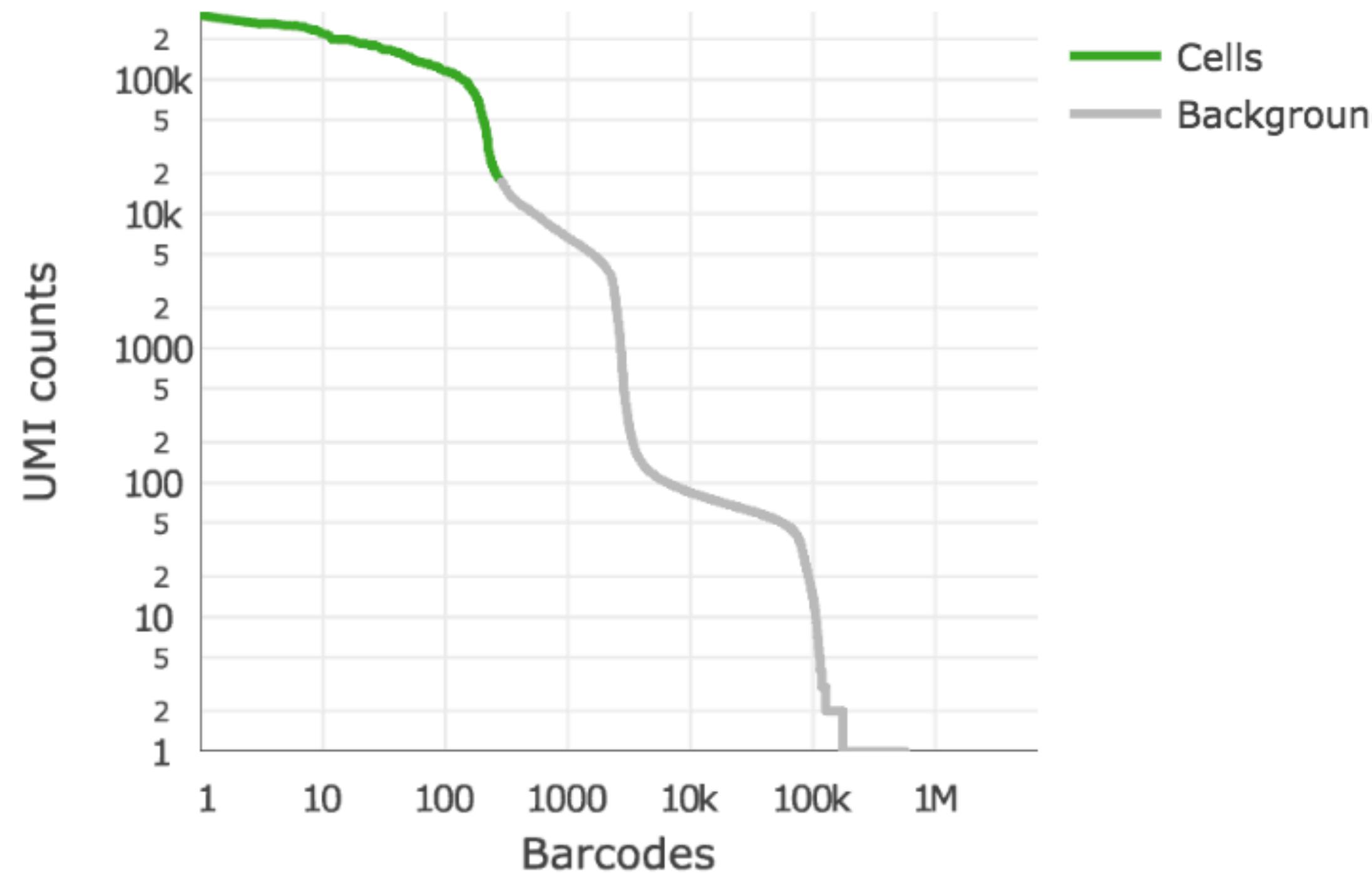


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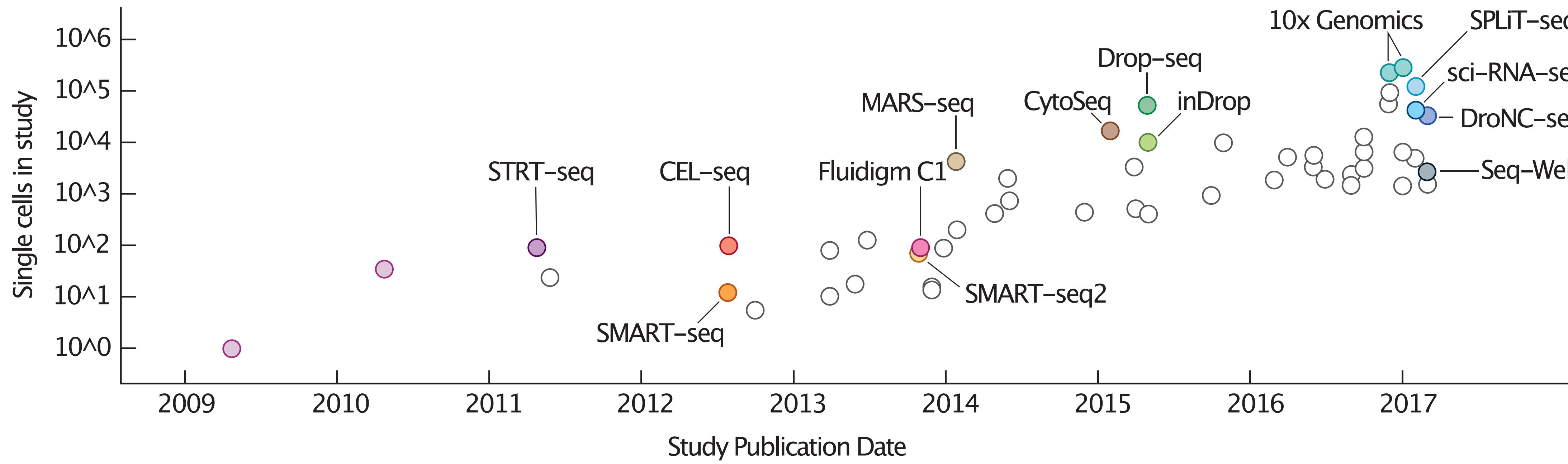
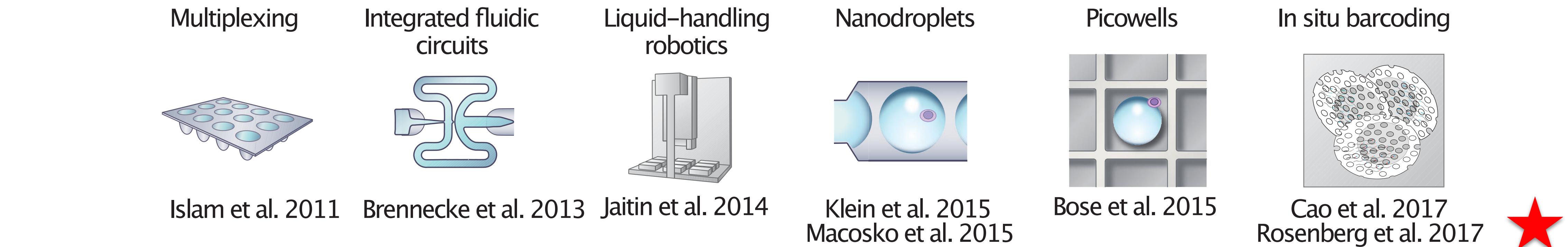


Challenges with droplet methods

- What do you call a cell?



scRNA-seq output has increased significantly

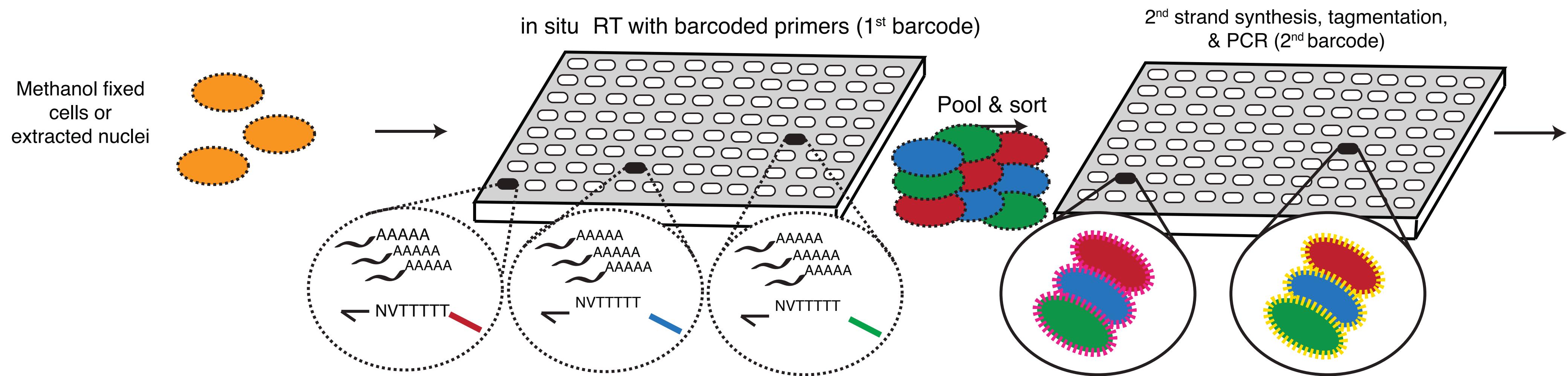


Svensson et al 2018

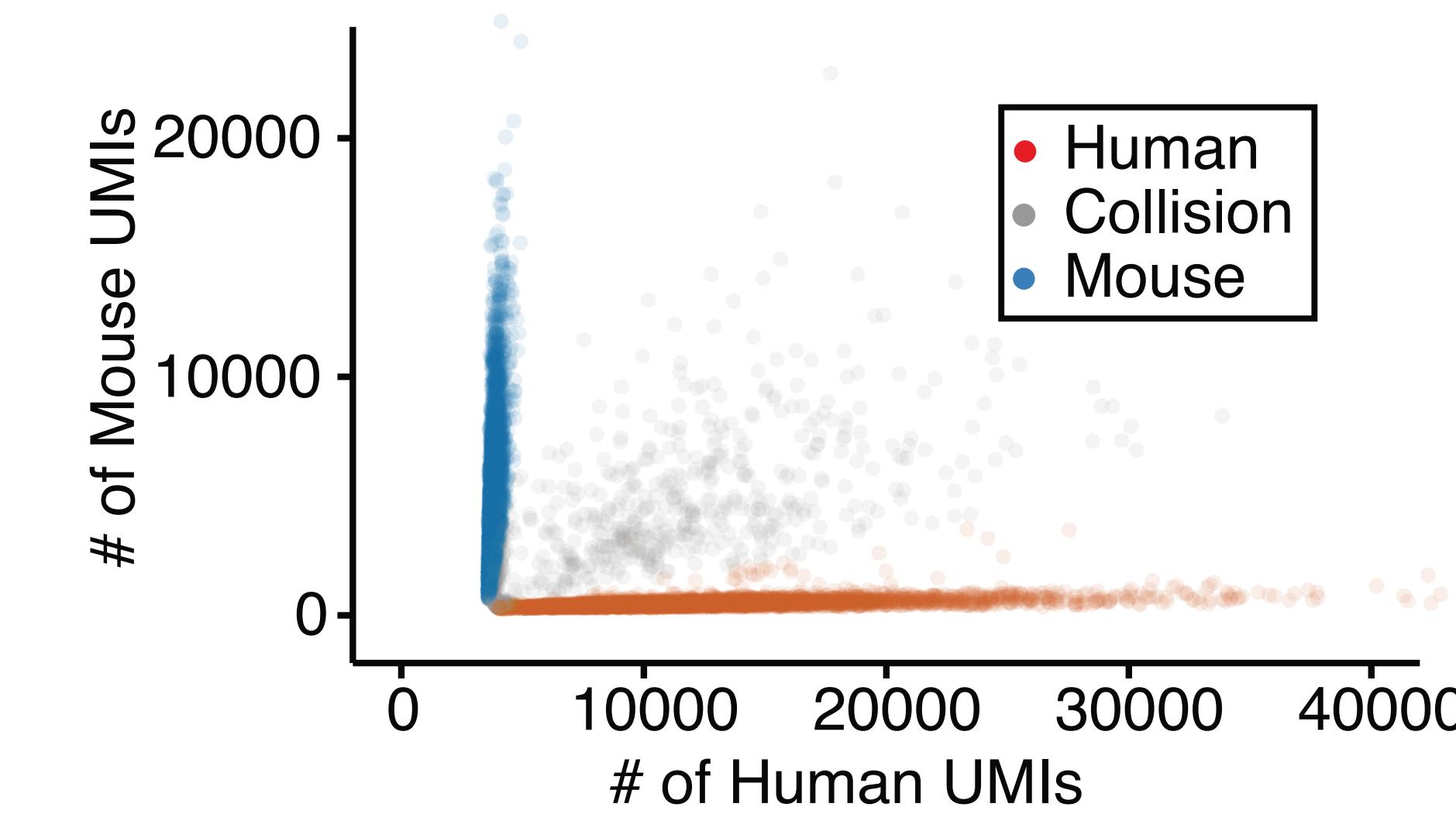
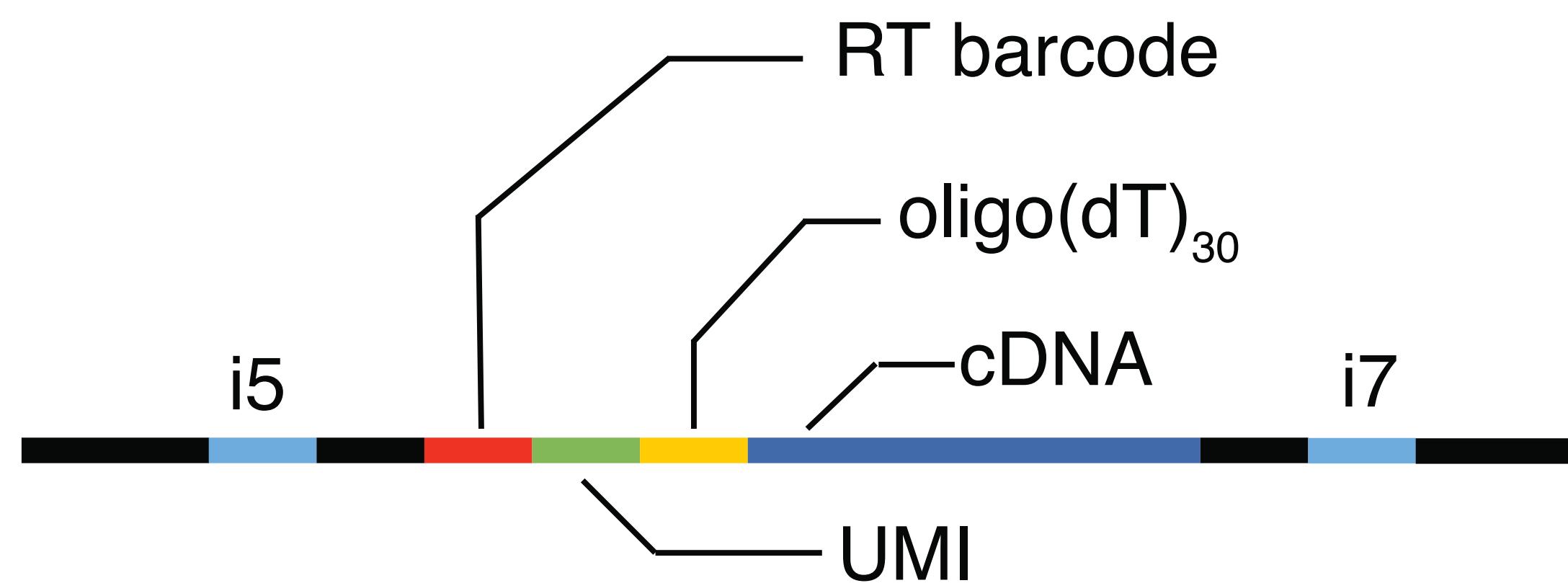
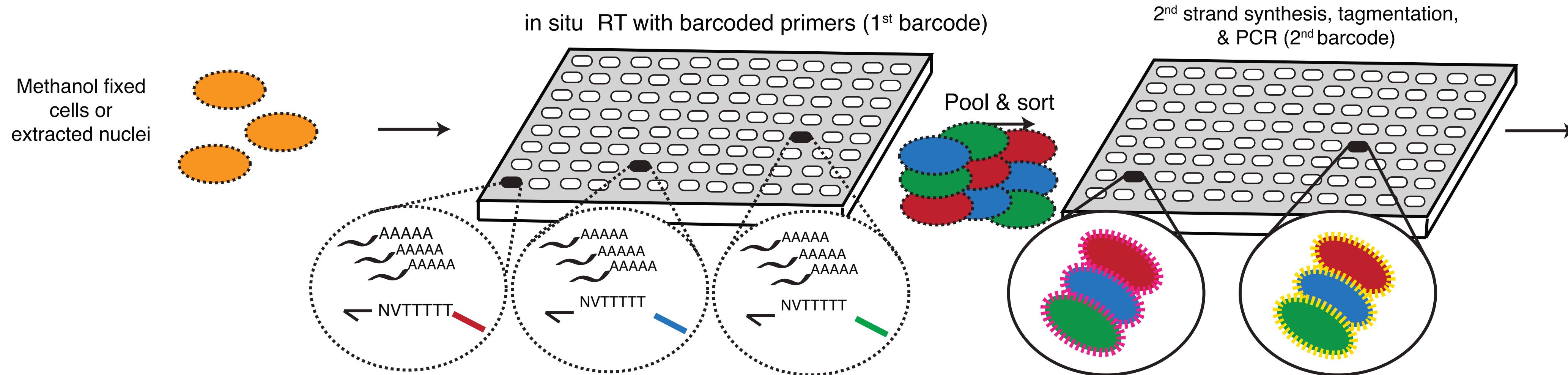
SCI-seq

- Single-cell Combinatorial Indexing
- In situ reactions that add barcodes
- Split pooling in between each step
- Many flavors: RNA-seq, ATAC-seq, Hi-C...

SCI-RNA-seq



SCI-RNA-seq



Cao et al 2017 Science

SCI-seq scales non-linearly

Barcode Combinations

1	96
96	9,216

- In most other platforms, increasing number of reactions scales linearly.
- With SCI-seq, increase is non-linear.
- Going from 2 ->3 barcode levels further increases combinations. Barcoded Nextera or ligation.

SCI-seq scales non-linearly

Barcode Combinations

1	96	384
96	9,216	36,864
384	36,864	147,456

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SCI-seq scales non-linearly

Barcode Combinations

1	96	384	1536
96	9,216	36,864	147,456
384	36,864	147,456	589,824
1536	147,456	589,824	2,359,296

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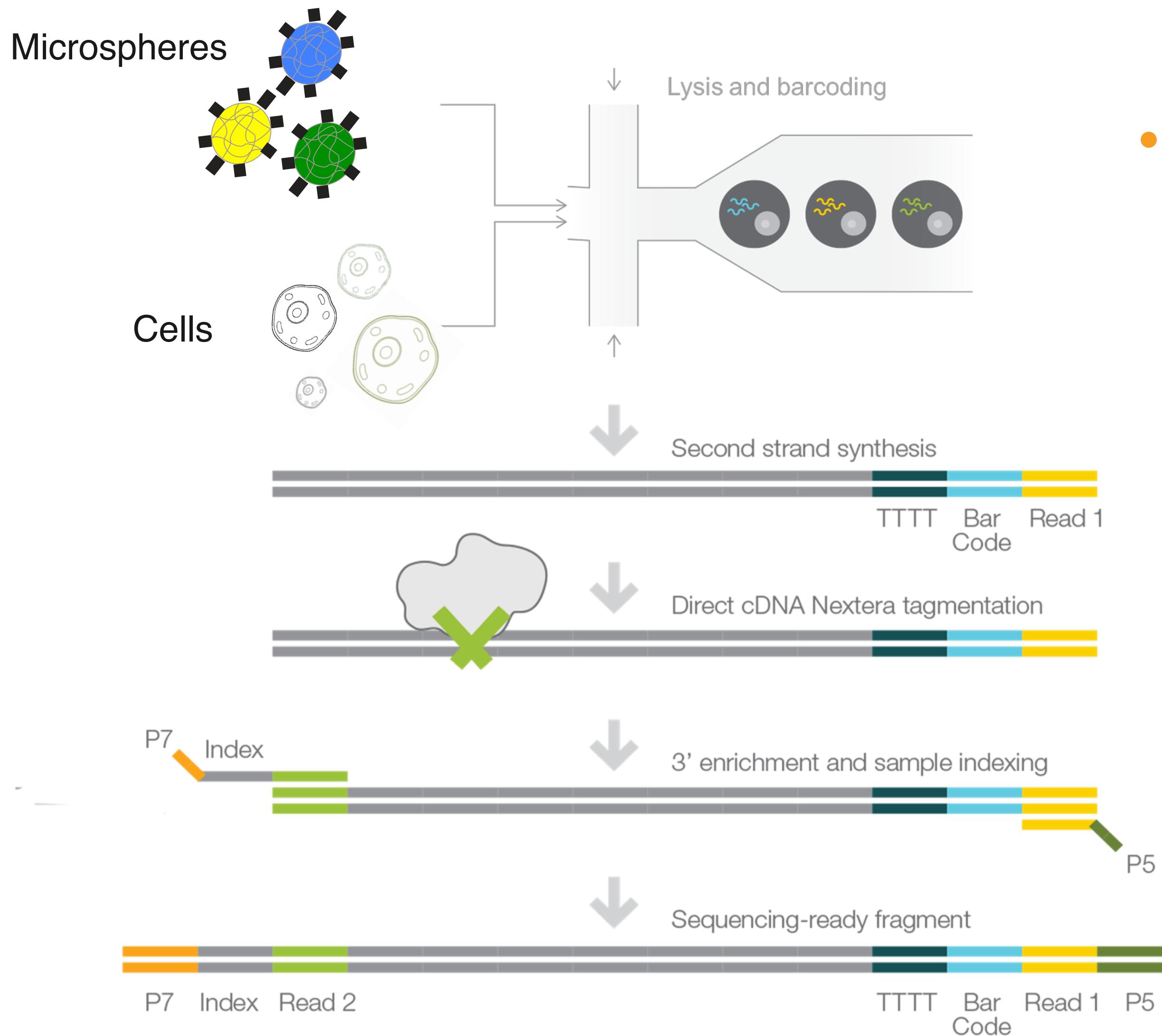
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96	96	384	1536
96	1	4	14
384	4	14	57
1536	14	57	226
384	96	384	1536
96	4	14	57
384	14	57	226
1536	57	226	906

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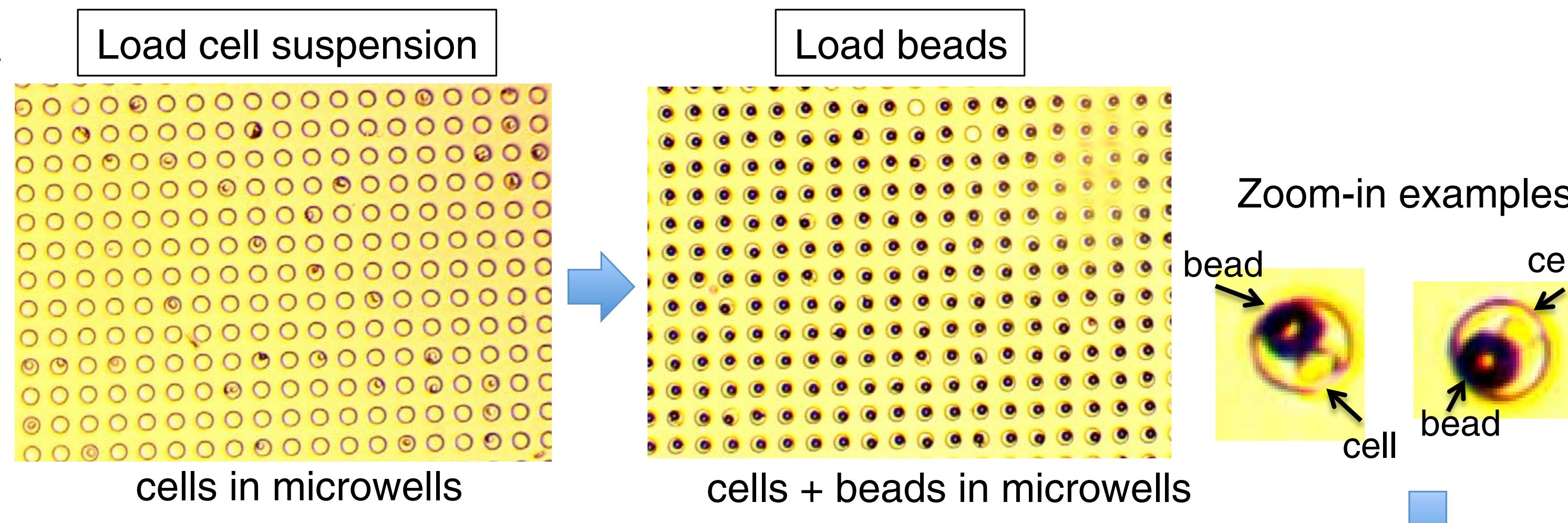
BioRad/Illumina ddSEQ



- 4 independent lanes on chip
~300-400 cells/lane
- Commercial DropSeq
- Double Poisson
- Basespace analysis + FlowJo app

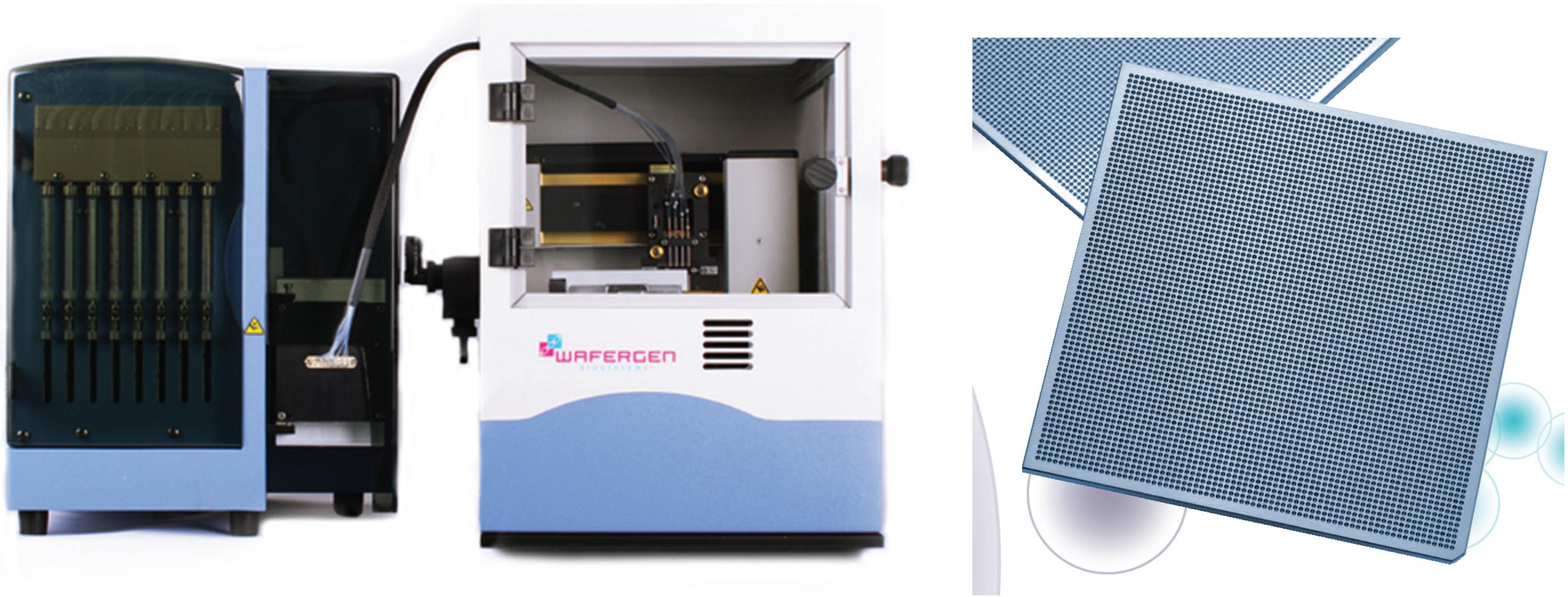
BD Resolve and Rhapsody

- Very similar to CelSee
- Precise - plate-based format with beads
 - Low Read 1 diversity after 16 bases (8 base cell/UMI)
- Rhapsody - Bead and cell settling in microwells.
 - Potentially thousands to tens of thousands of cells
 - A|A

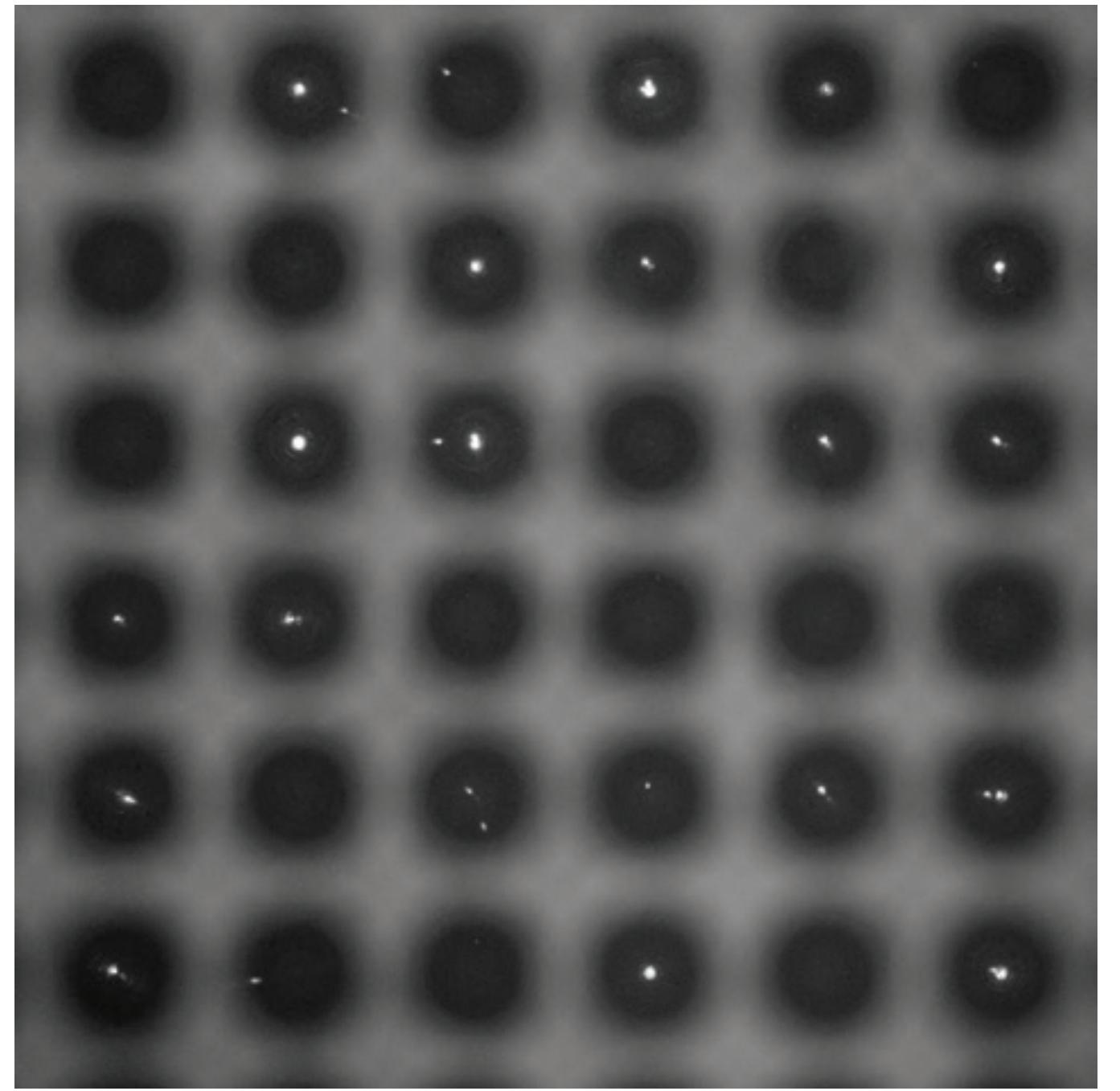


CelSee

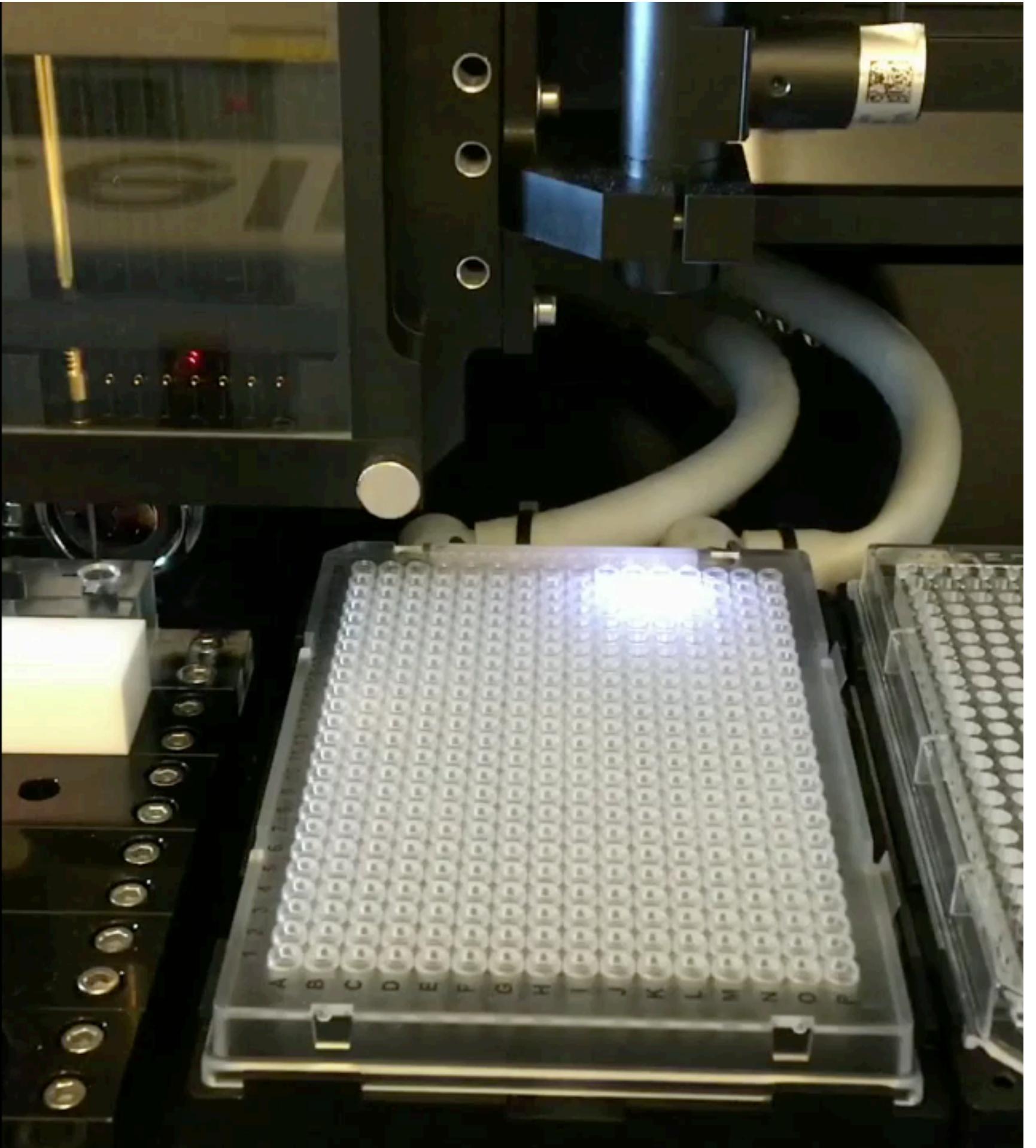
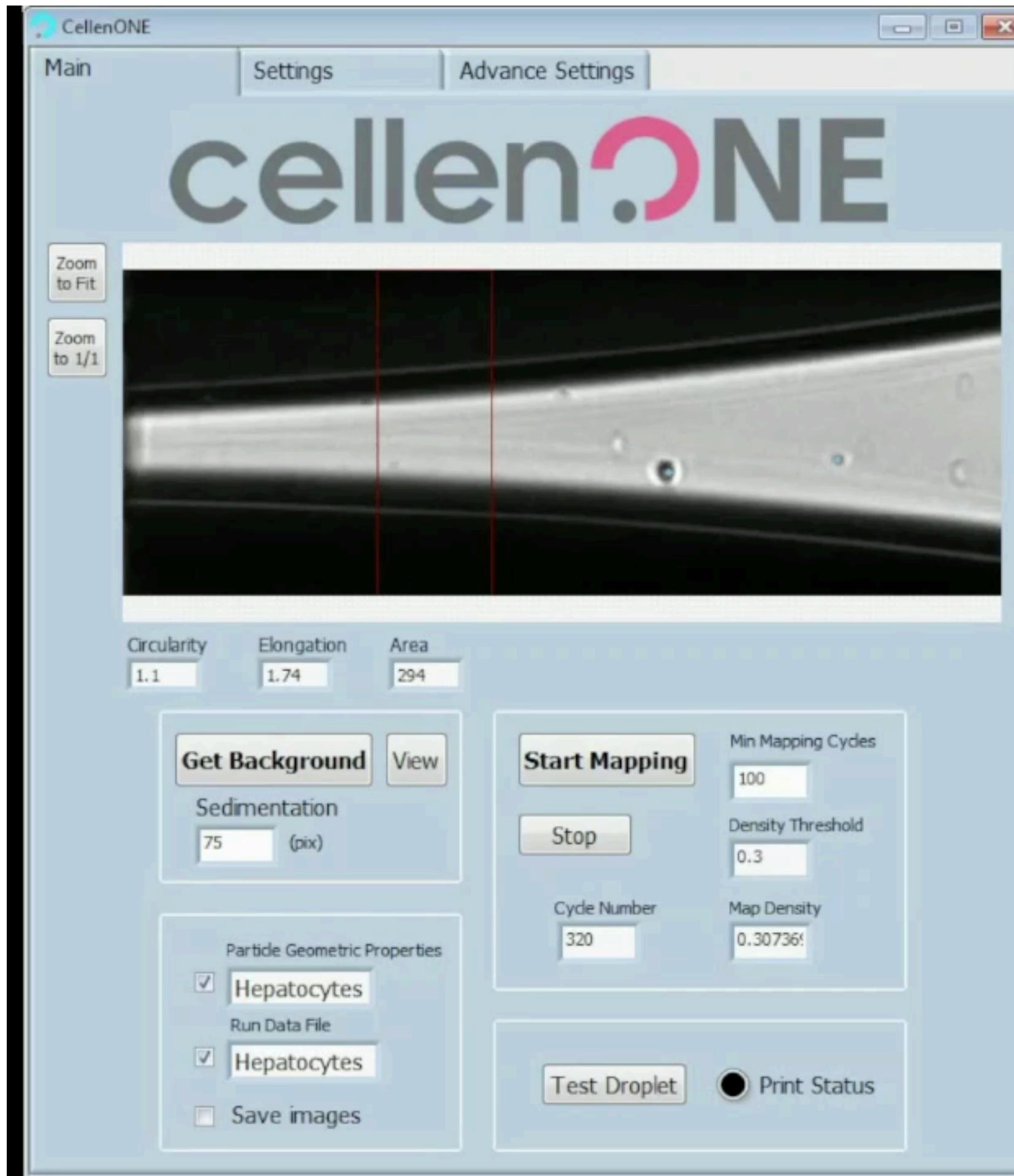
Wafergen/Takara iCell8



- Dispense into a 5184 well plate with barcoded oligos
- Add cells to wells and identify singlets by imaging
- ~1800 single cells/chip due to Poisson distribution
- 3' sequencing

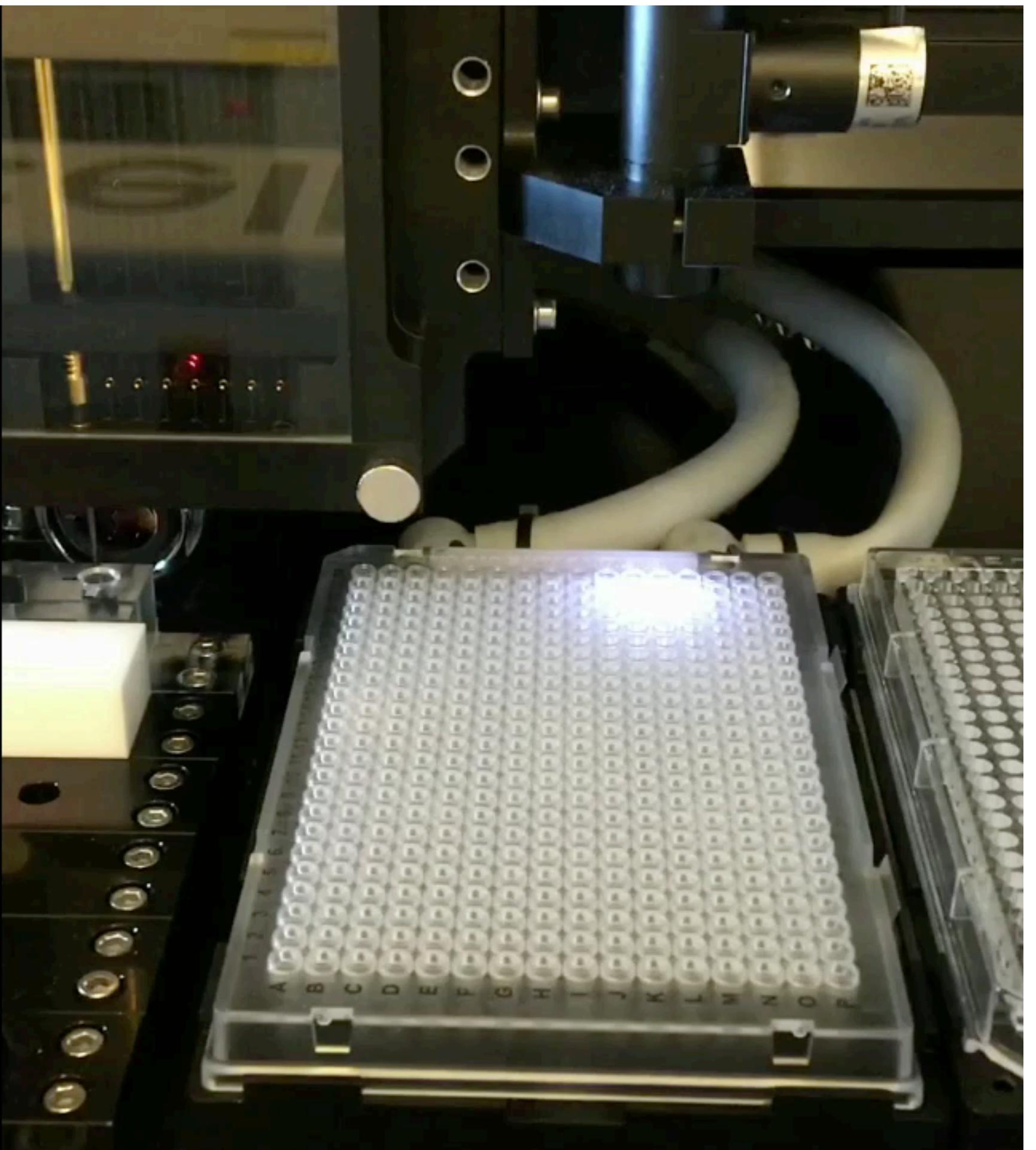
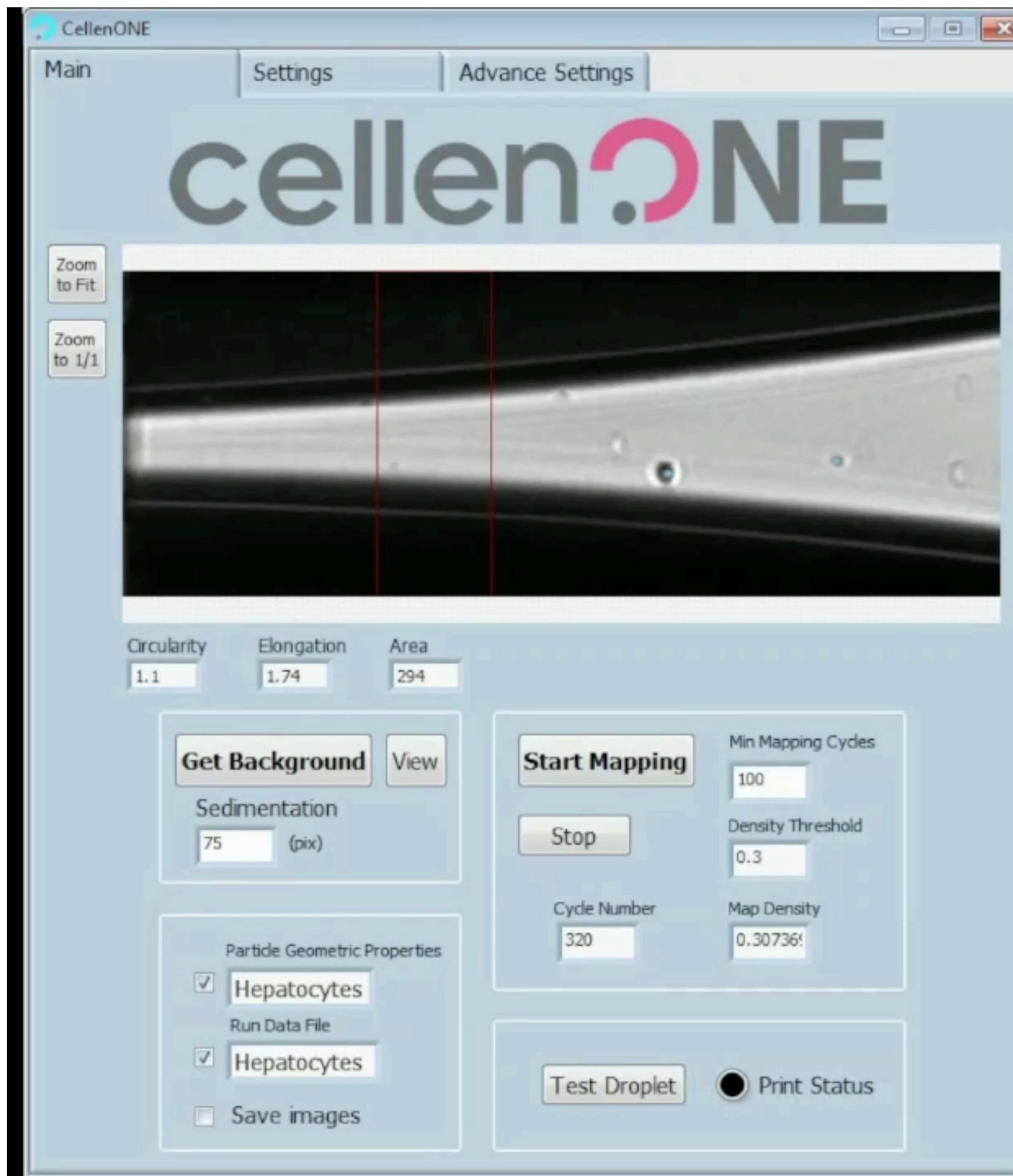


Scienion dispenser



- Beats Poisson
- High recovery for low-cell numbers (<97%)
- 96 cells <4 minutes
- Multiple destination types: 96/384/1536, slides...
- Low cell # samples, ie CSF, vitreous fluid,...

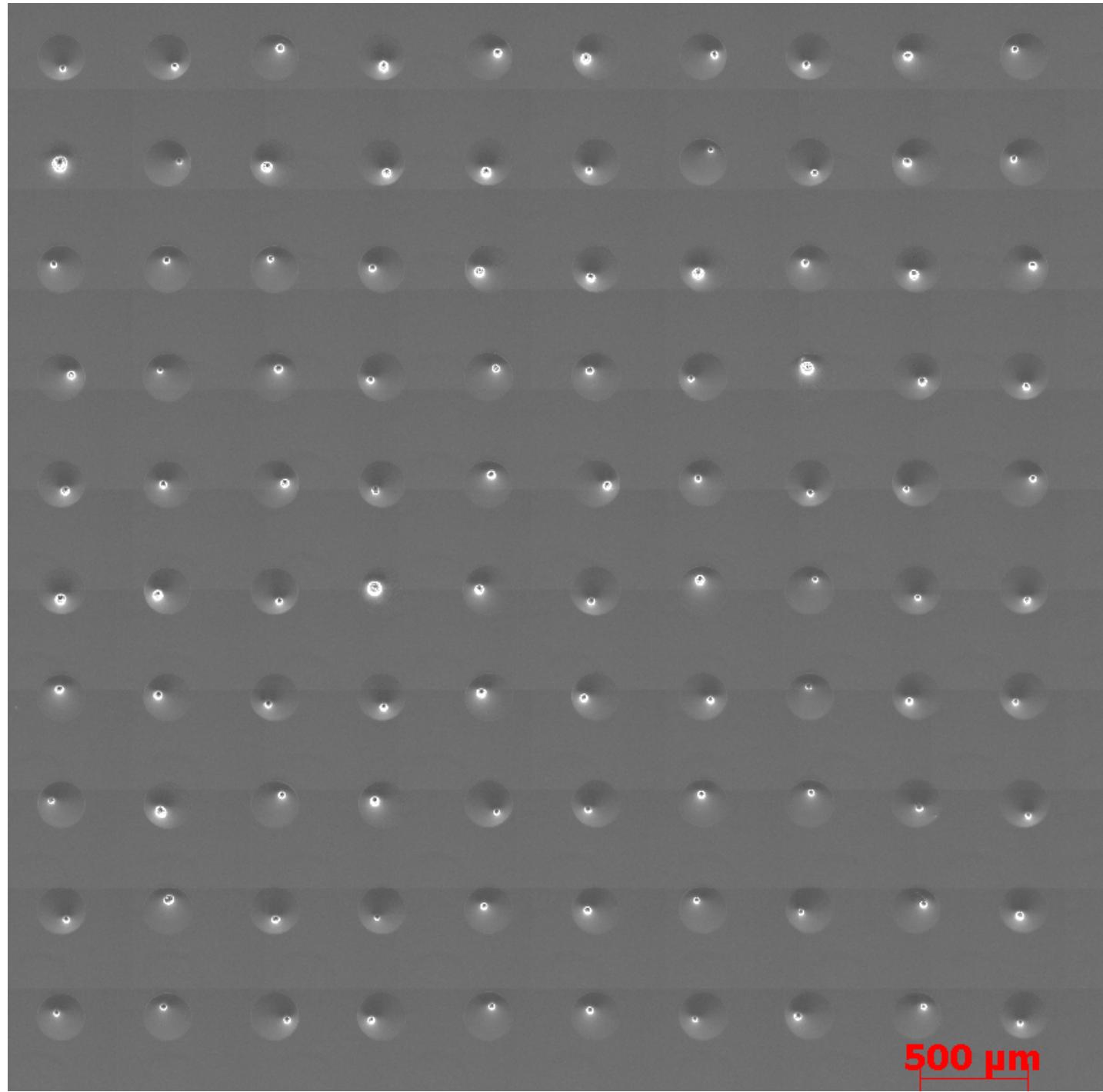
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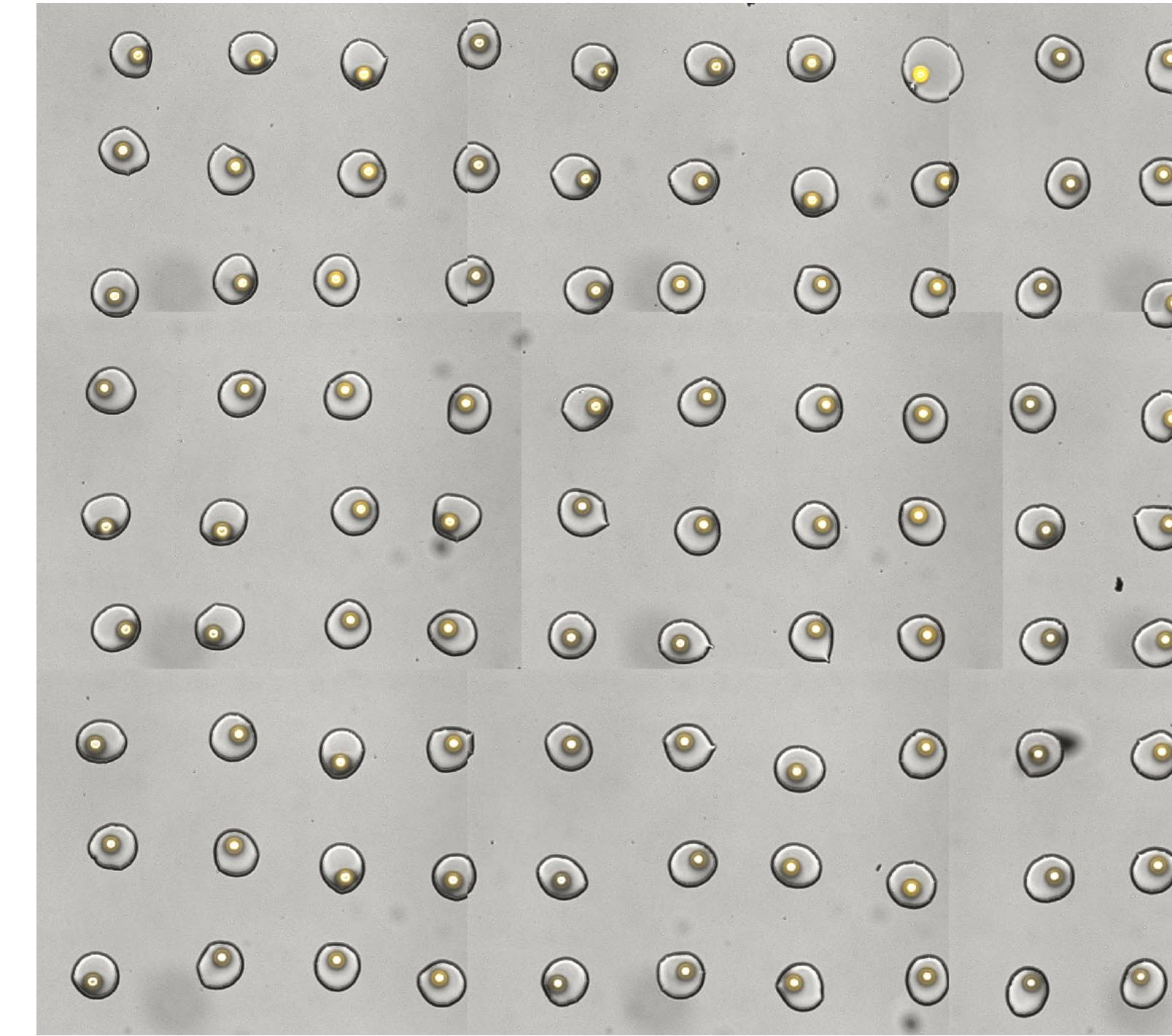
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Single-particle dispensing

Dissociated
Lung-cancer spheroid



21 um beads

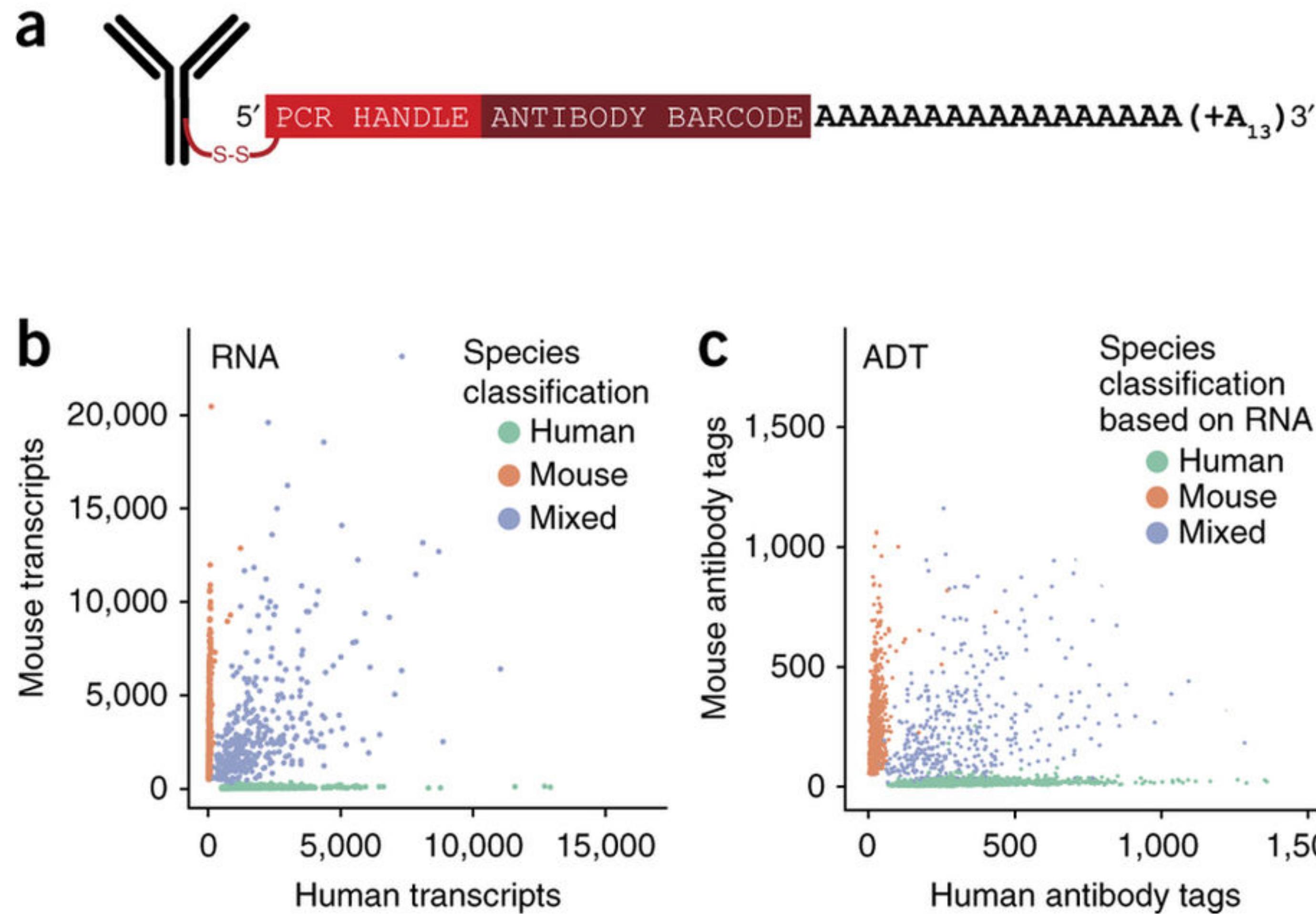


- Unselected and doublet cells can go into a tube and redisensed
- Fluorescence selection in future version

Single Cell Sequencing not restricted to RNA

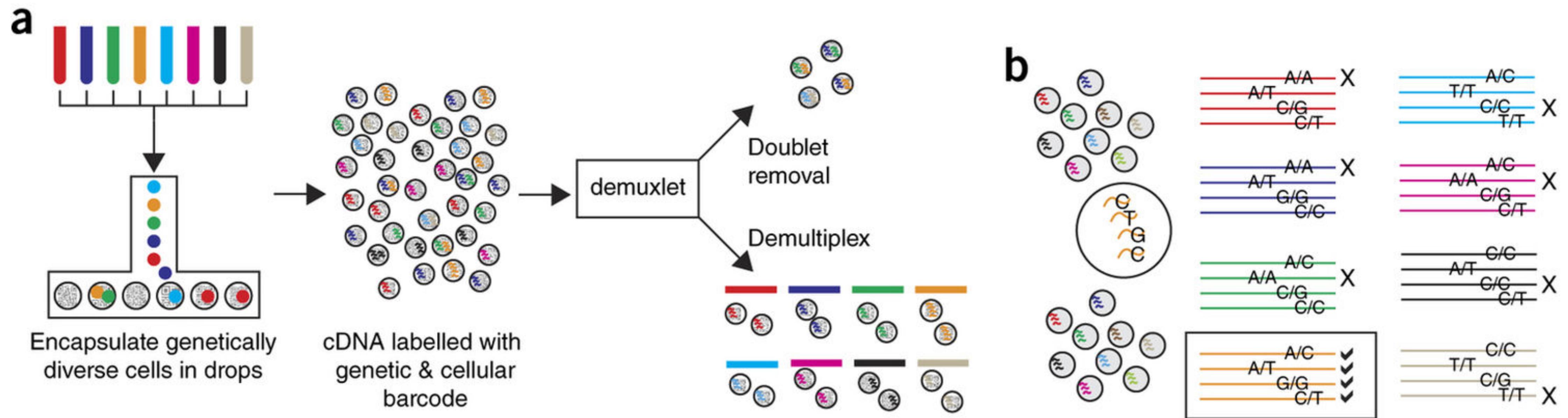
- Targeted single cell DNA sequencing
- Immune Repertoire and antigen sequencing
- Protein
- ATAC-seq
- Spatially resolved sequencing

Quantifying proteins - CITE-seq

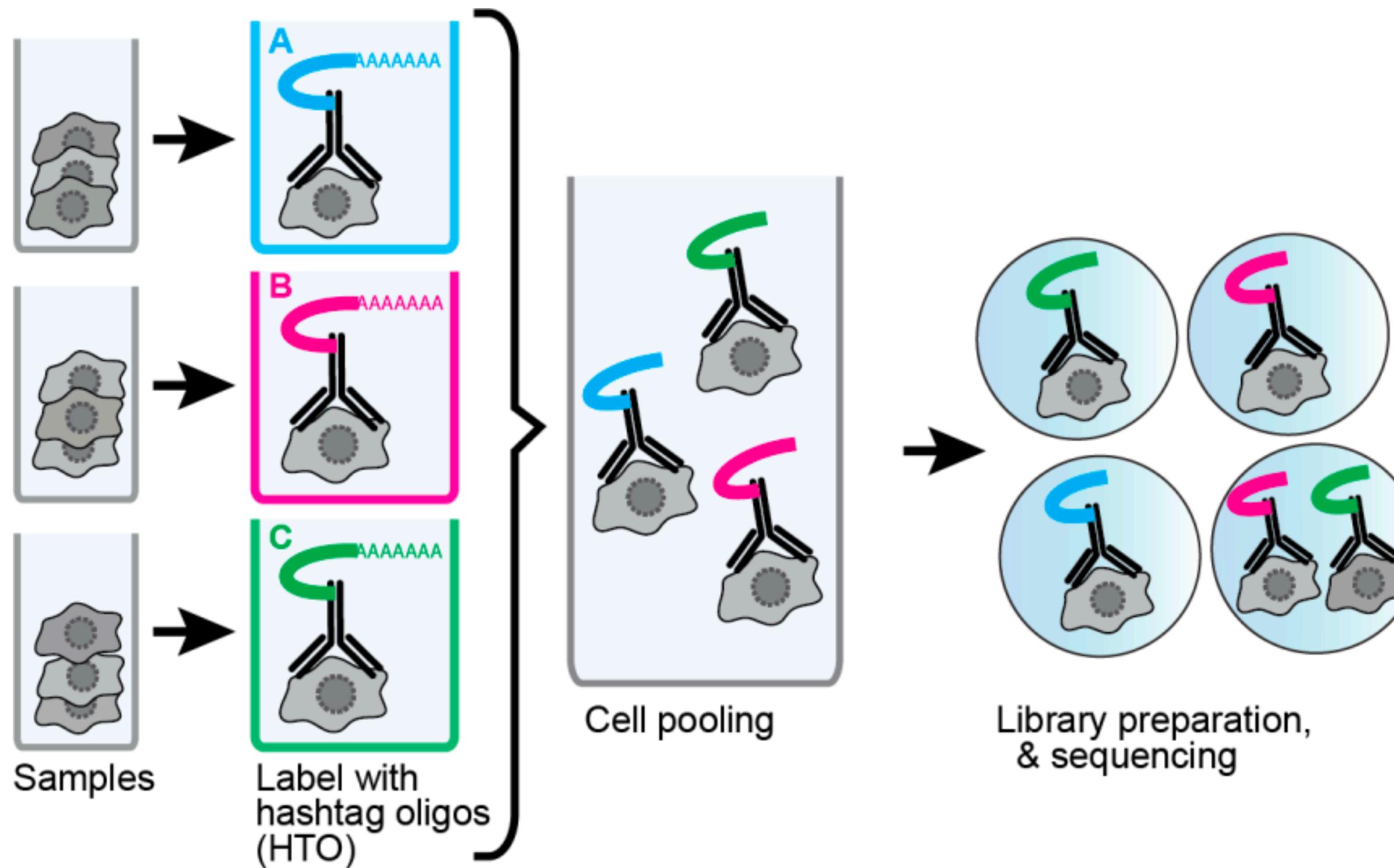


- Cellular Indexing of Transcriptomes and Epitopes.
- Compatible with most sc-RNA-seq systems
- BD and BioLegend have dozens of barcoded antibodies
- Restricted to cell surface

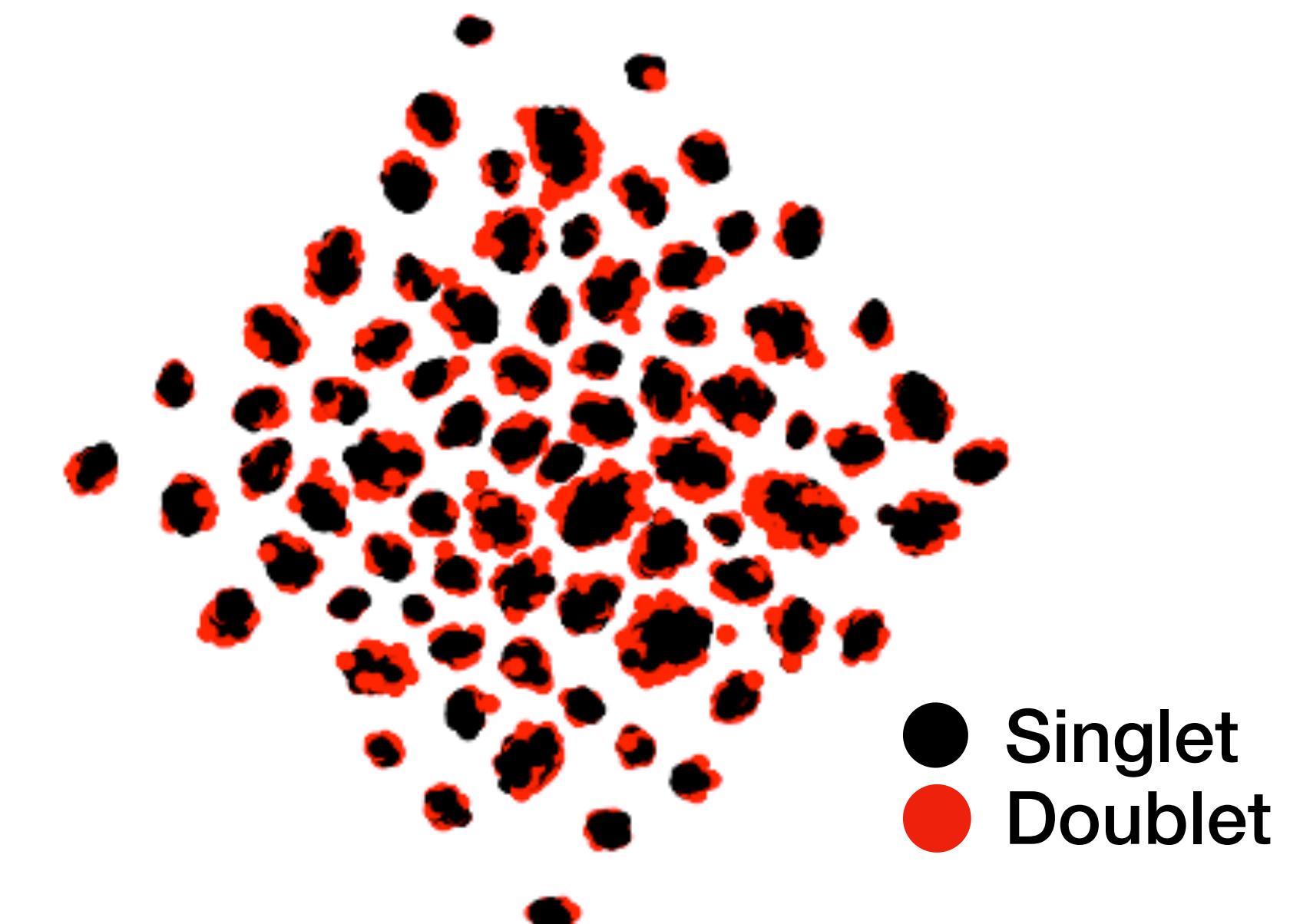
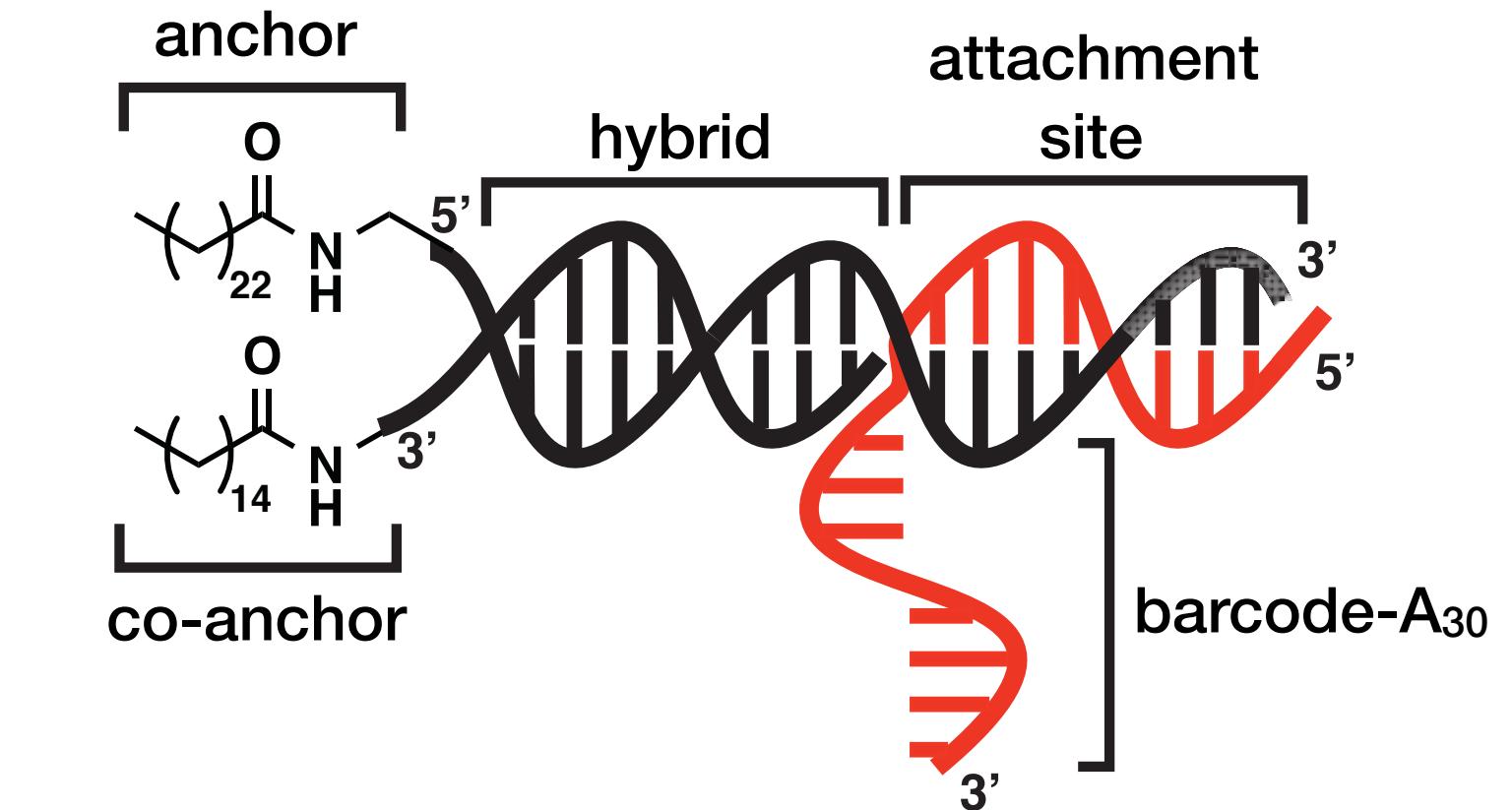
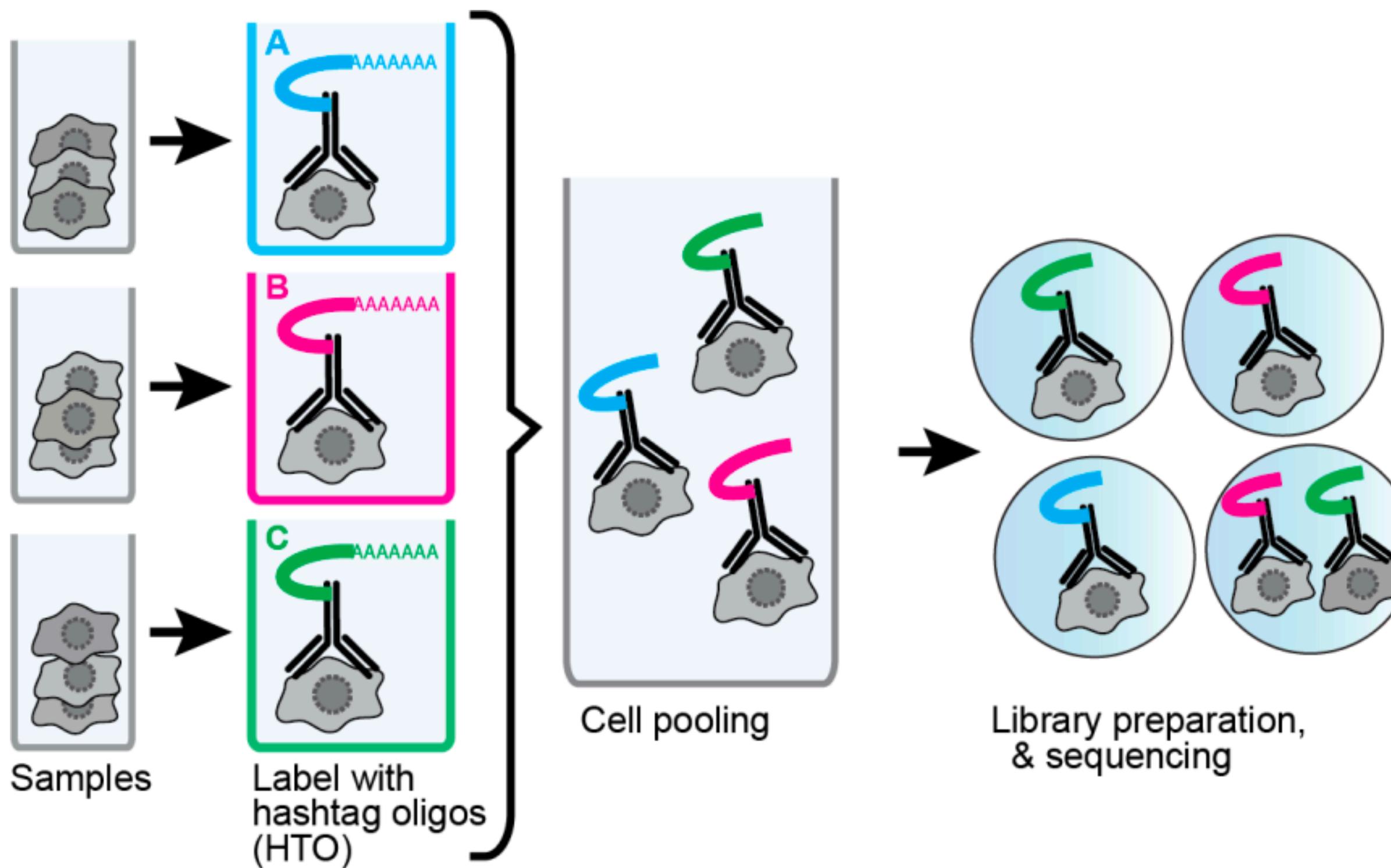
demuxlet - Multiplexing by genotype



Non-genetic multiplexing strategies



Non-genetic multiplexing strategies

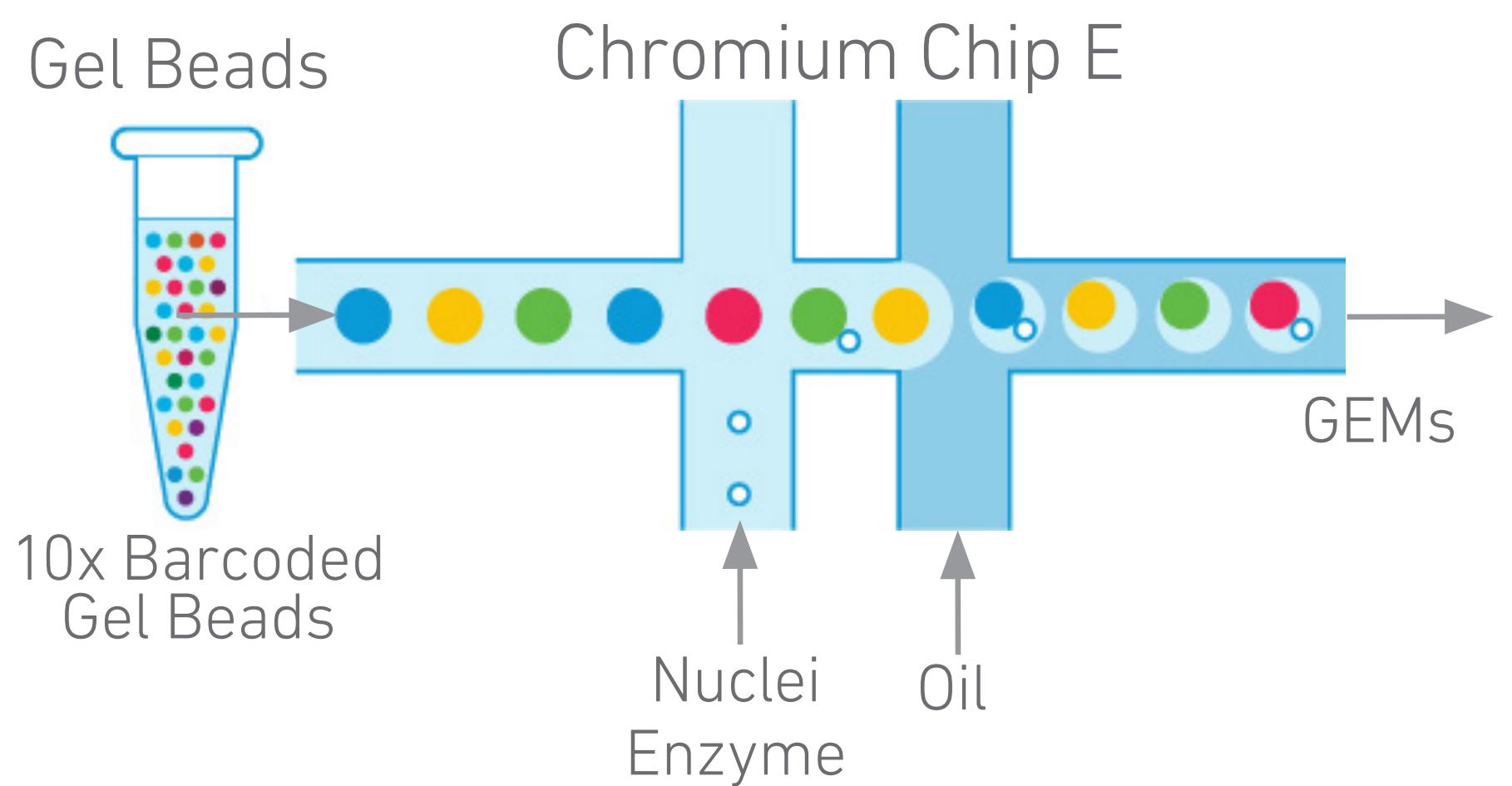
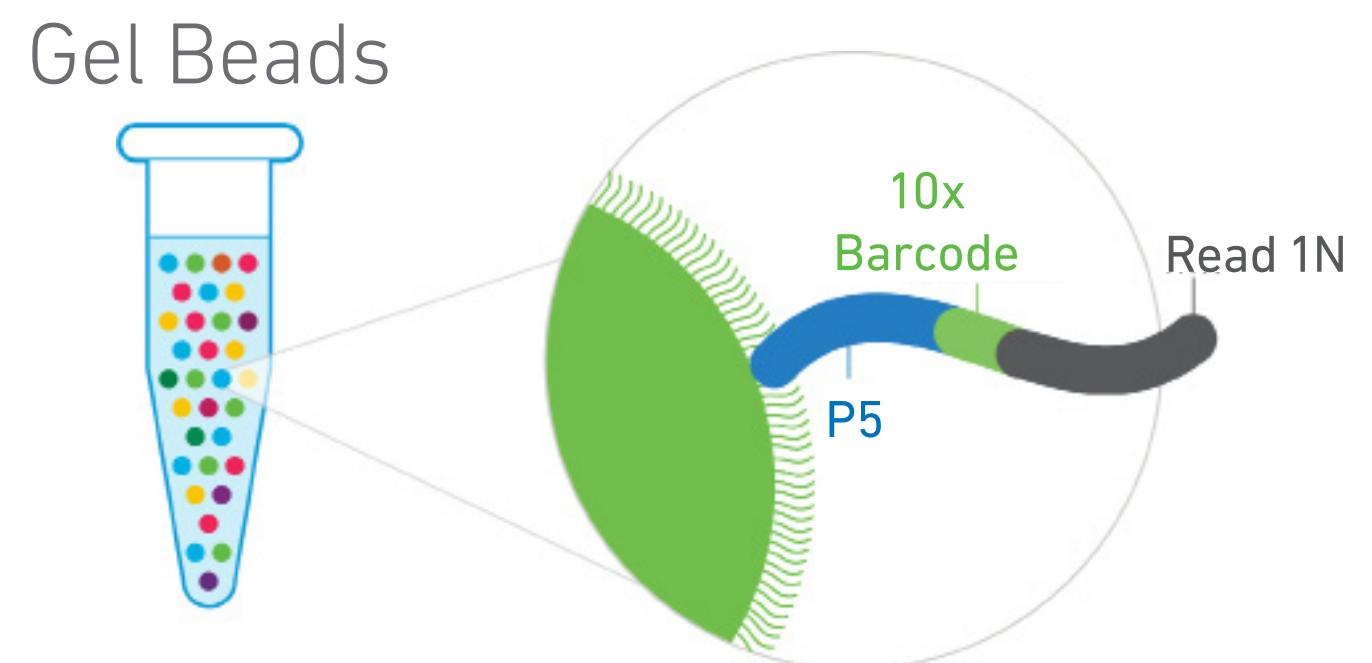


scATAC

Prepare and tagment nuclei in bulk

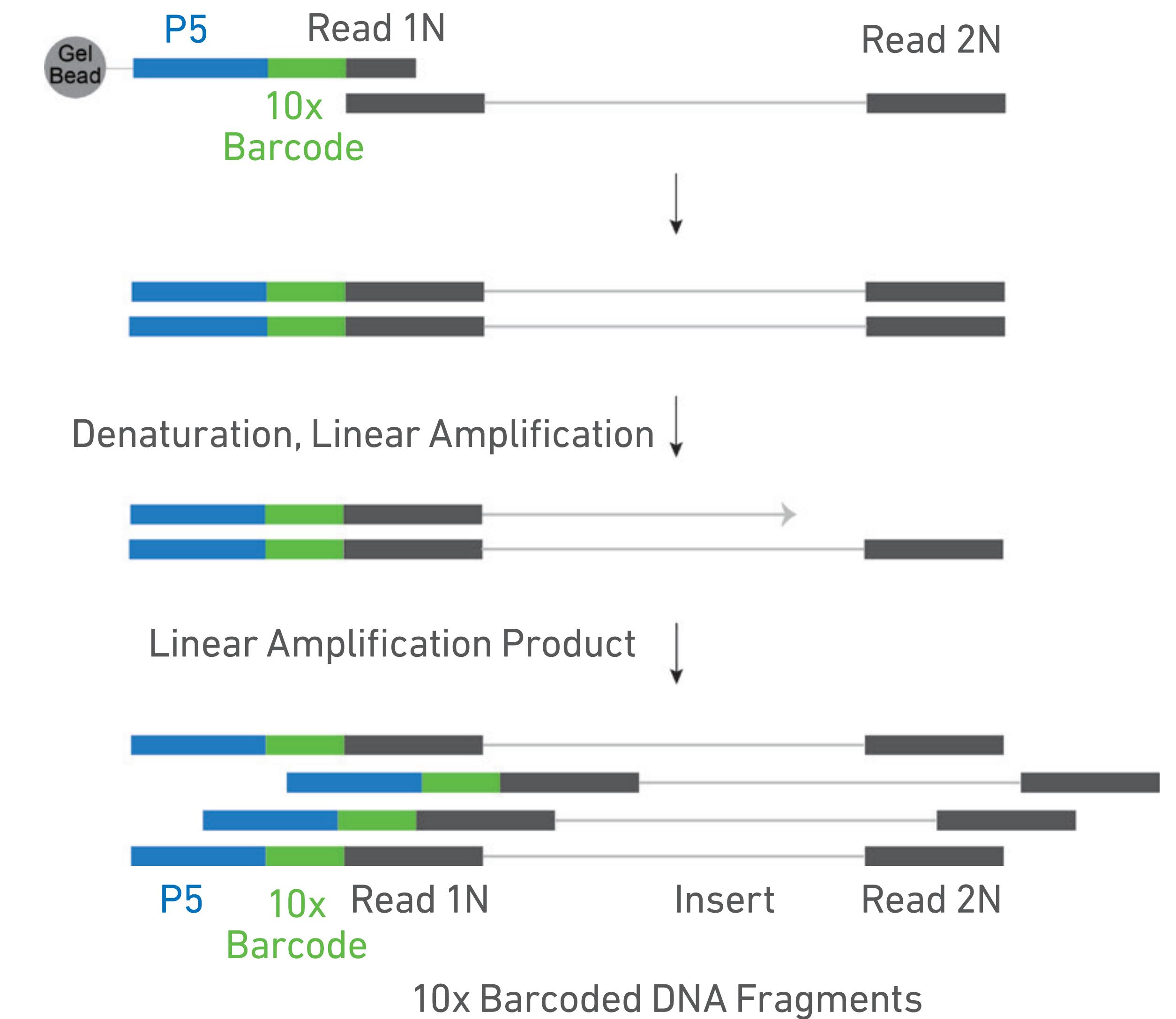
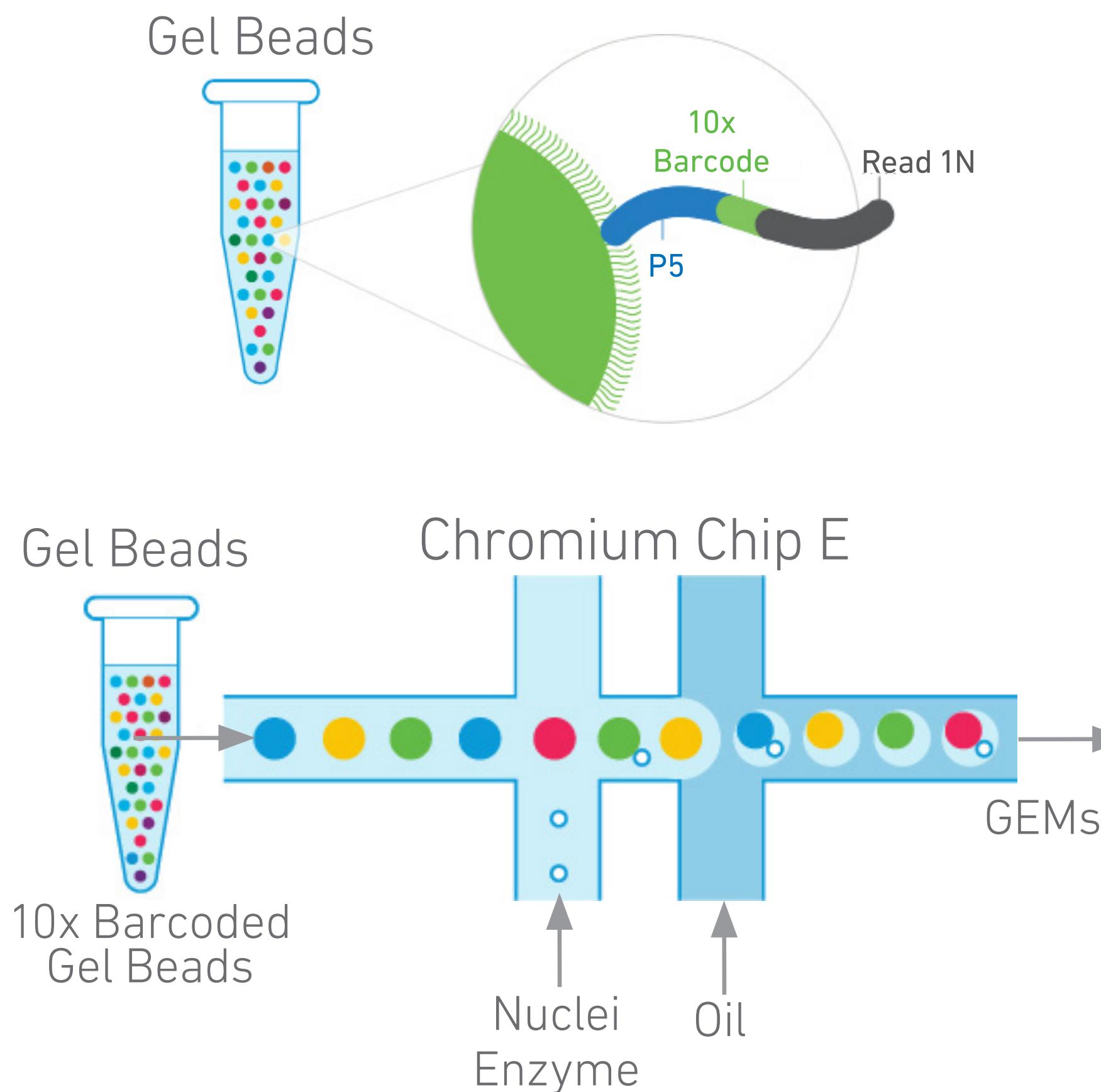
scATAC

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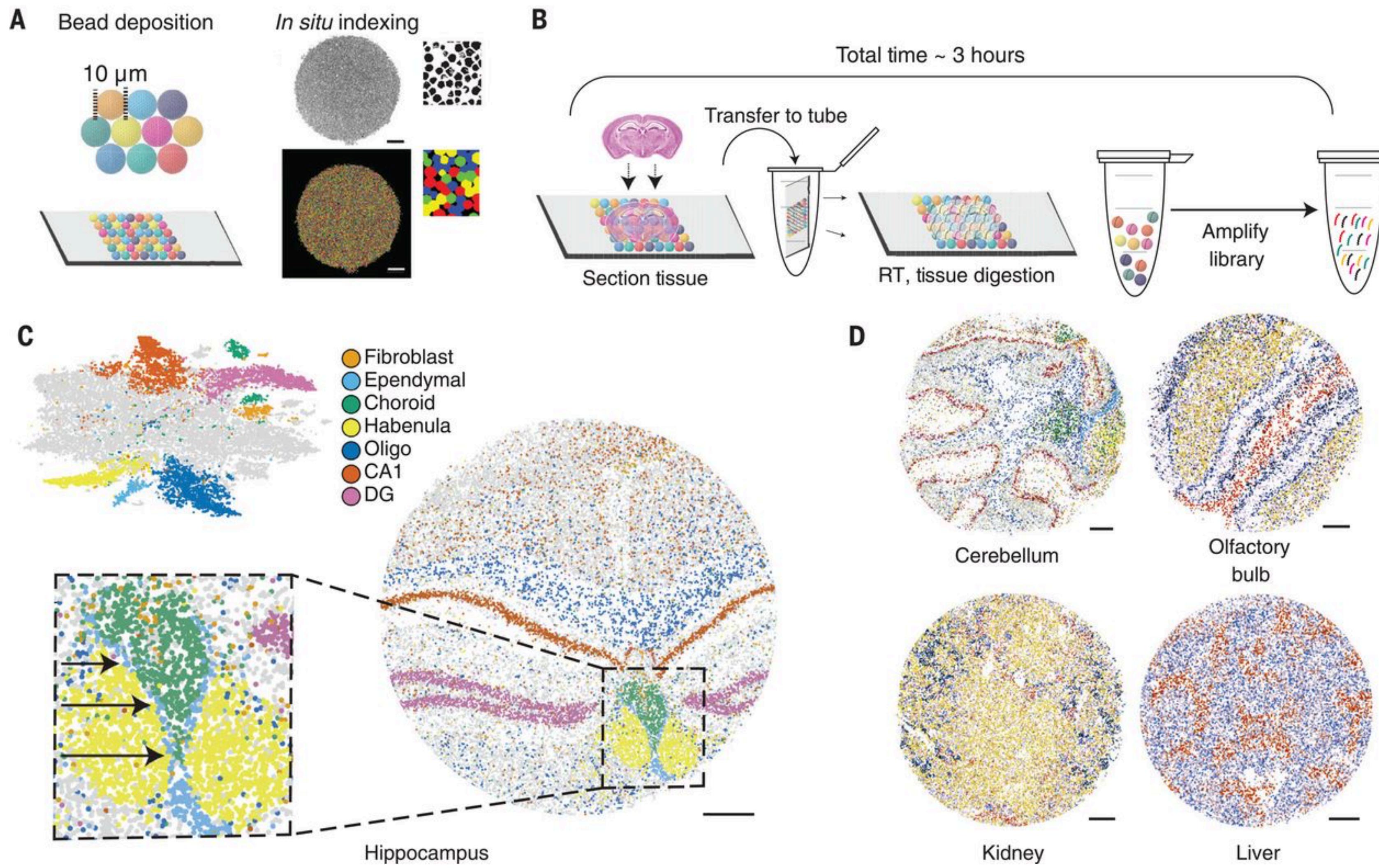


scATAC

Prepare and tagment nuclei in bulk



SLIDE-seq spatially resolved transcriptomes



Conclusion

- Single cell sequencing is a powerful and rapidly changing field
 - Driven by several factors: lower sequencing costs, increased demand, **preprints**
- Don't disregard non-sequencing methods - CyTOF, MIBI, SeqFISH, STAR-Map
- See a shift from descriptive studies to experimental studies
 - Perturb and CROP-Seq
 - Drug screens
- Future will tie in multiomic data (protein, chromatin accessibility, space, time)
- Questions?