Single-cell Sequencing Platforms

UC Davis Single Cell Analysis Workshop
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December 18, 2017

Methods covered today

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

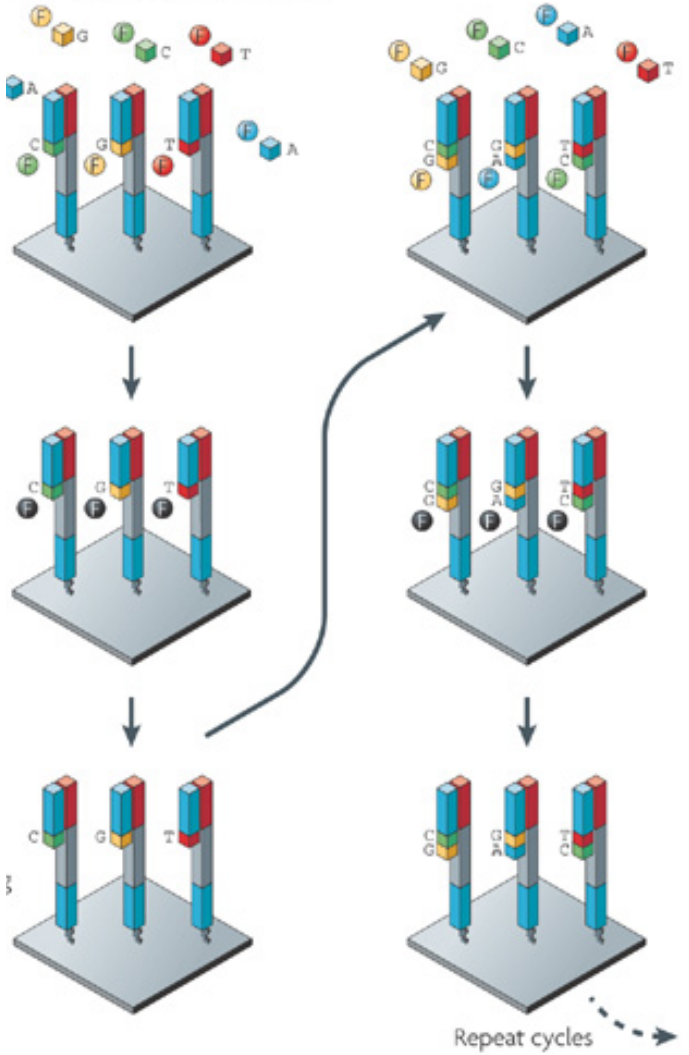
Methods not covered

- CEL-seq
- SPLIT-seq
- inDrops
- STRT-seq
- Many others
- Useful resource: https://teichlab.github.io/scg_lib_structs/

Illumina Sequencing

Top: CATCGT
Bottom: CCCCCC

- Not single molecule! ~1000 copies per spot
- Reads limited to 100-300 bases
- Get tons of reads -> good for counting



Illumina sequencing

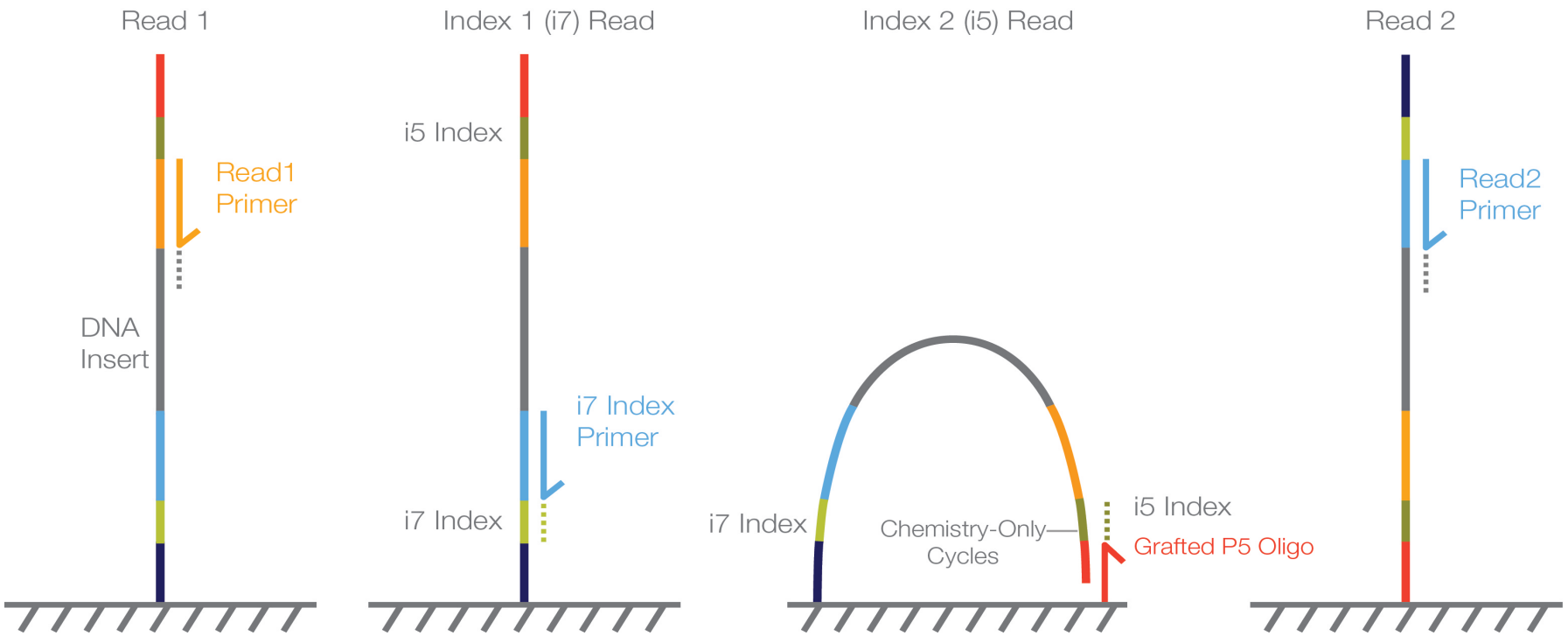
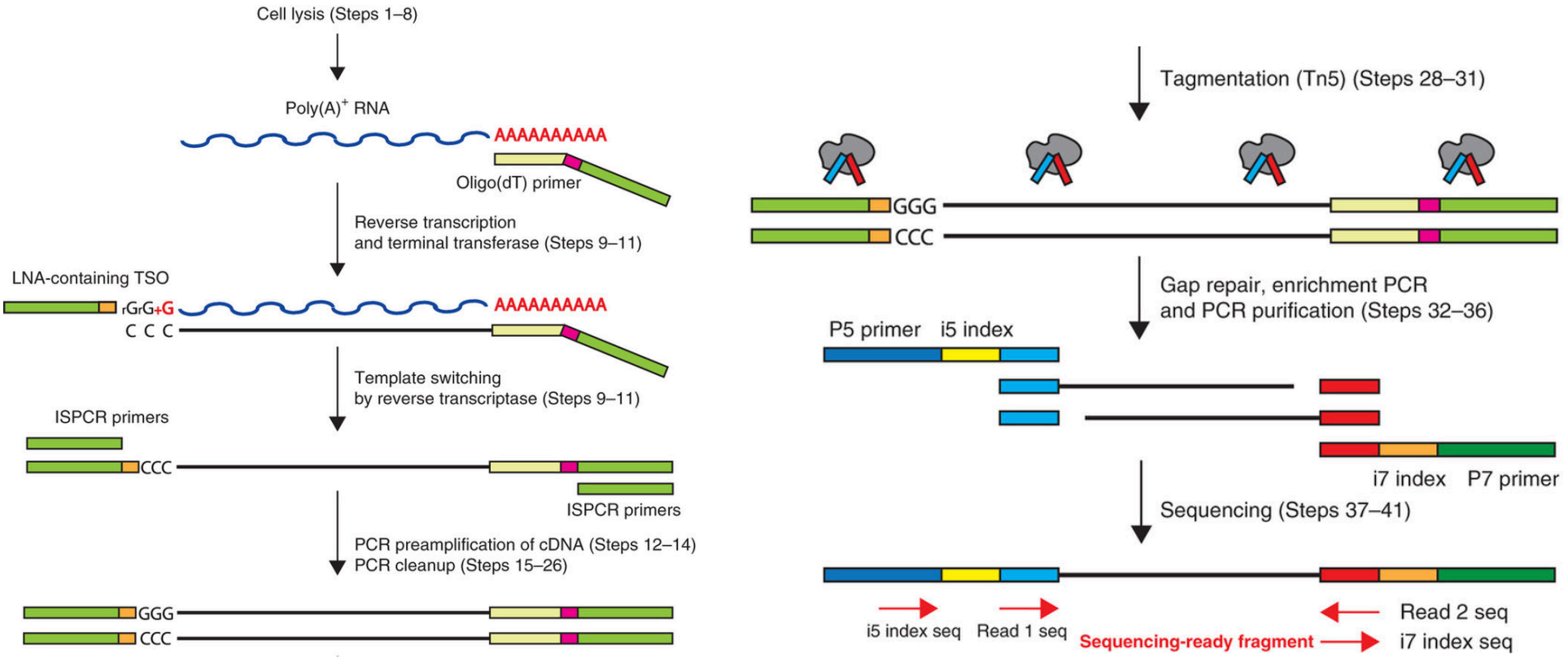
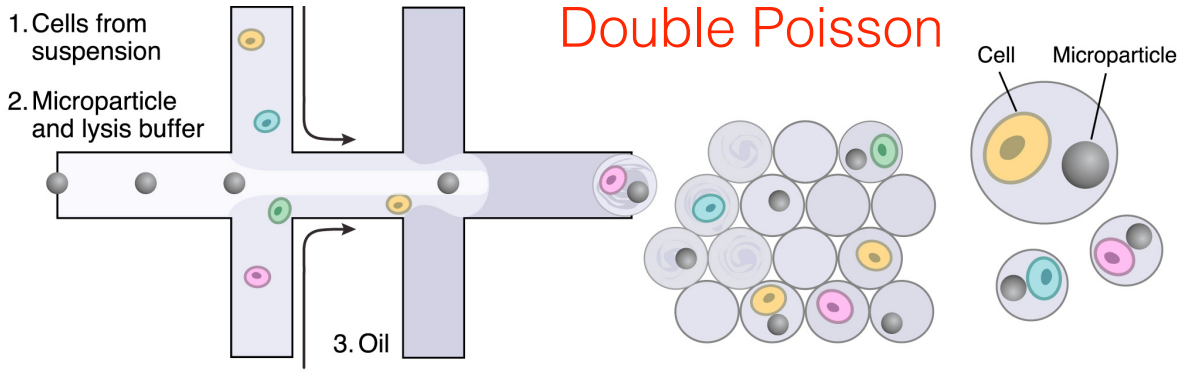


Plate-based SMART-seq

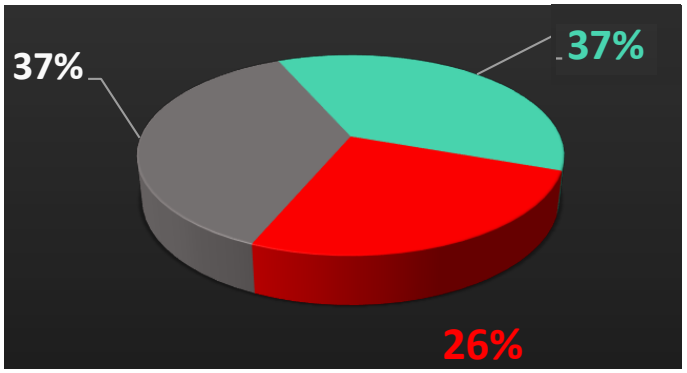


DropSeq

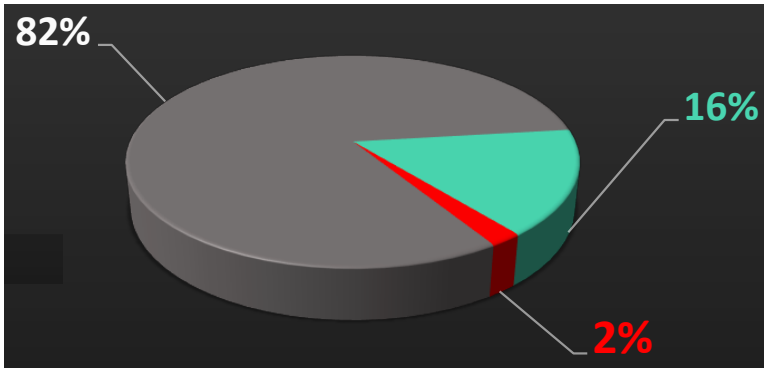


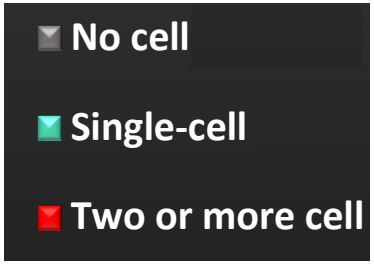
Read 1: only 20 bases of diversity.

Poisson distribution



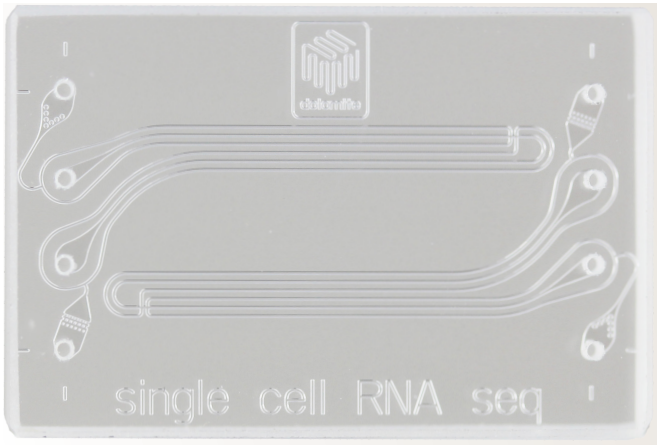
- 1/10 droplets contain a bead
- 1/20 droplets contain a cell (~5% doublet rate)



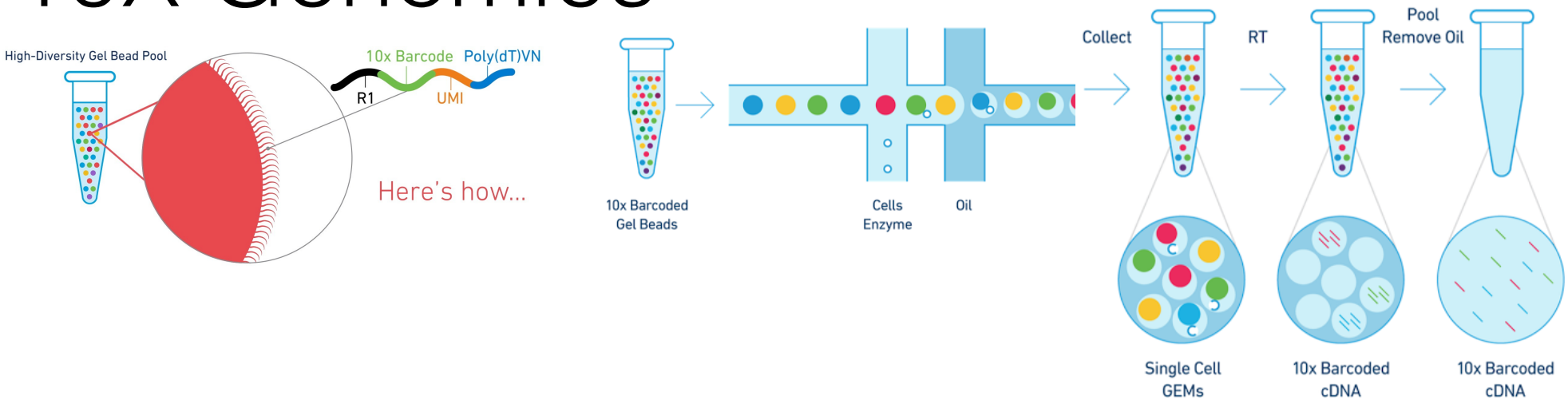


PDMS alternative

- Dolomite Bio sells a system that uses glass chips and pressure pumps
- Easier than PDMS but system costs more
- Chips can be connected to syringe pumps
- Sample loop for beads, no need for stirrer

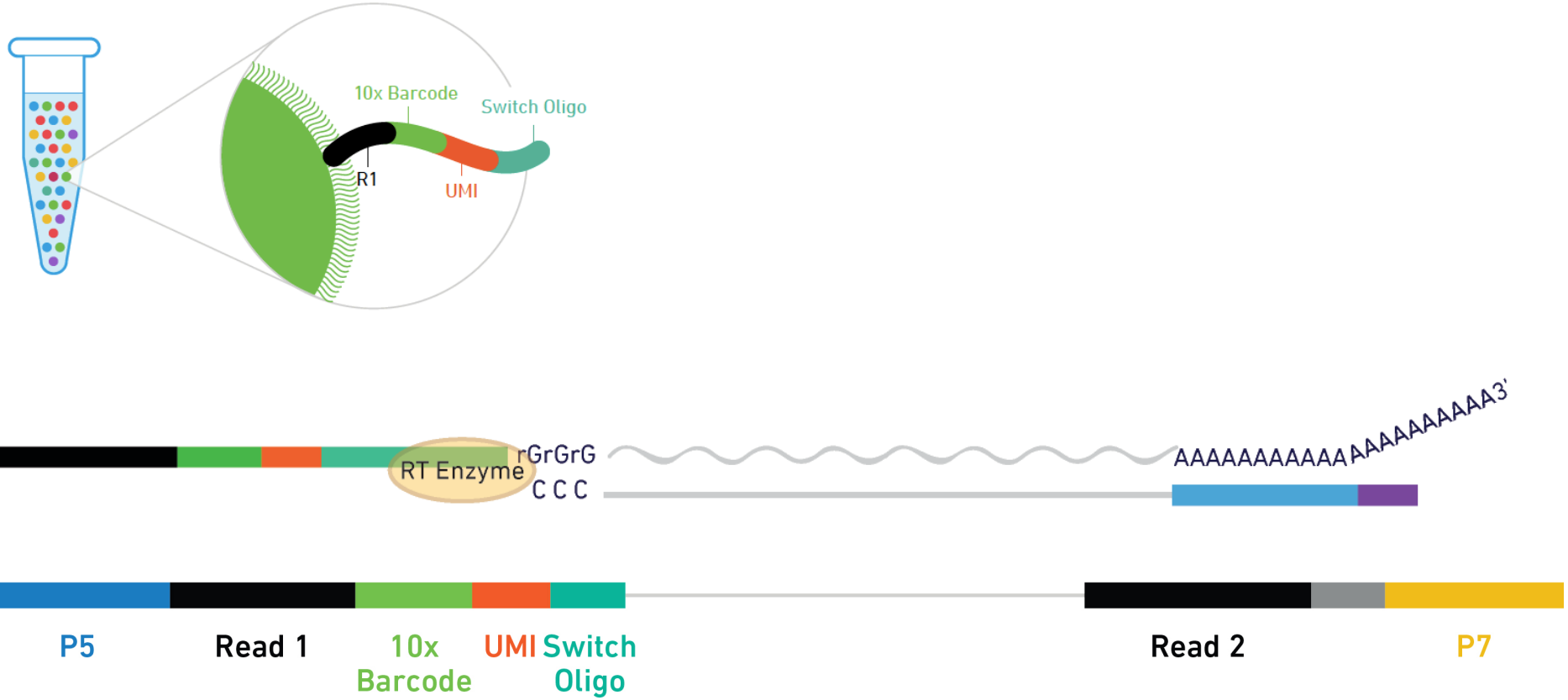


10X Genomics



- ~10-12K cells/lane
- Higher Cost ($20K for 16 preps)

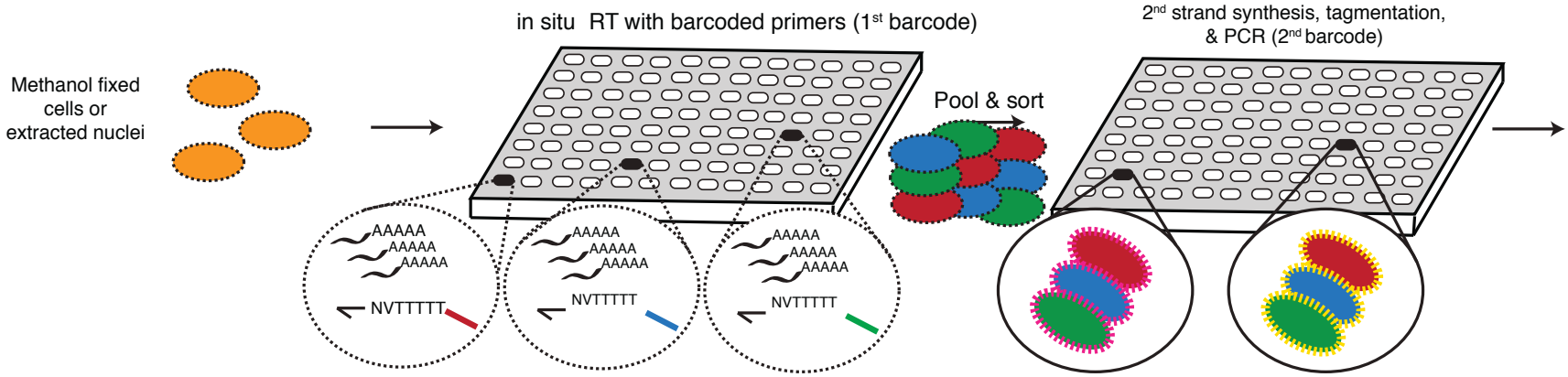
10X 5’ and TCR/BCR kits



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


Methanol fixed
cells or
extracted nuclei
in situ RT with barcoded primers (1st barcode)
Pool & sort
2nd strand synthesis, tagmentation,
& PCR (2nd barcode)
AAAAA
AAAAA
AAAAA
NVTTTTT

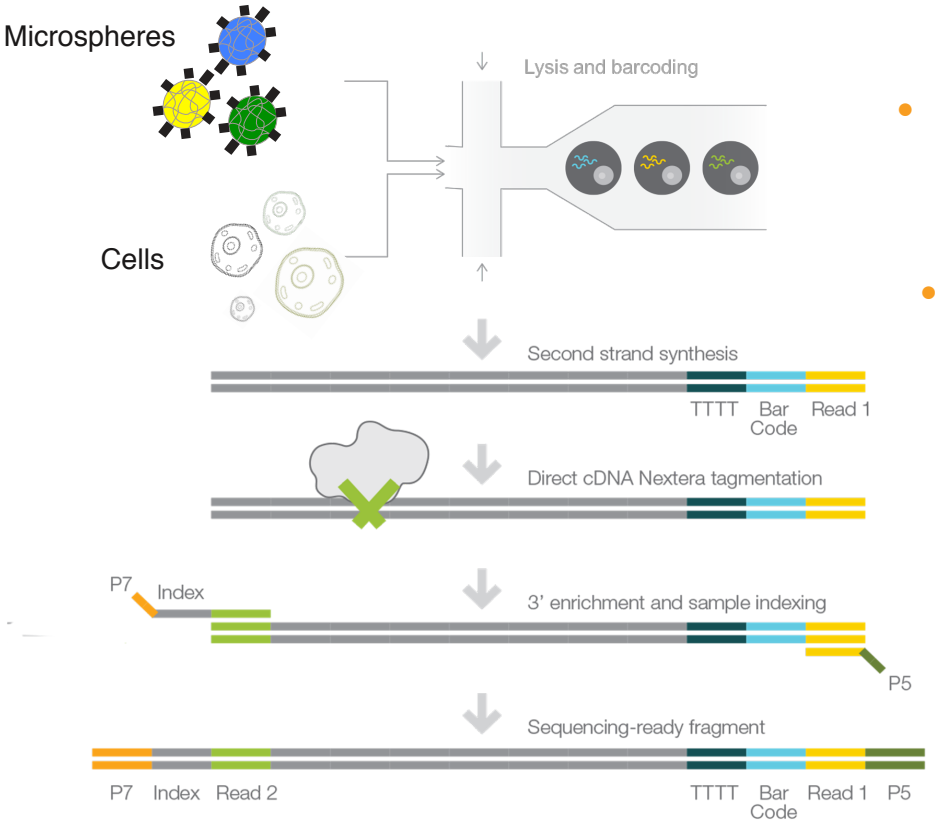
SCI-seq scales non-linearly

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 barcodes further increases combinations. Barcoded Nextera or SSS step.

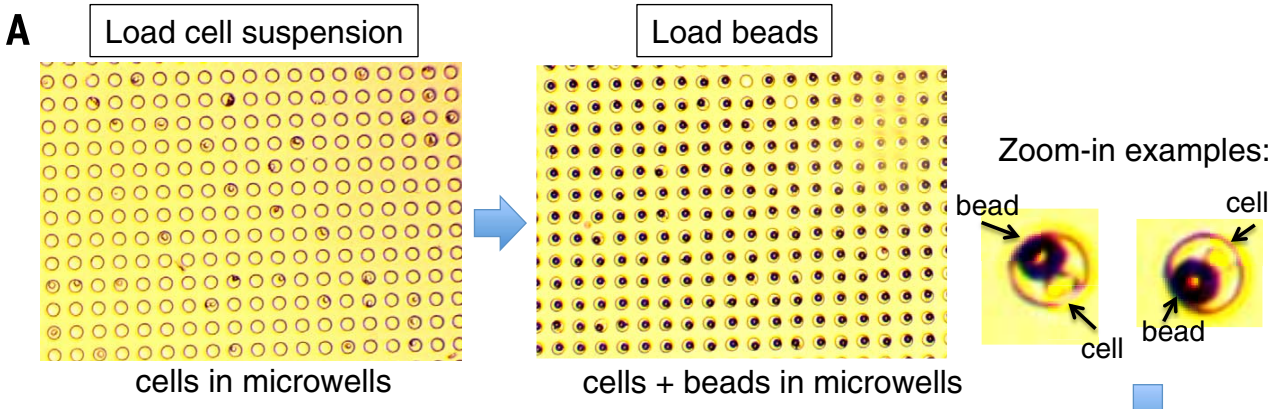
BioRad/Illumina ddSEQ



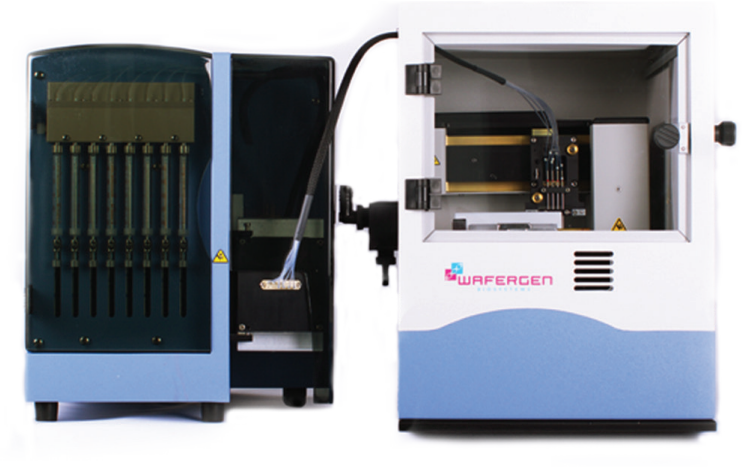
- 4 independent lanes on chip ~300-400 cells/lane
- Possible better capture
- 68 base Read 1 for cell barcode and UMI.
- 75 base Read 2
- Double Poisson
- 5.8% doublet with 1300 cell prep
- Basespace analysis + FlowJo app

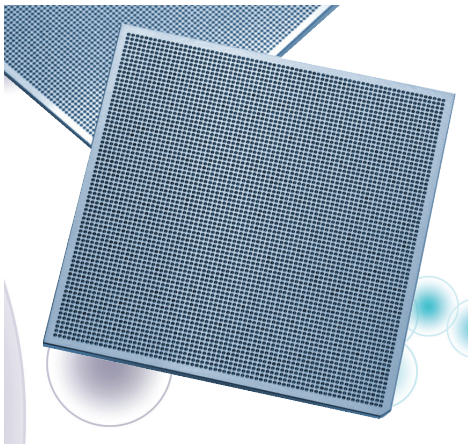
BD Precise and Resolve

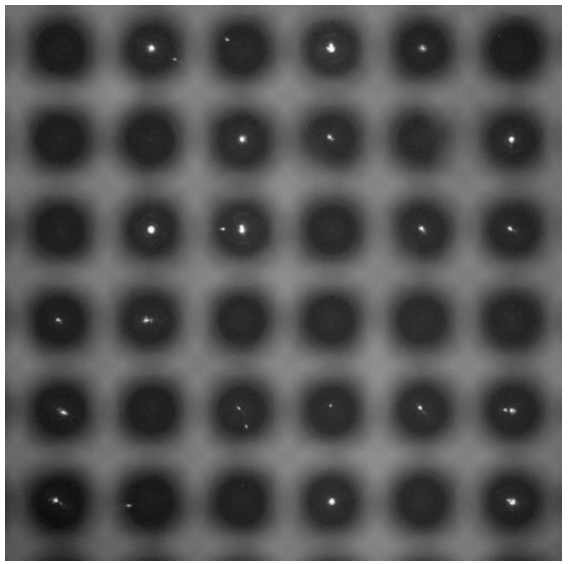
- Precise - plate-based format with beads
  - Low Read 1 diversity after 16 bases (8 base cell/UMI)
- Resolve - Bead and cell settling in microwells.
  - Potentially thousands to tens of thousands of cells
  - Also has read 1 diversity issues.



Wafergen/Takara ICell8

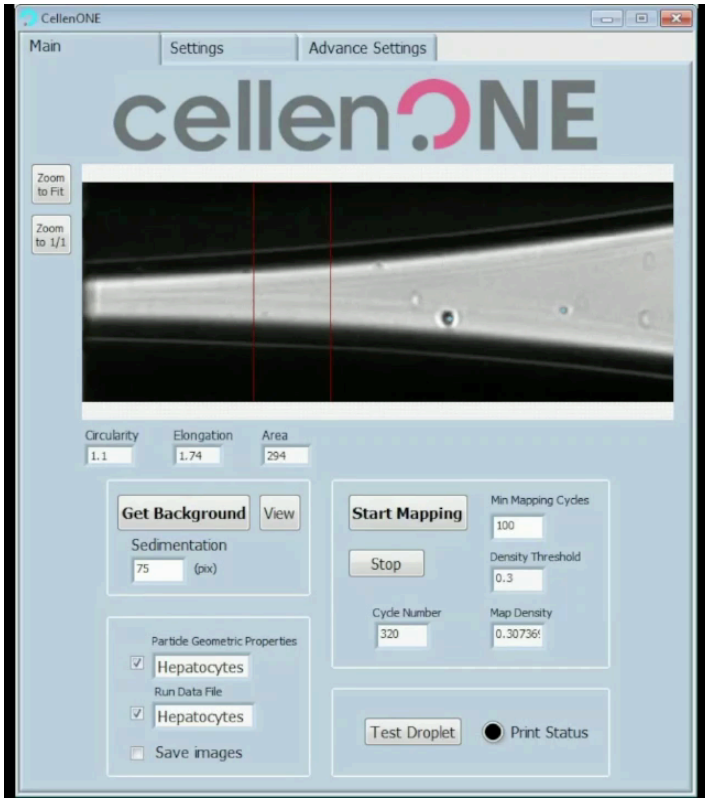


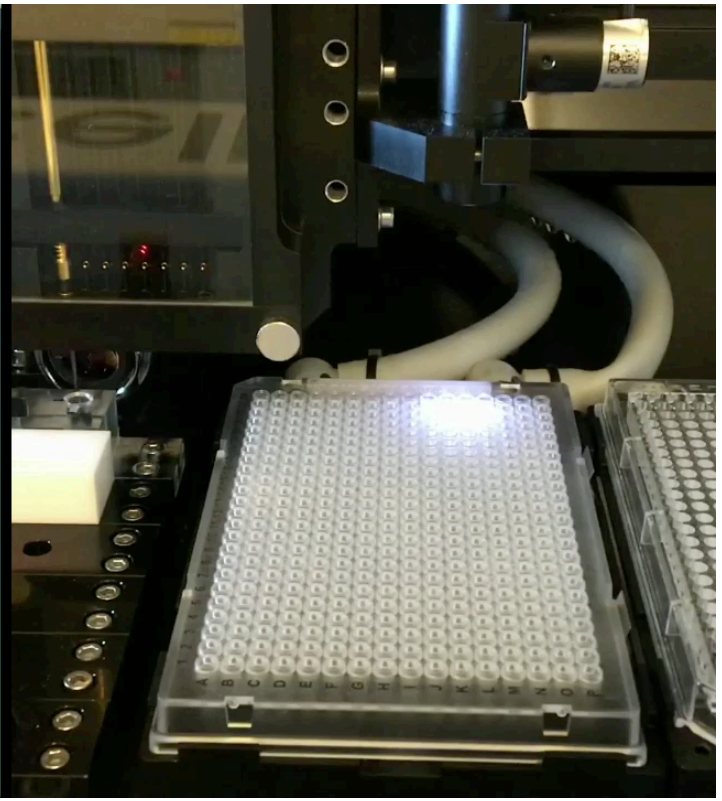




- Dispense into a 5184 well plate with barcoded oligos
- Add cells to wells and identify singlets by imaging
- ~1800 single cells/chip due to Poisson distribution
- 3’ sequencing

Scienion dispenser

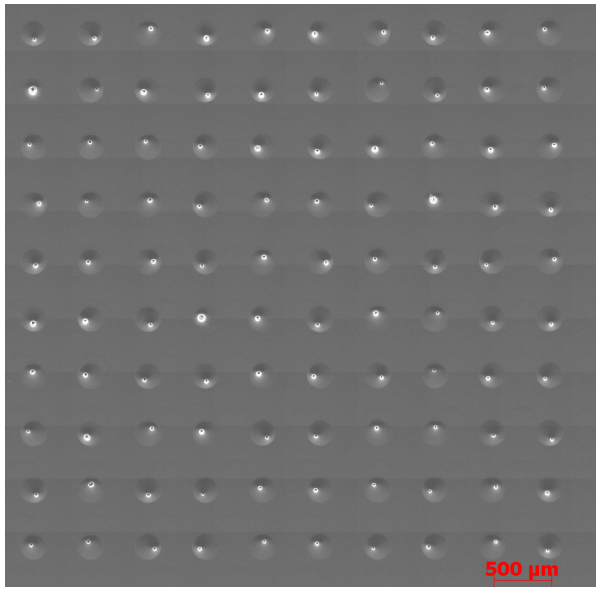




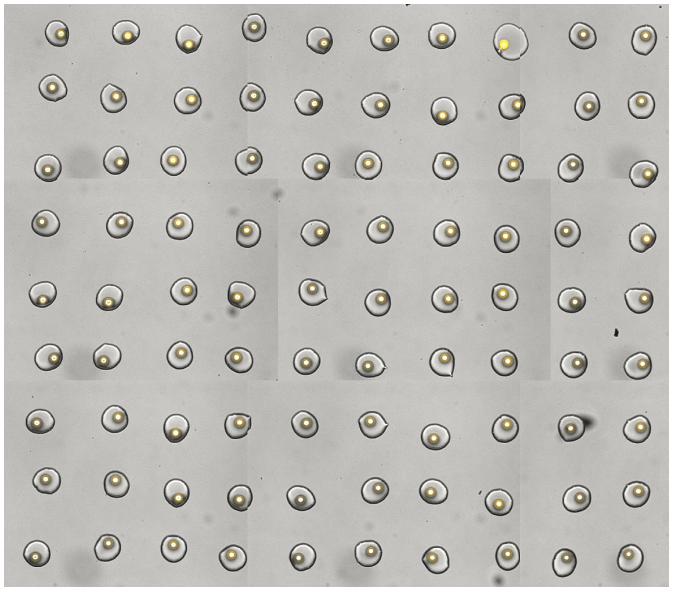
- Beats Poisson
- High recovery for low-cell numbers (<97%)
- 96 cells <4 minutes
- Multiple destination types: 96/384/1536, slides…
- Low cell # samples, ie CSF, vitreous fluid,…

Single-particle dispensing

Dissociated
Lung-cancer spheroid

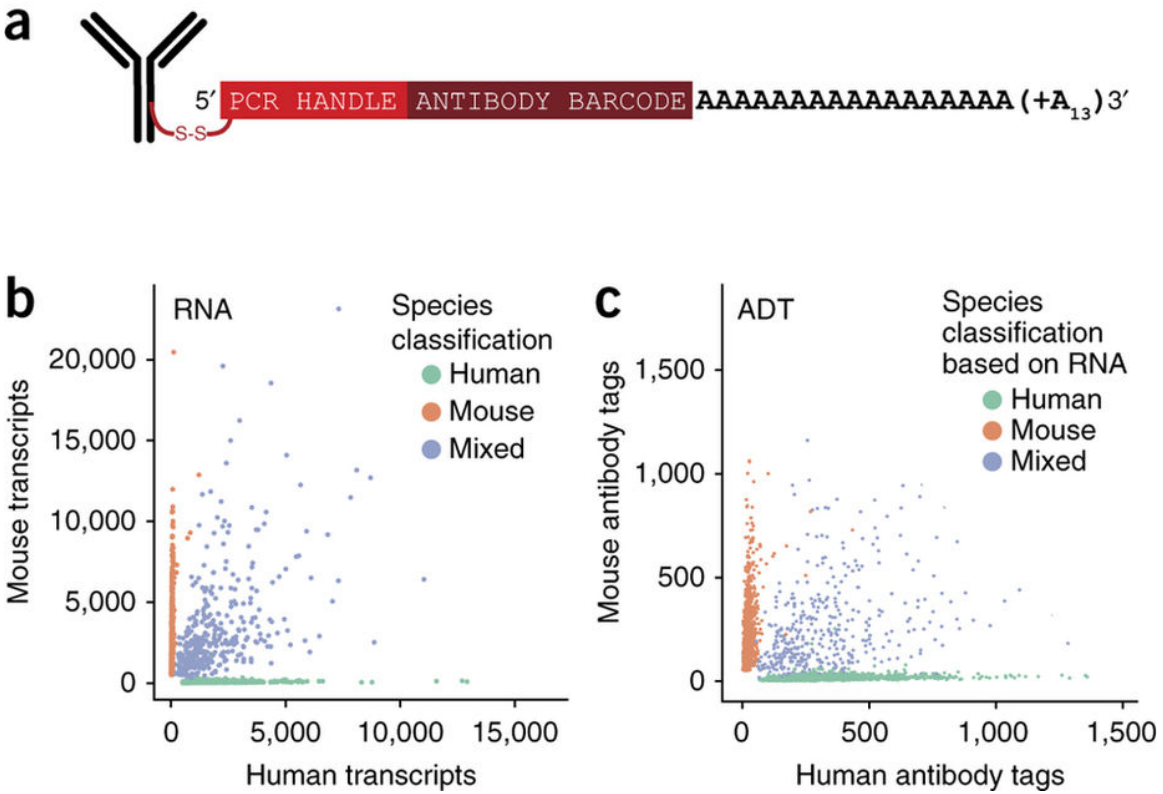


21 um beads



- Unselected and doublet cells can go into a tube and redispensed

Quantifying proteins - CITE-seq



- Cellular Indexing of Transcriptomes and Epitopes.
- Mouse or human anti-CD29
- Compatible with most sc-RNA-seq systems
- BD Antibodies and labeling kits coming soon

Multiplexing Samples - demuxlet