

Droplet-based single cell RNA sequencing (scRNAseq)

Miao-Ping Chien

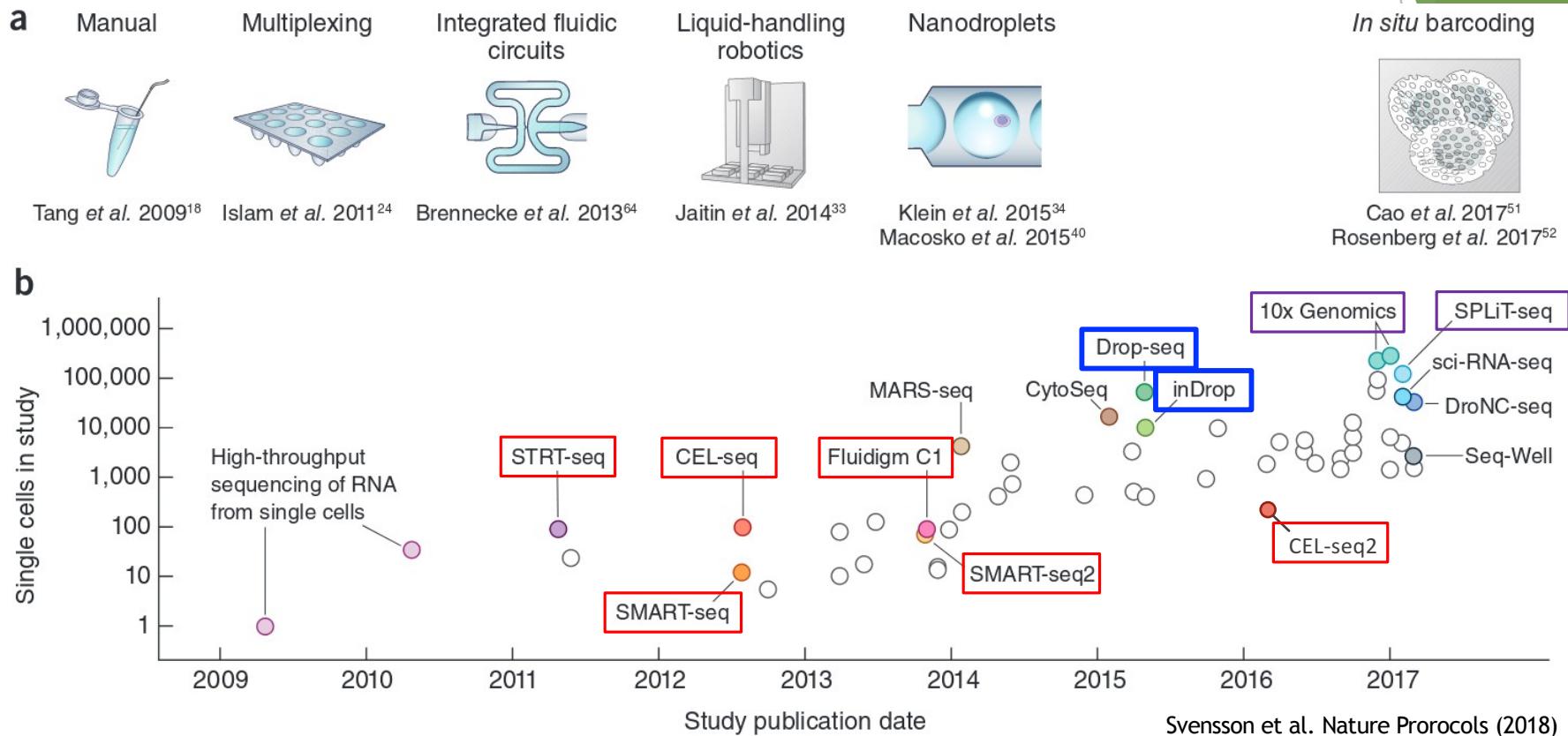
Erasmus MC, Group leader

2020 Single Cell Analysis Workshop, 2020/10/19

Outline

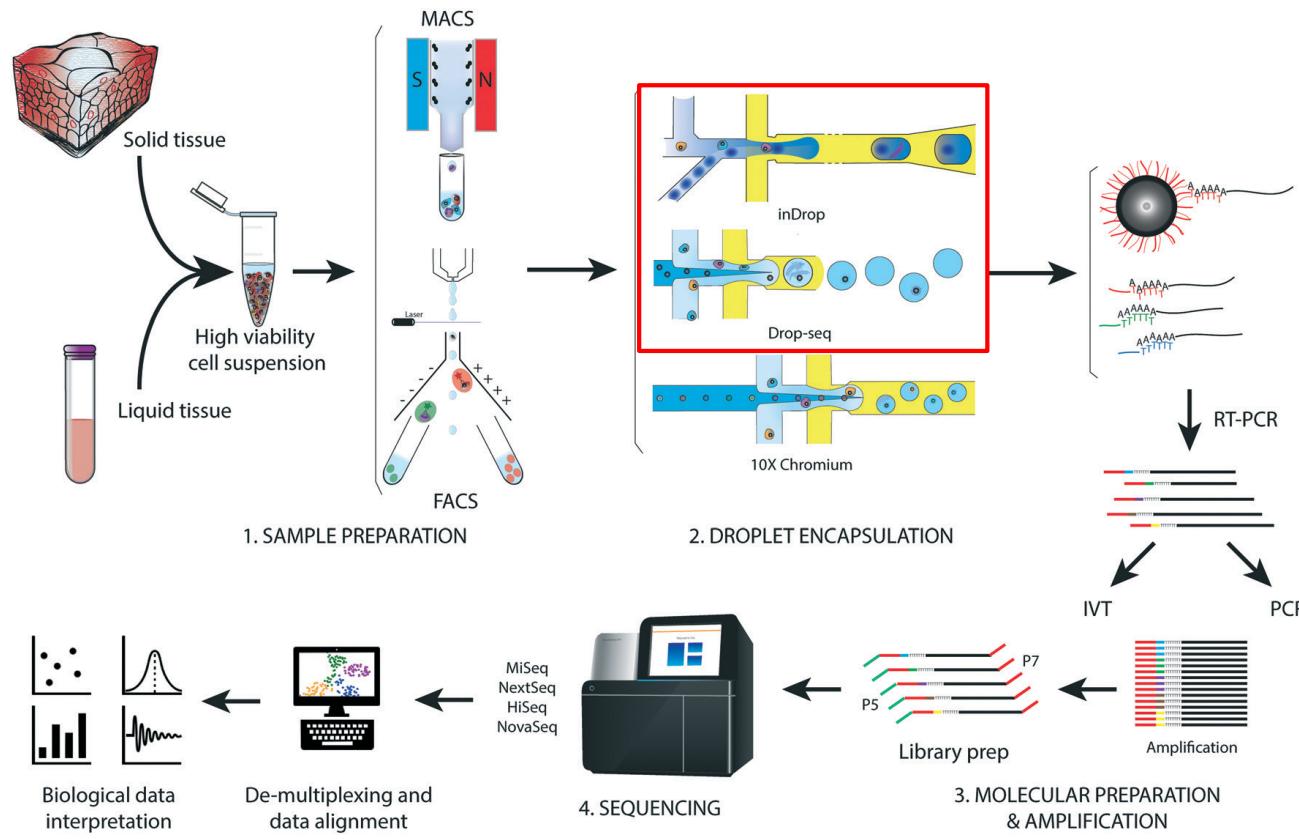
- General introduction about droplet-based scRNAseq
- Different types of droplet-based scRNAseq
- Workflow of different droplet-based scRNAseq

Evolution of scRNAseq techniques



- 100s cells thanks to **multiplexing (barcode)**
- 10,000s cells thanks to random cell captures techniques with **nanodroplets (manual)**
- 100K cells thanks to **10X Genomics** and **in situ barcoding**

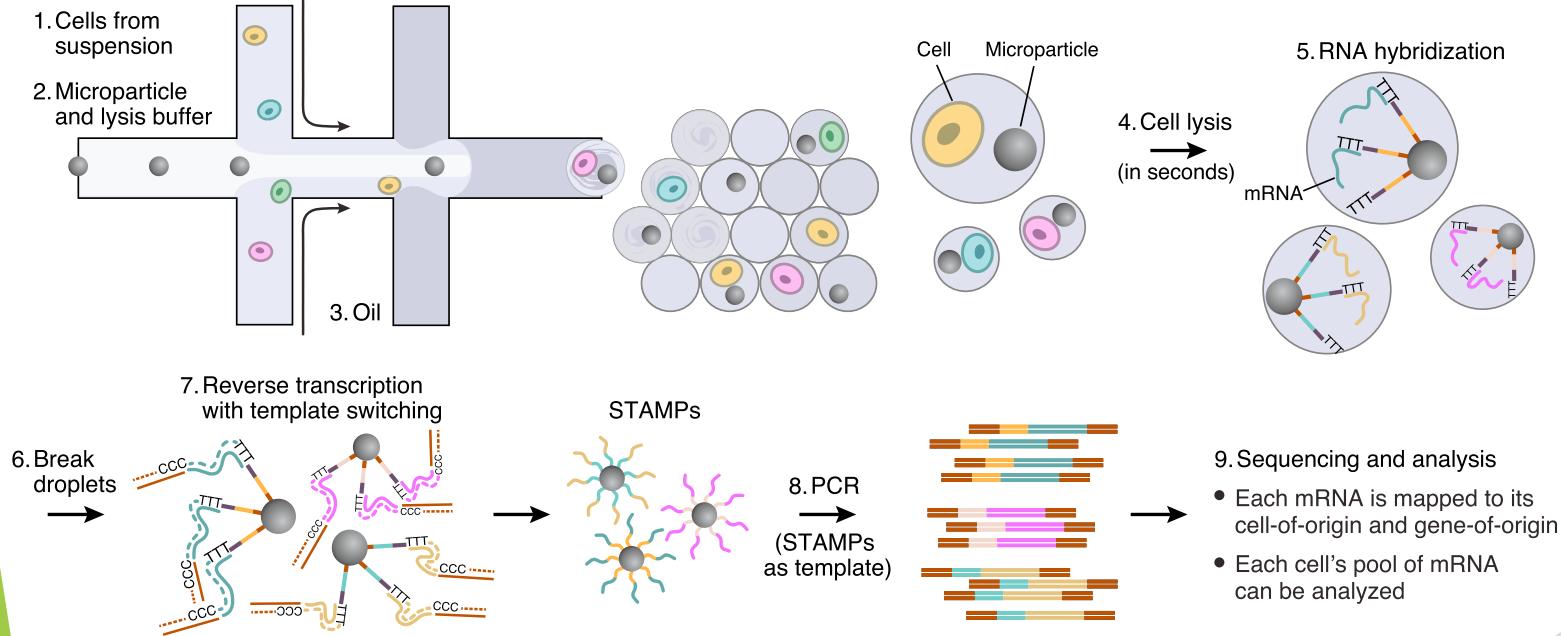
Typical workflow of droplet-based scRNASeq



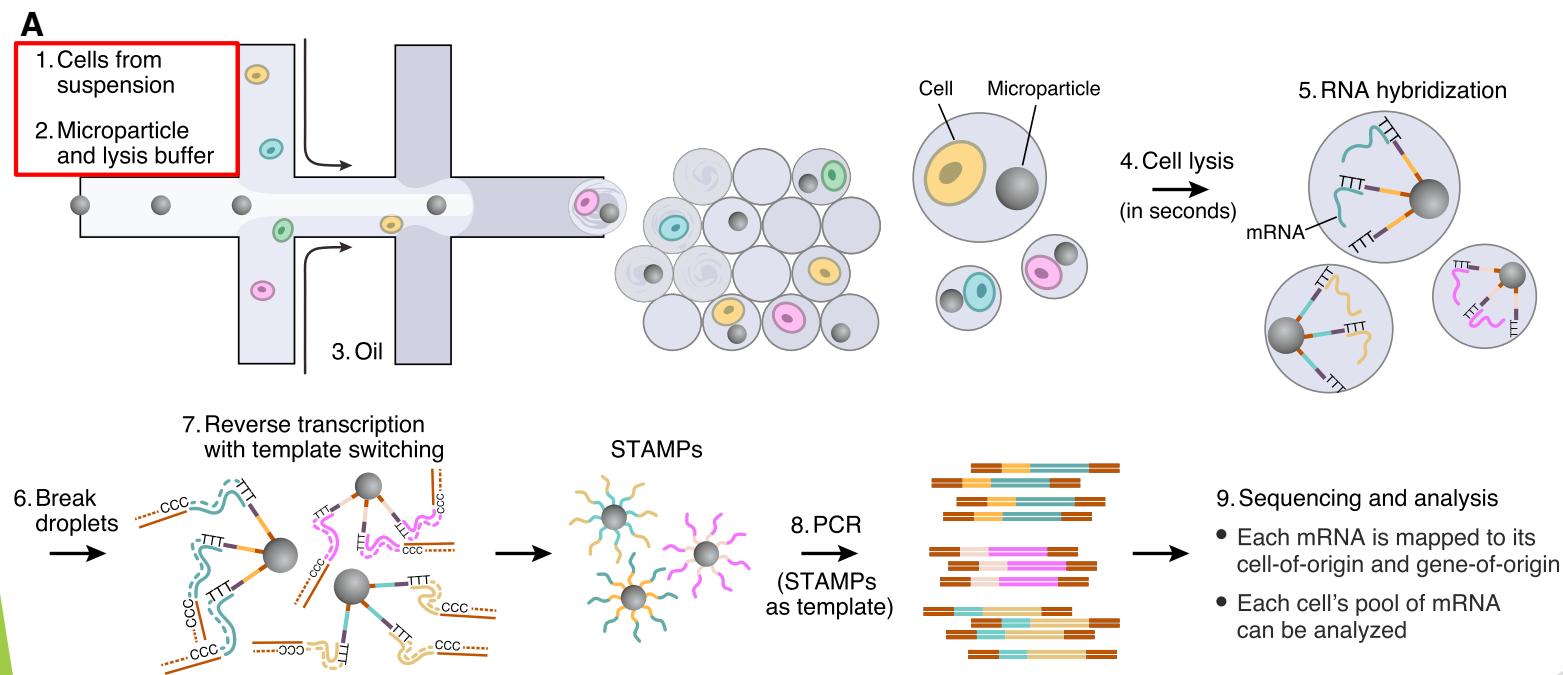
What's the difference between DropSeq and InDrop?

DropSeq overview

A



DropSeq overview

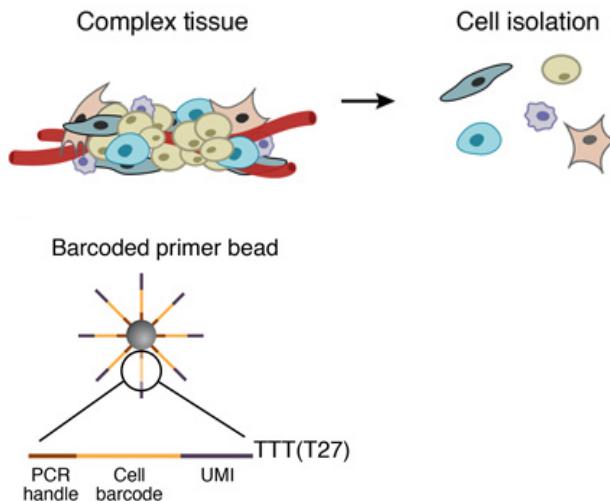


DropSeq workflow

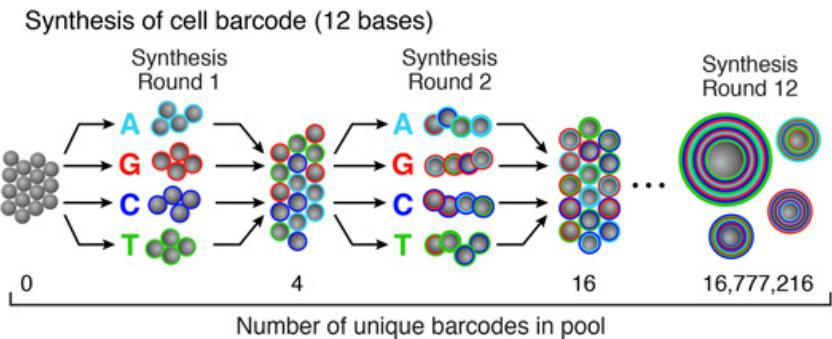
1. PREPARE A SINGLE-CELL SUSPENSION FROM A COMPLEX TISSUE

2. PRIMER SYNTHESIS

Sequence of primers on the microparticle



Split-and-pool synthesis of the cell barcode



Cell, 2015, 161, 1202-1214

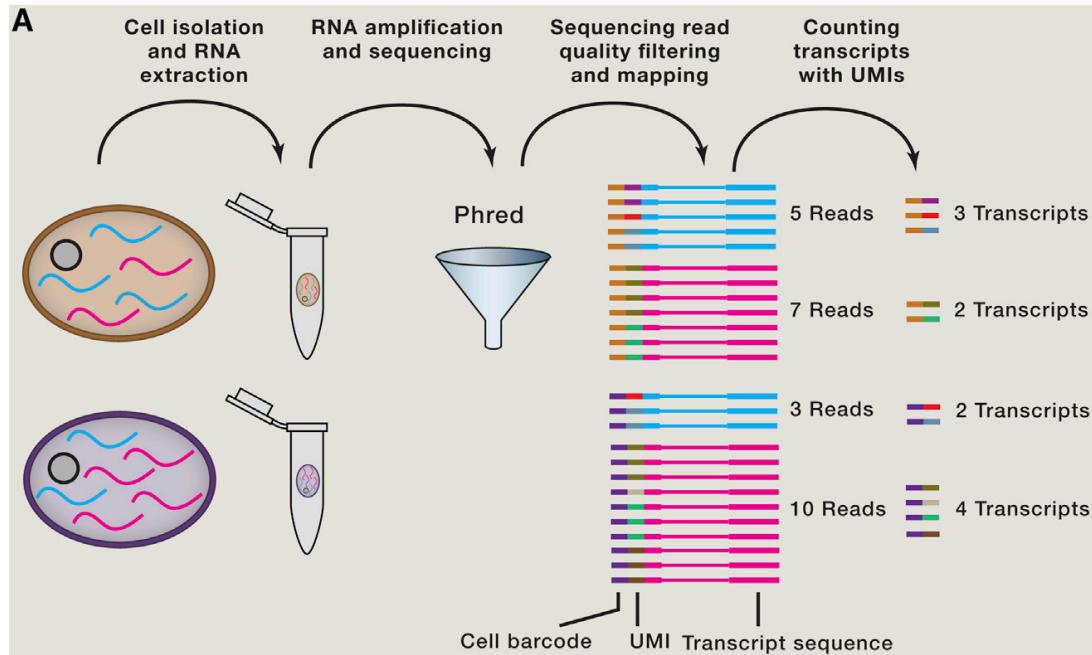
Synthesis of a unique molecular identifier (UMI)

Synthesis of UMI (8 bases)



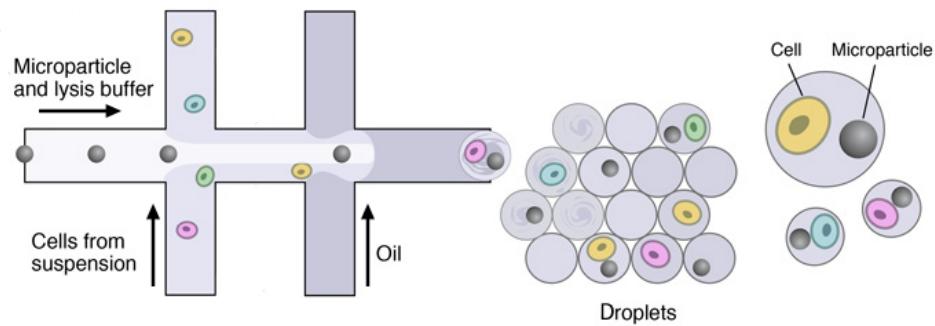
- Millions of the same cell barcode per bead
- 4^8 different molecular barcodes (UMIs) per bead

Quantification of mRNA Expression with unique molecular identifier UMIs

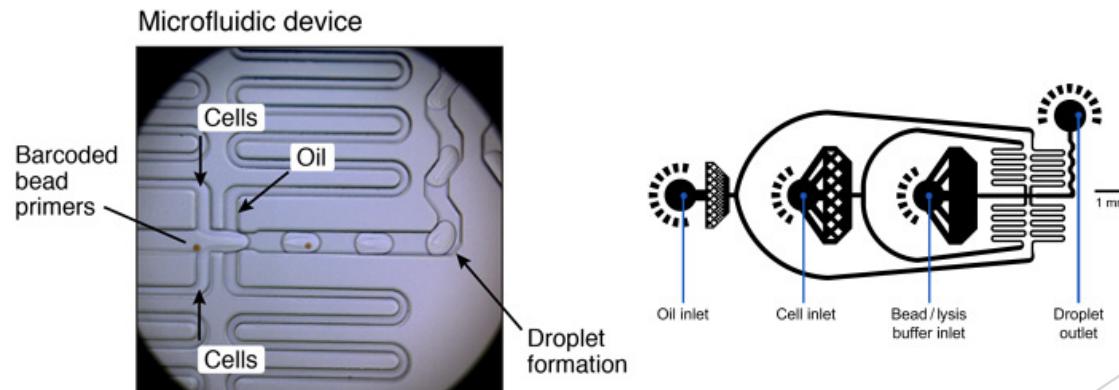


DropSeq workflow

3. MICROFLUIDIC DEVICE



Microfluidic setup

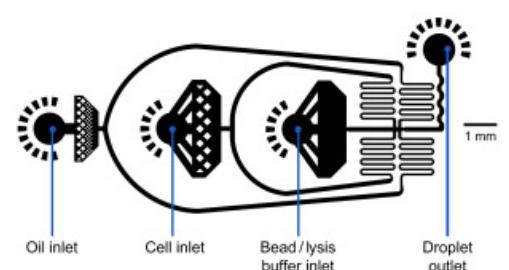
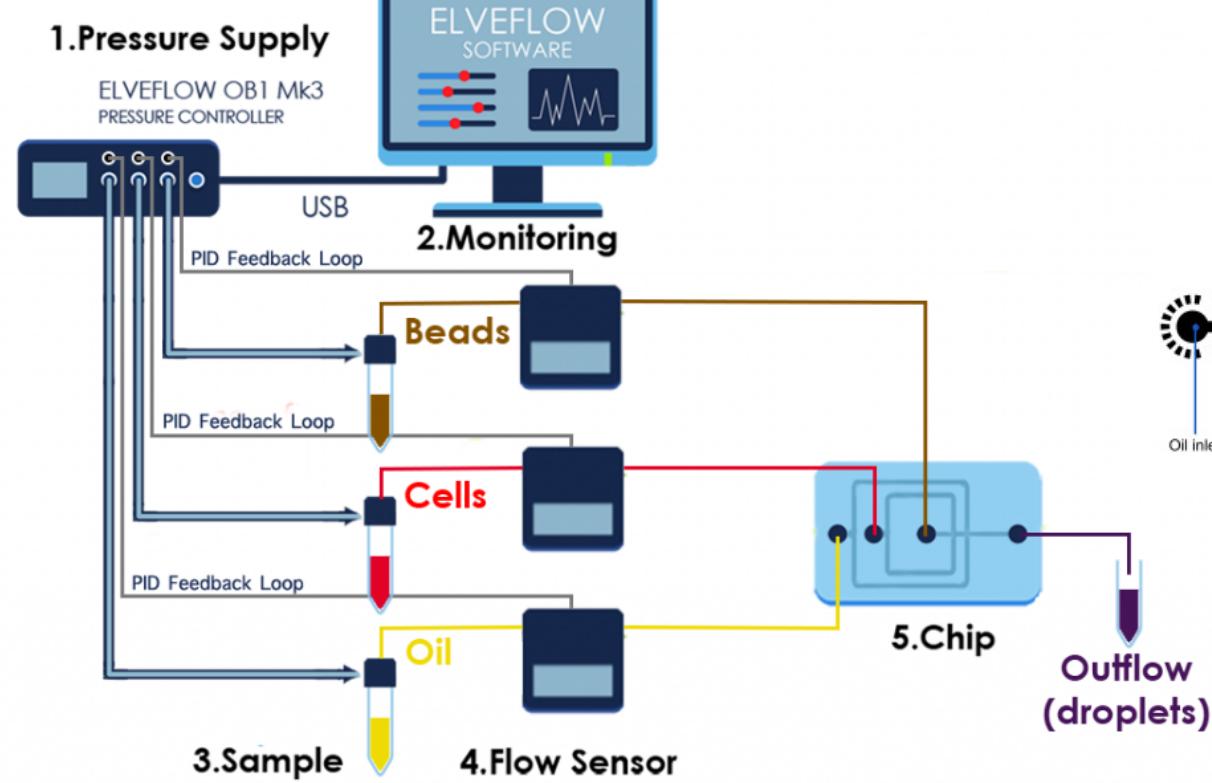


Cell, 2015, 161, 1202-1214

DropSeq workflow

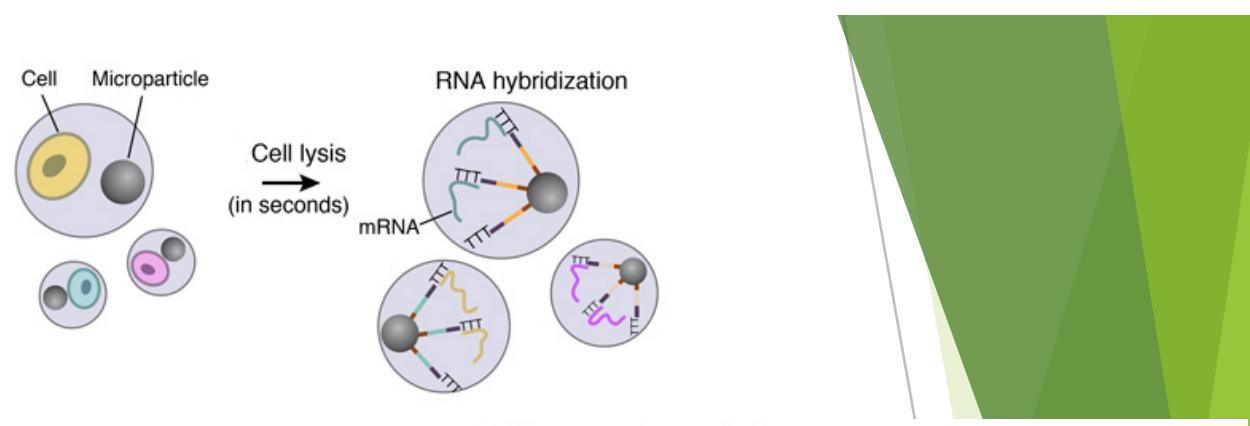
3. MICROFLUIDIC DEVICE

Set-up Diagram



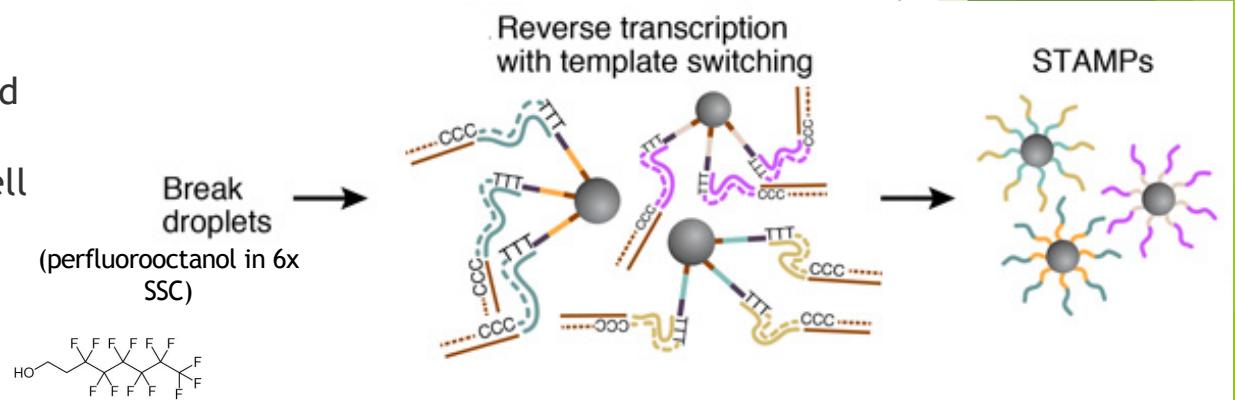
DropSeq workflow

4. CELL LYSIS AND RNA HYBRIDIZATION



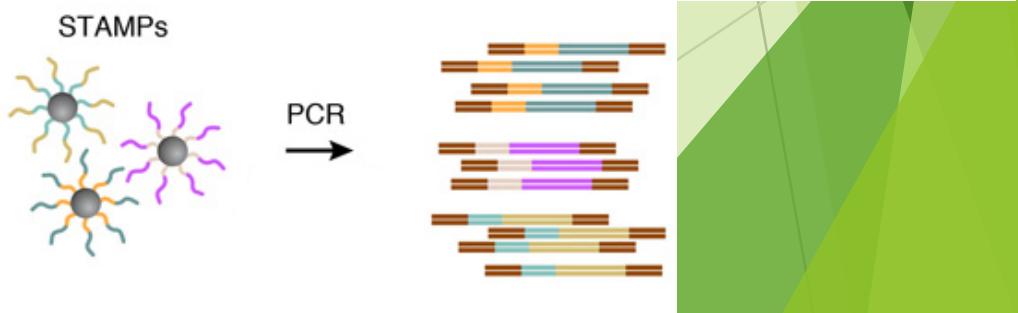
5. STAMPS GENERATION

The mRNAs are then reverse-transcribed into cDNAs together in one reaction, forming a set of beads called “single-cell transcriptomes attached to microparticles” (STAMPs).



6. AMPLIFICATION OF STAMPS

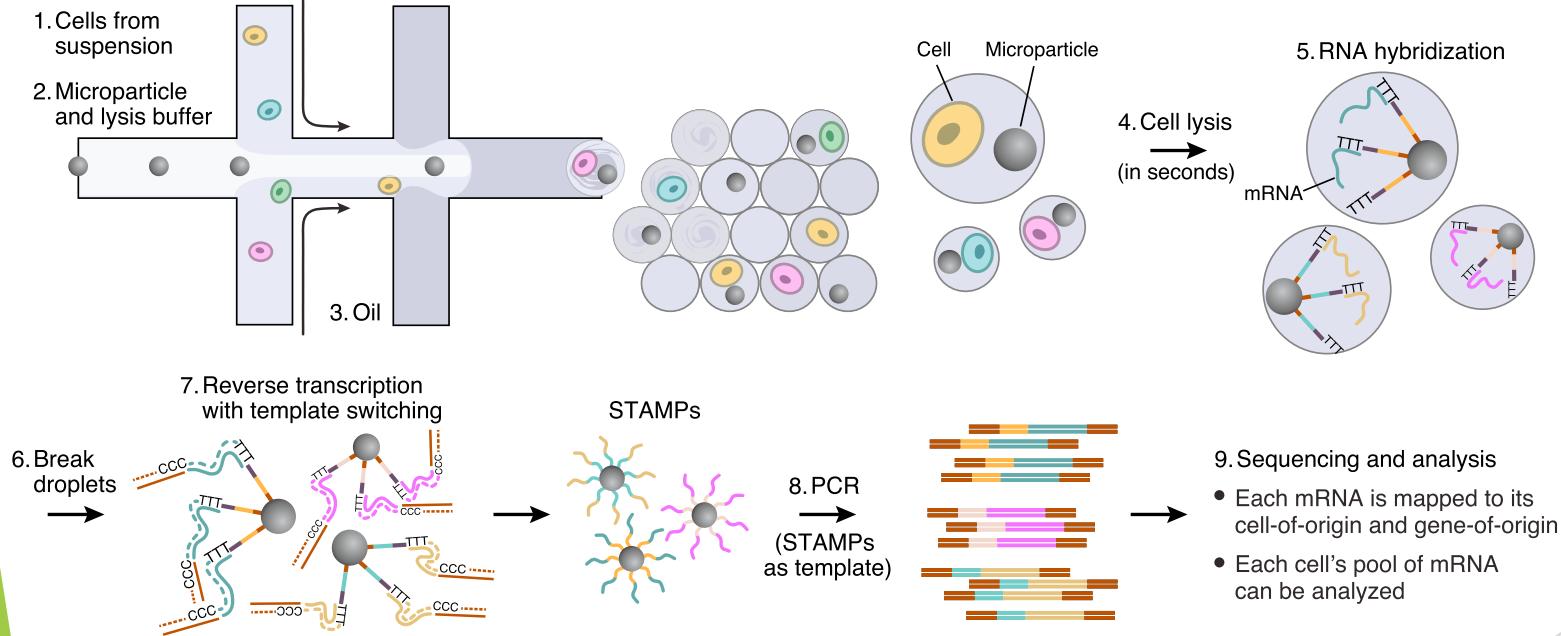
The barcoded STAMPs can then be amplified in pools by PCR reaction for high-throughput mRNA-sequencing, to analyze any desired number of individual cells.



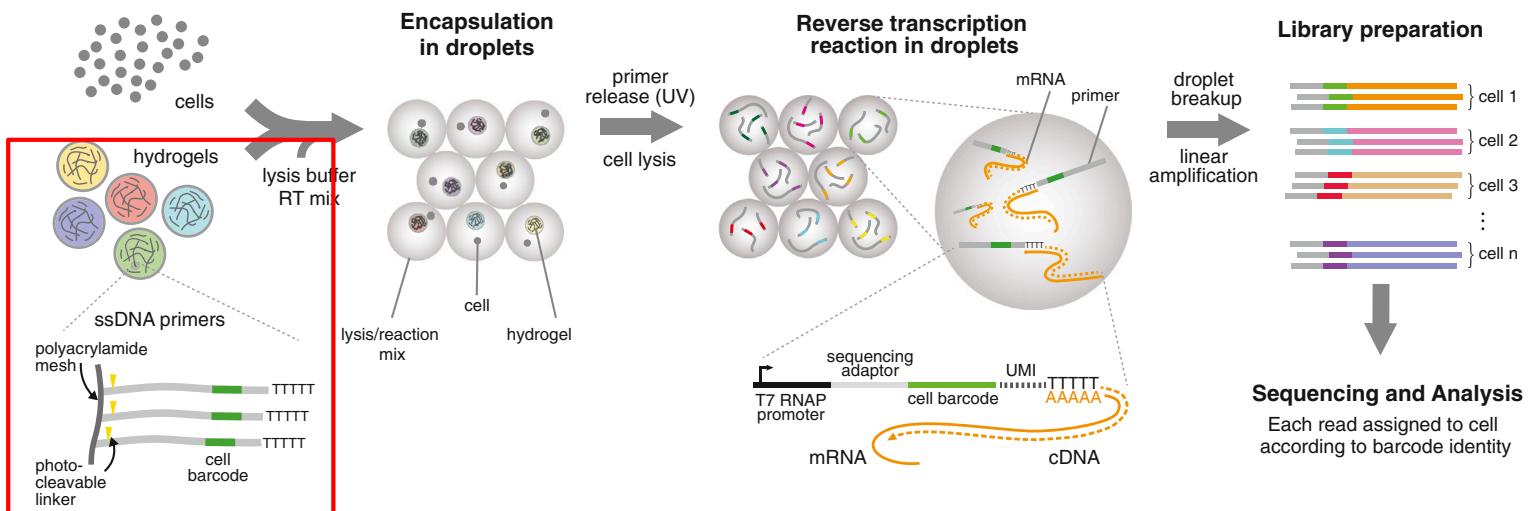
Cell, 2015, 161, 1202-1214

DropSeq overview

A



InDrop overview

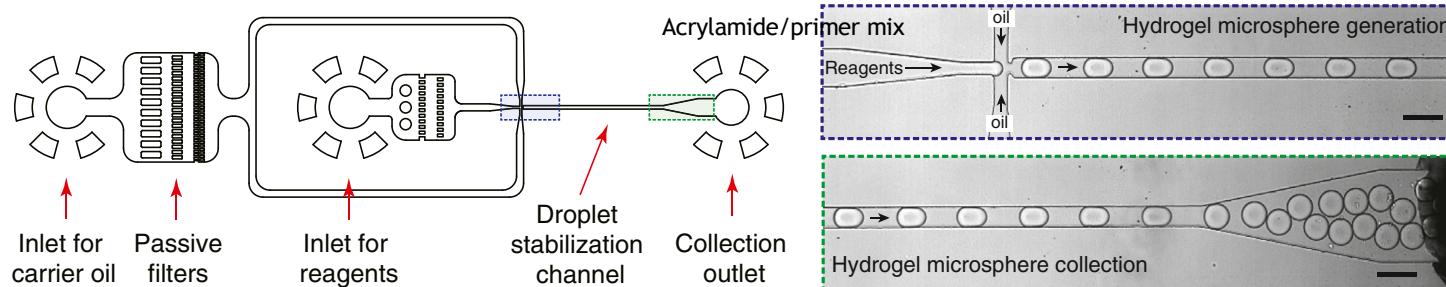


Cell, 2015, 161, 1187-1201

I) Synthesis of barcoding hydrogel beads

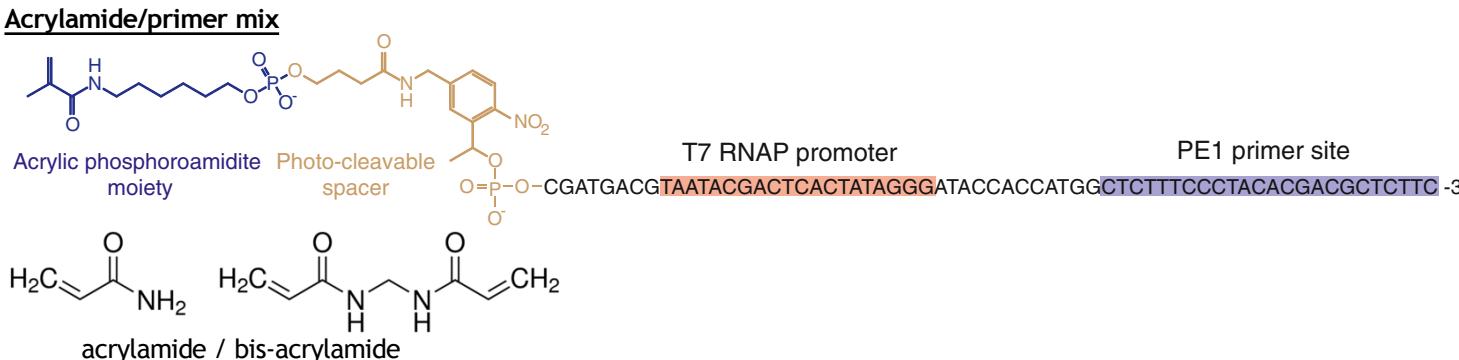


A



Droplet collection and off-chip polymerization

B



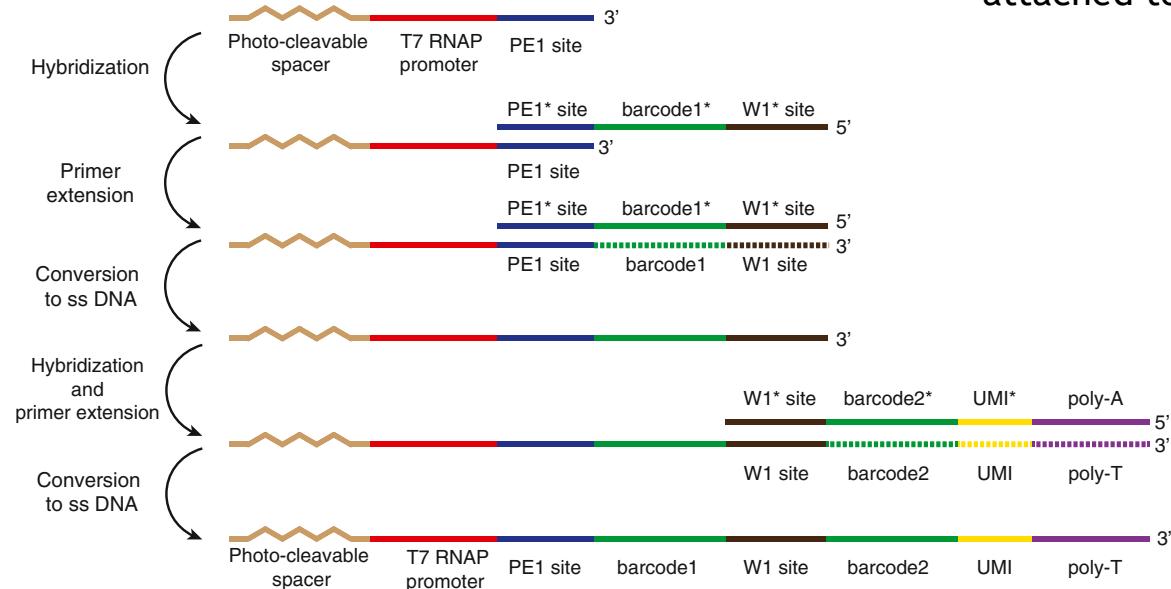
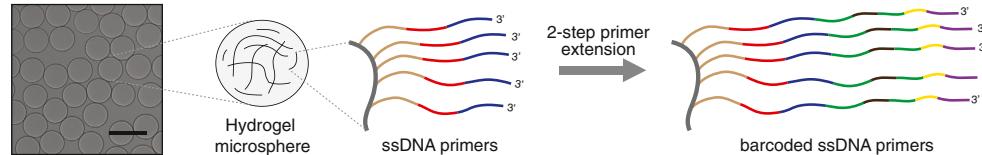
(A-B) Hydrogel bead generation and collection. An aqueous acrylamide/bis-acrylamide solution carrying acrydite-modified DNA oligonucleotide is emulsified using a microfluidic device to yield highly monodispersed droplets, which are collected off-chip and polymerized into hydrogel beads. Scale bars, 100 μ m.

Cell, 2015, 161, 1187-1201

1CELLBIO

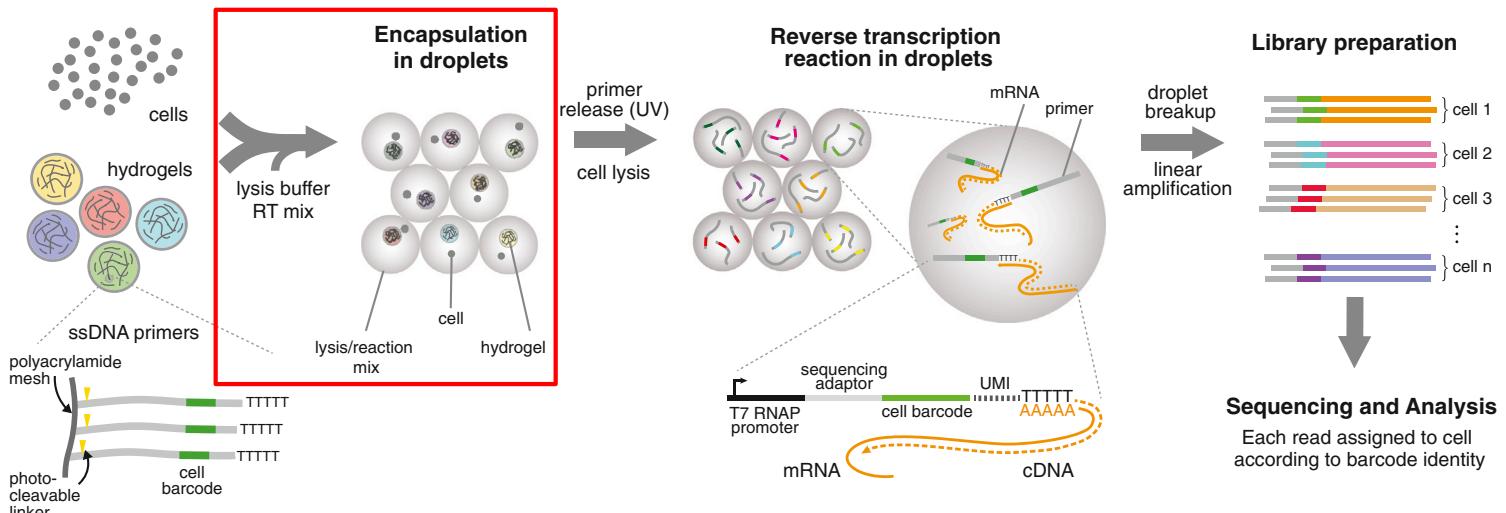
I) Synthesis of barcoding hydrogel beads

c)



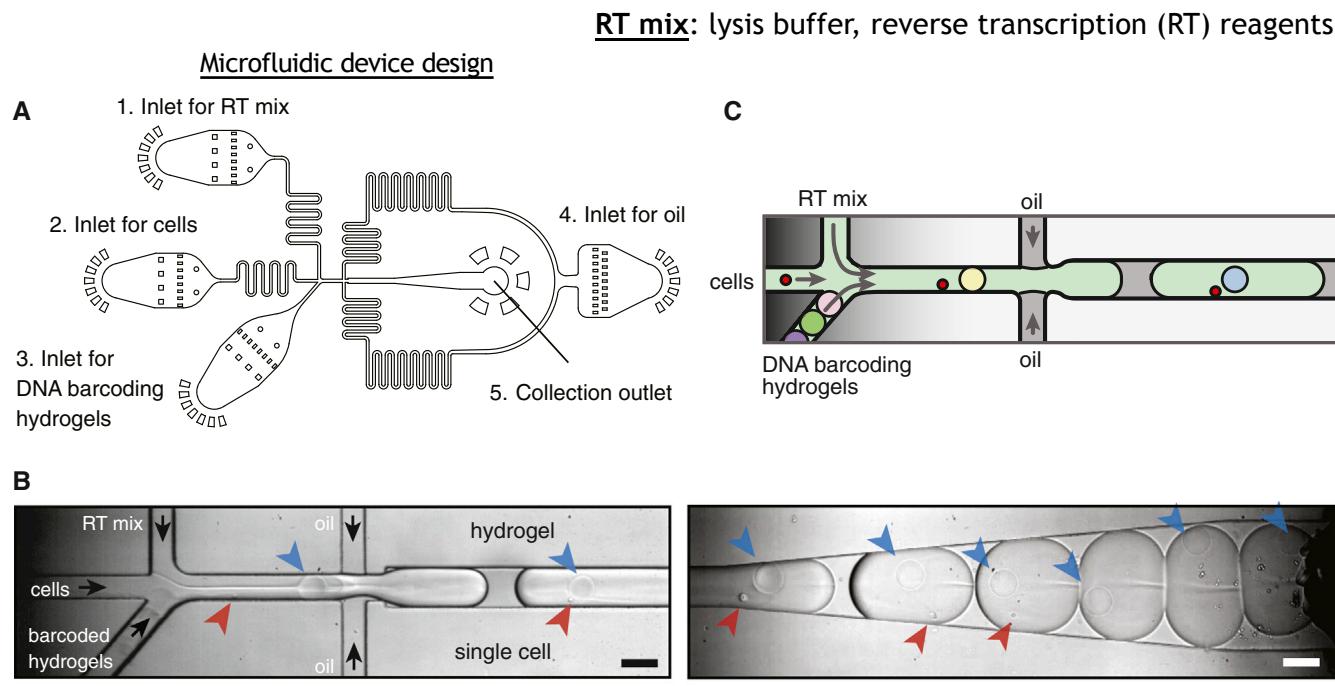
C) Primer extension reaction to incorporate barcode sequences into DNA oligonucleotides attached to hydrogel beads

InDrop overview

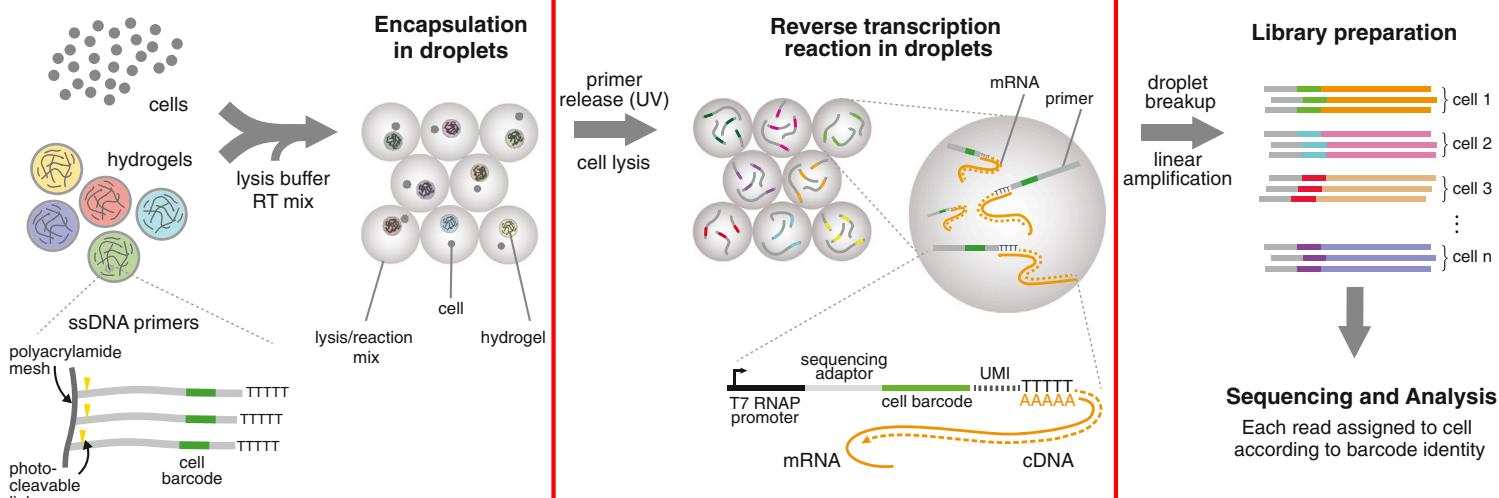


Cell, 2015, 161, 1187-1201

II) Encapsulation in droplets

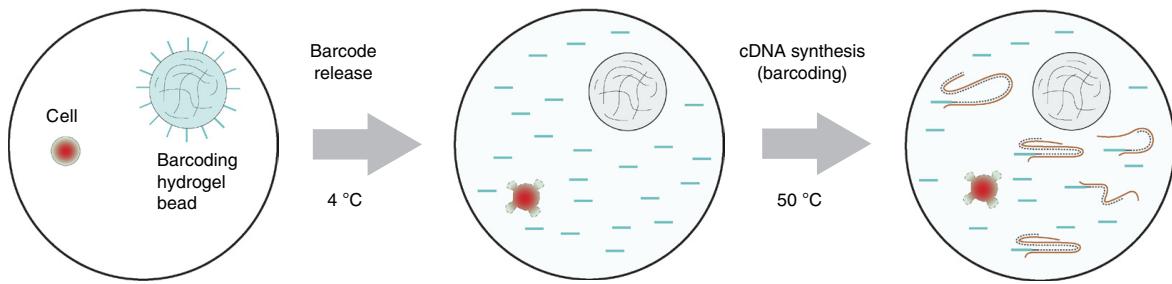


InDrop overview

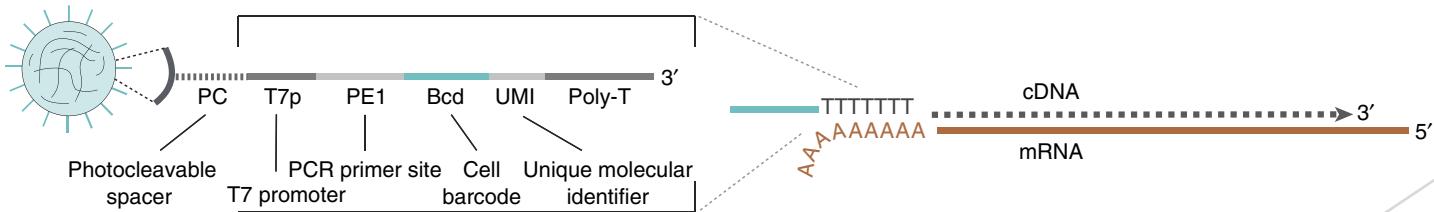


III) Reverse transcription in droplets

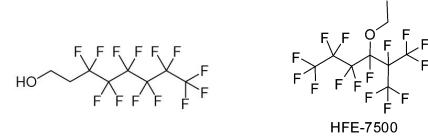
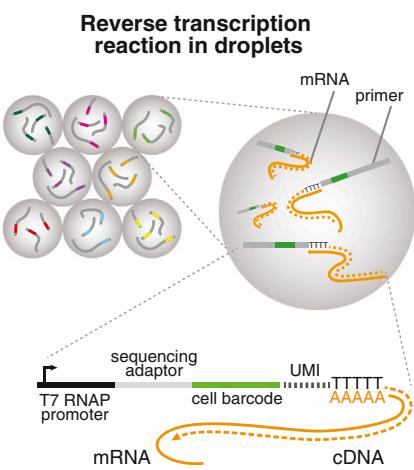
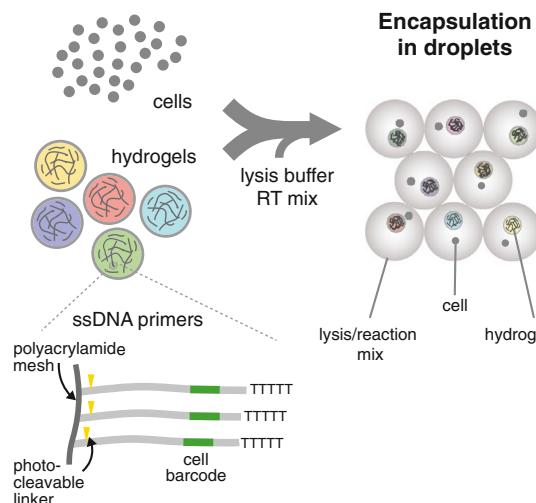
- After cell and hydrogel bead encapsulation, the barcoding cDNA primers are released from the beads using **365 nm UV light** (~10 mW/cm²; which is not damaging to DNA/RNA), followed by mRNA capture and reverse transcription



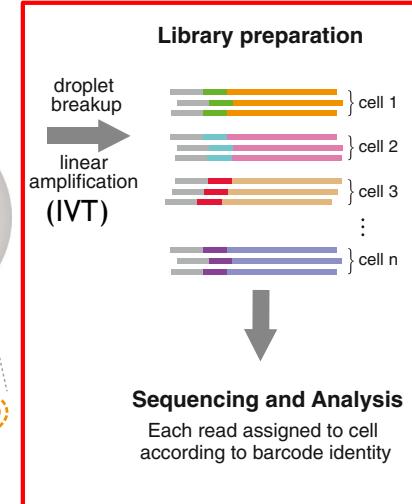
- mRNA capture and reverse transcription



InDrop overview

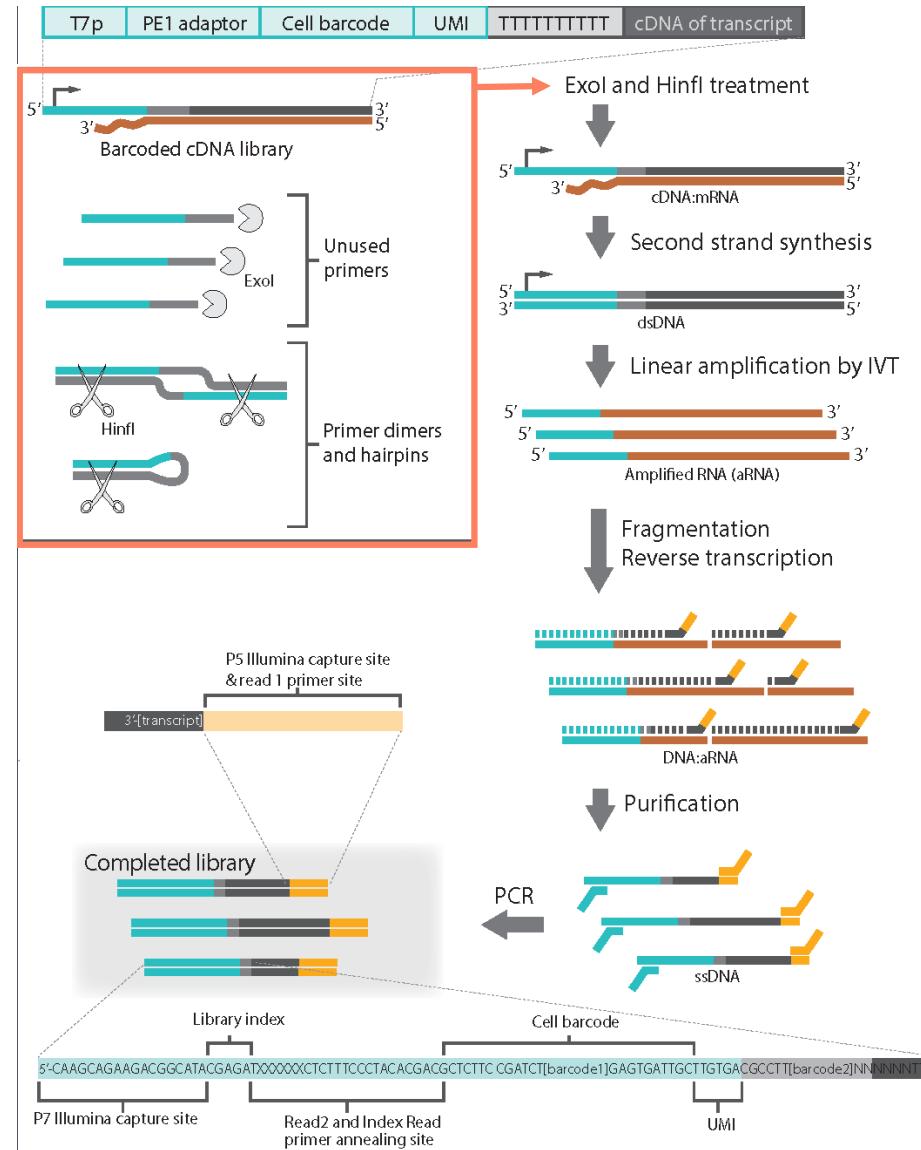


20% (v/v) perfluorooctanol + 80% (v/v) HFE-7500

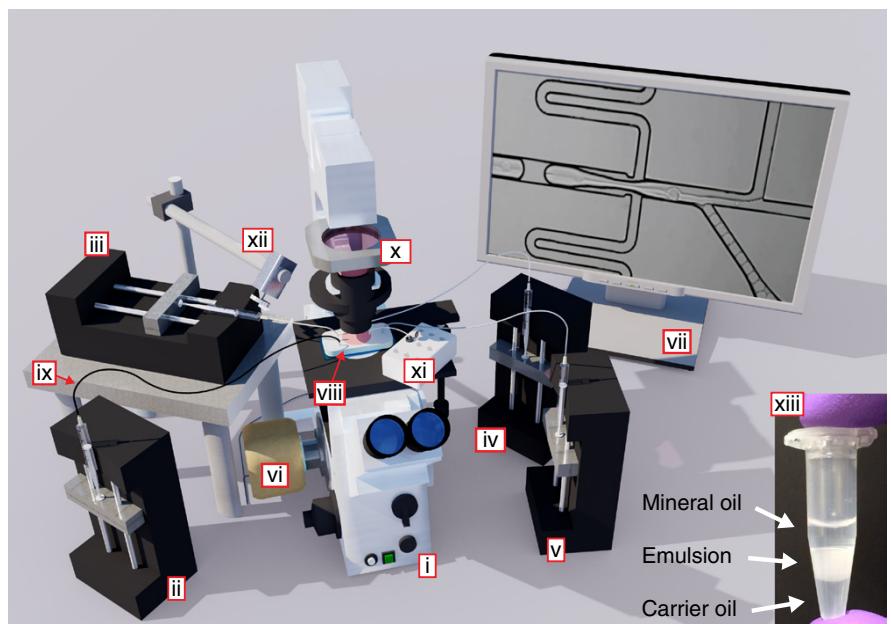


IV) InDrop library prep

- 2nd strand synthesis to make full length dsDNA
- In vitro transcription (IVT) back to RNA off T7 promoter from primer
- Fragmentation RNA (heat/base)
- RT with random hexamer primer containing adaptor
- PCR off adaptors to add index and illumina adaptor

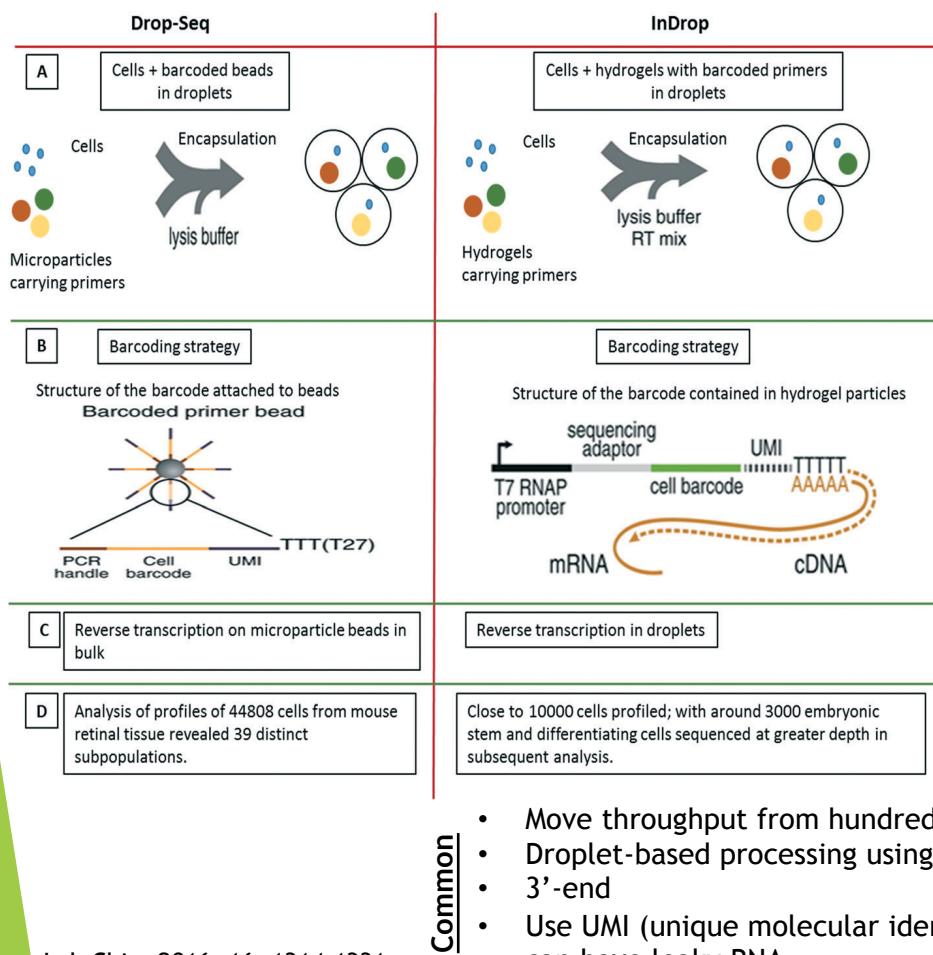


The inDrops platform



- (i) Inverted bright-field microscope;
- (ii-v) syringe pumps;
- (vi) fast speed camera;
- (vii) computer;
- (viii) microfluidic chip;
- (ix) barcoding hydrogel bead containing tubing, protected from ambient light by insertion into a second, opaque tubing, or alternatively, wrapped in aluminum foil;
- (x) red band-pass filter (≥ 600 nm);
- (xi) ice-cold rack containing the collection tube;
- (xii) cell mixer;
- (xiii) photograph of the collection tube showing three distinct phases

DropSeq vs InDrop



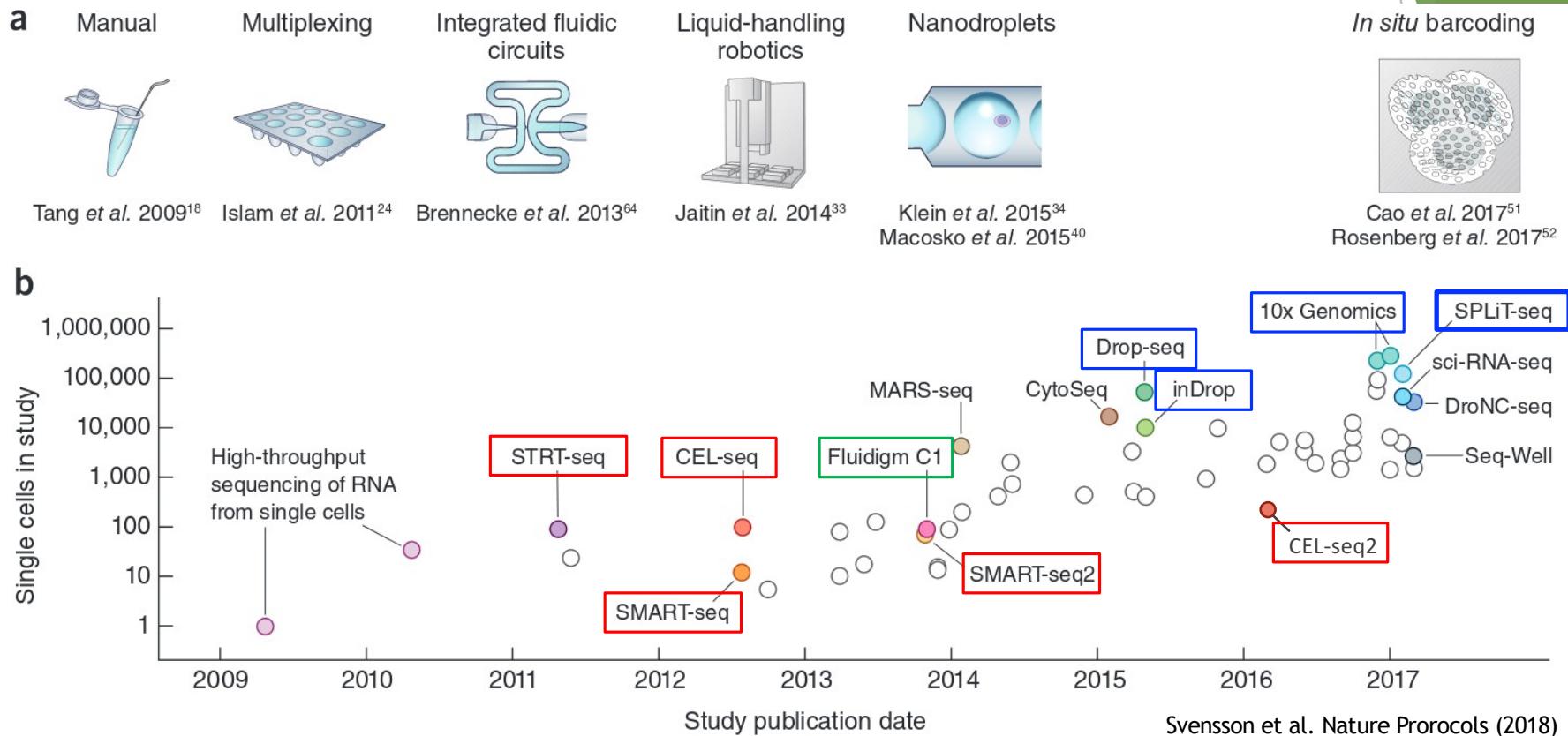
DropSeq

- Droplets are broken and reverse transcription (RT)/template switching occurs on beads in pool
- STAMP: single cell transcriptomes attached to microparticles

InDrop

- Reverse transcription (RT) in droplets (not in pool)

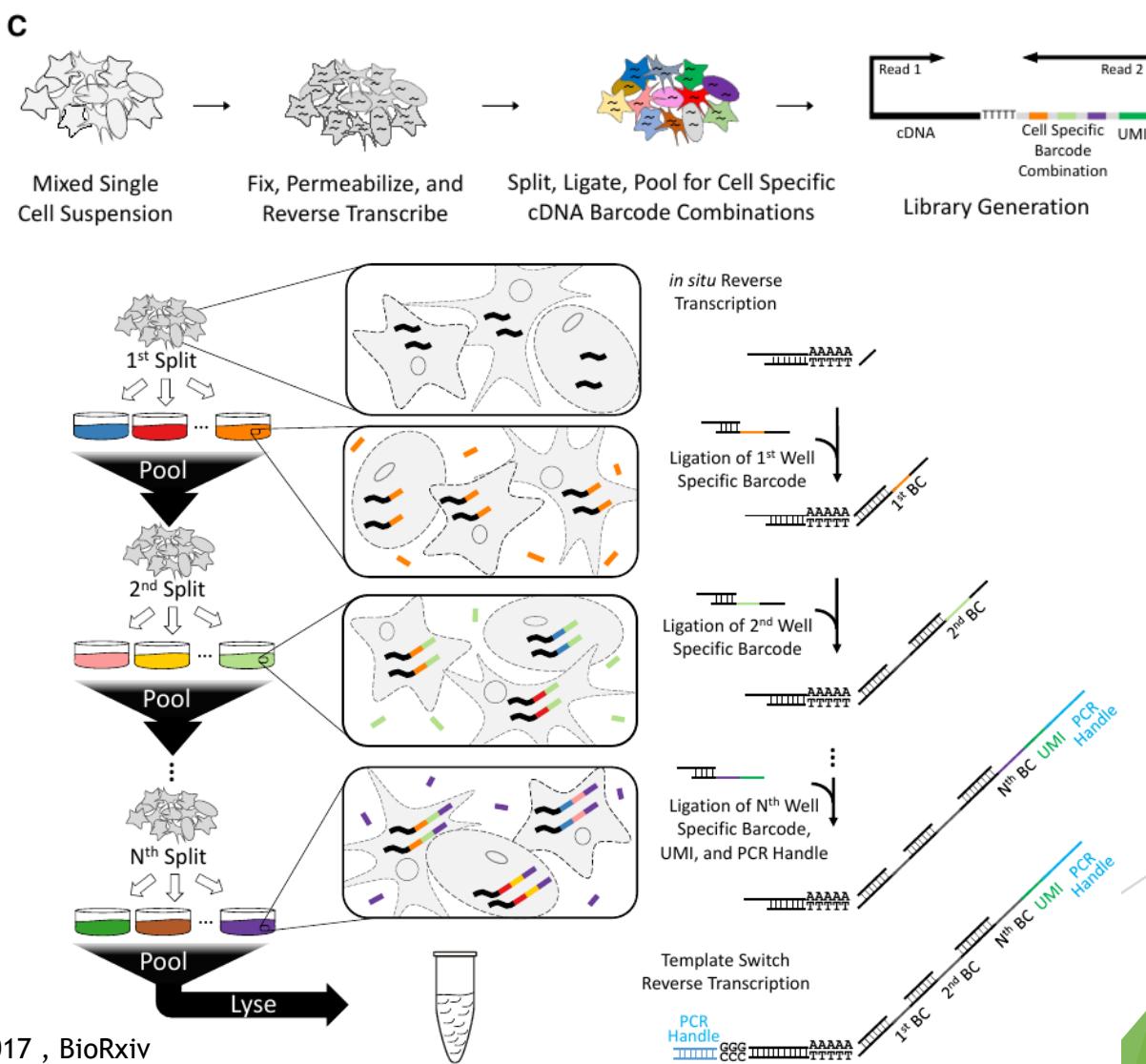
Evolution of scRNAseq techniques



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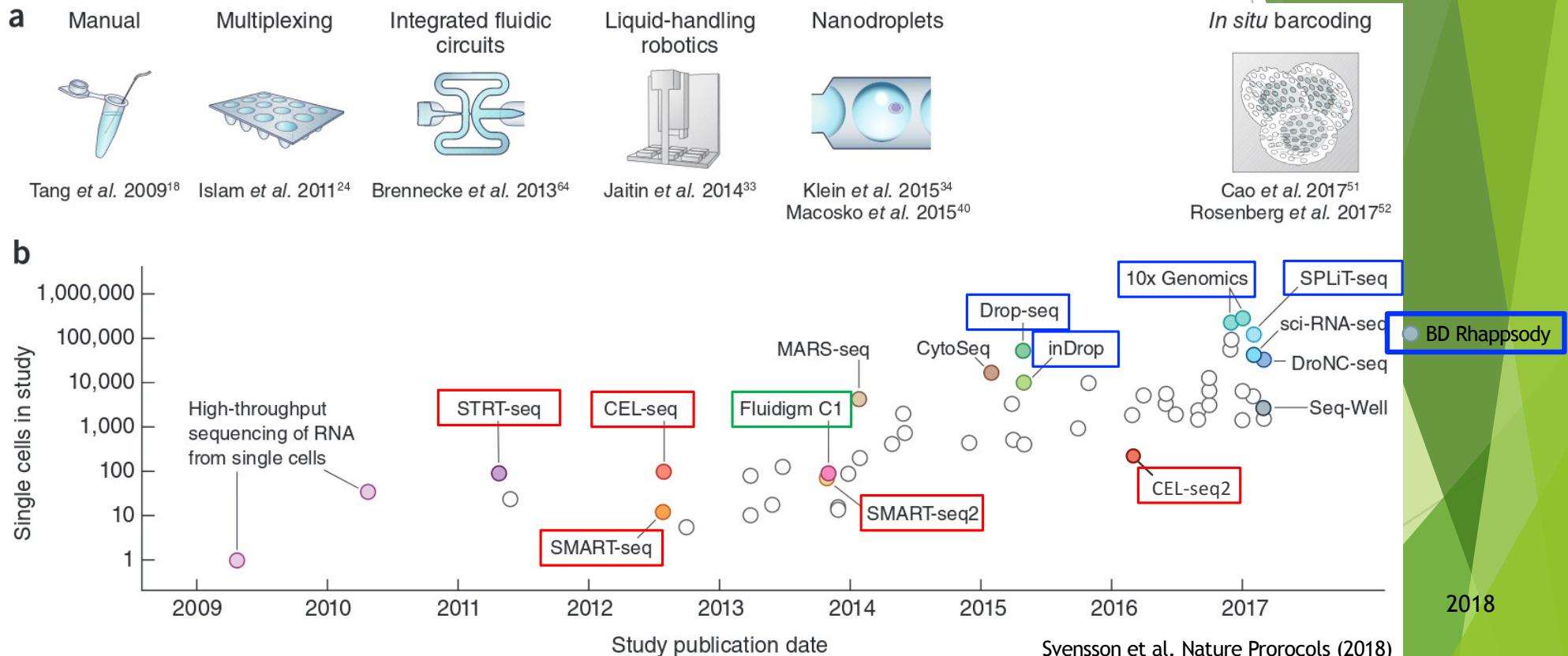
In situ barcoding

SPLIT-seq:
Split Pool
Ligation-based
Transcriptome
sequencing



Rosenburg et al, 2017 , BioRxiv

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BD Rhapsody™ Instrument

Rhapsody imaging
Scanner for QC
Measurements

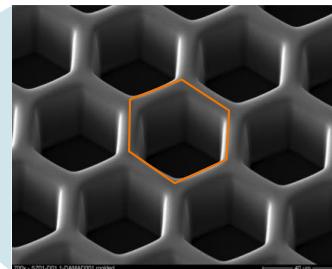


Rhapsody Express
For manipulating fluidics
from cell capture and processing

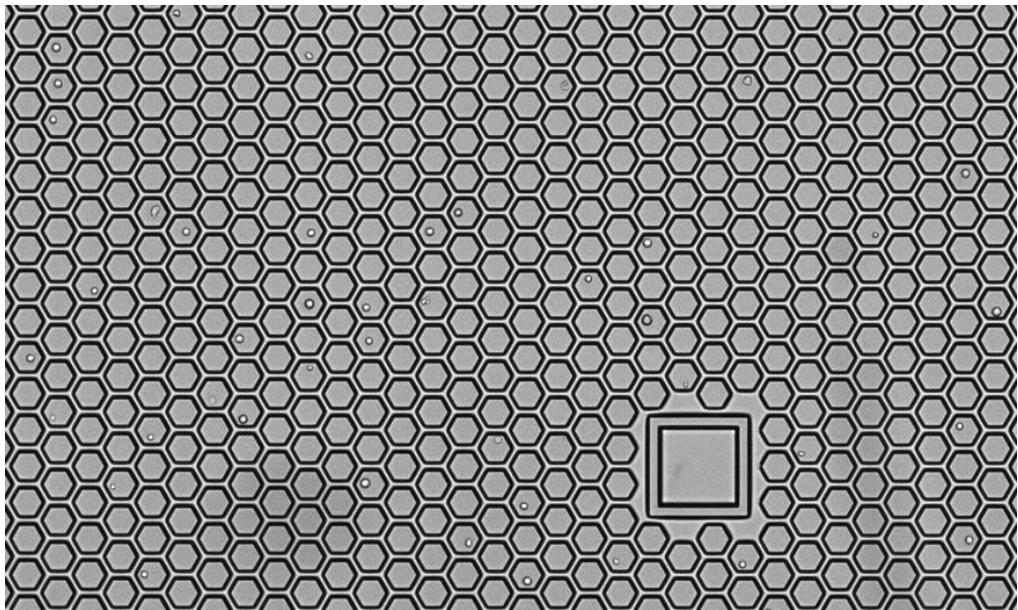


Micowell Cartridge

One system for analyzing
both protein and RNA at a
single cell level



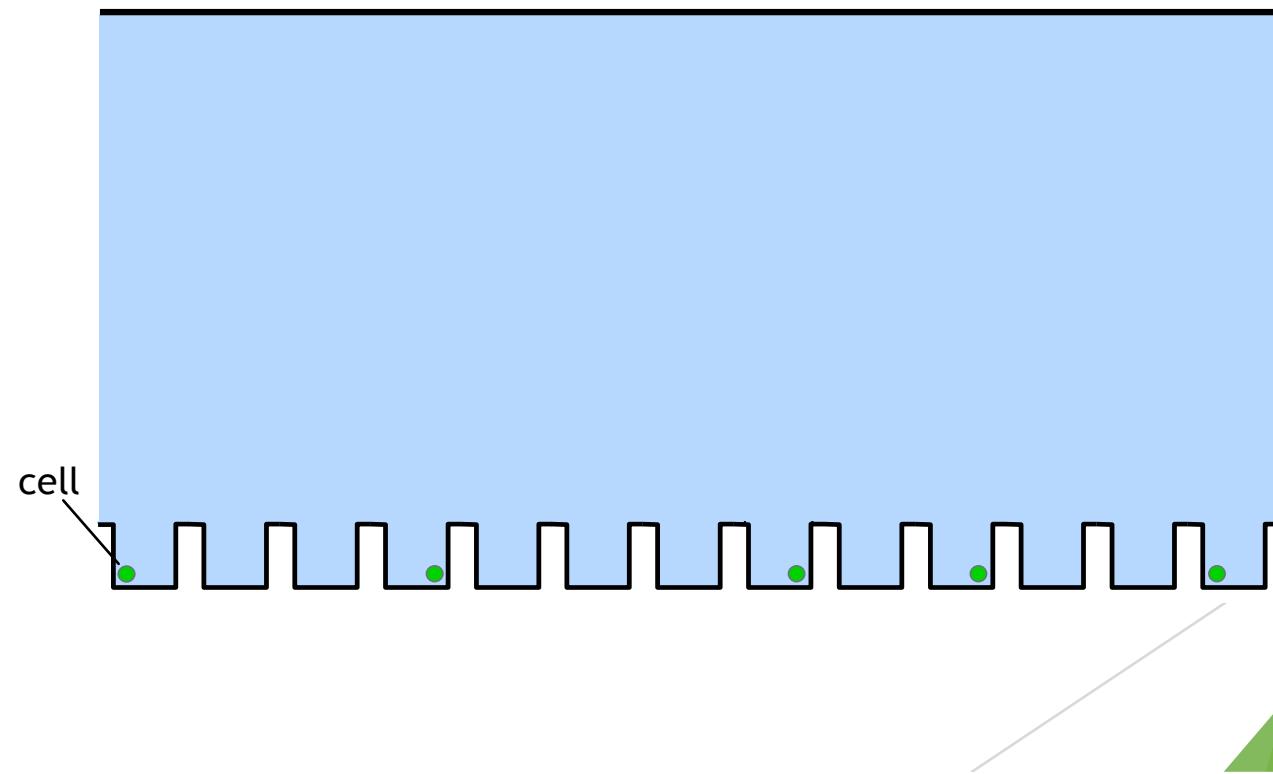
BD Rhapsody - microwell cartridge



- Sparse cell loading
- Multiplet rate predicted by Poisson distribution
 - ~ 2% multiplets in 10k cells
 - ~4-5% multiplets in 20k cells
 - ~10% multiplets in 40k cells

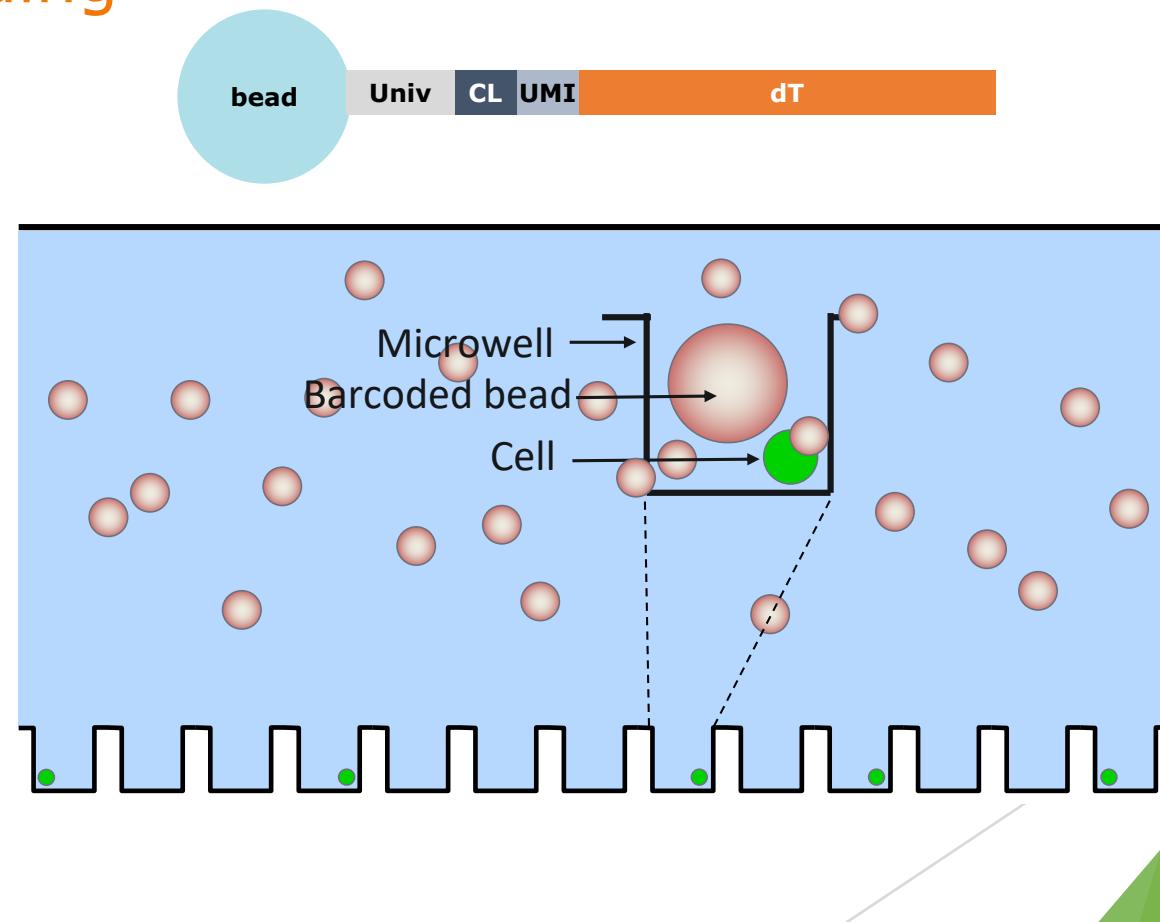
BD Rhapsody - microwell cartridge workflow

Cell loading



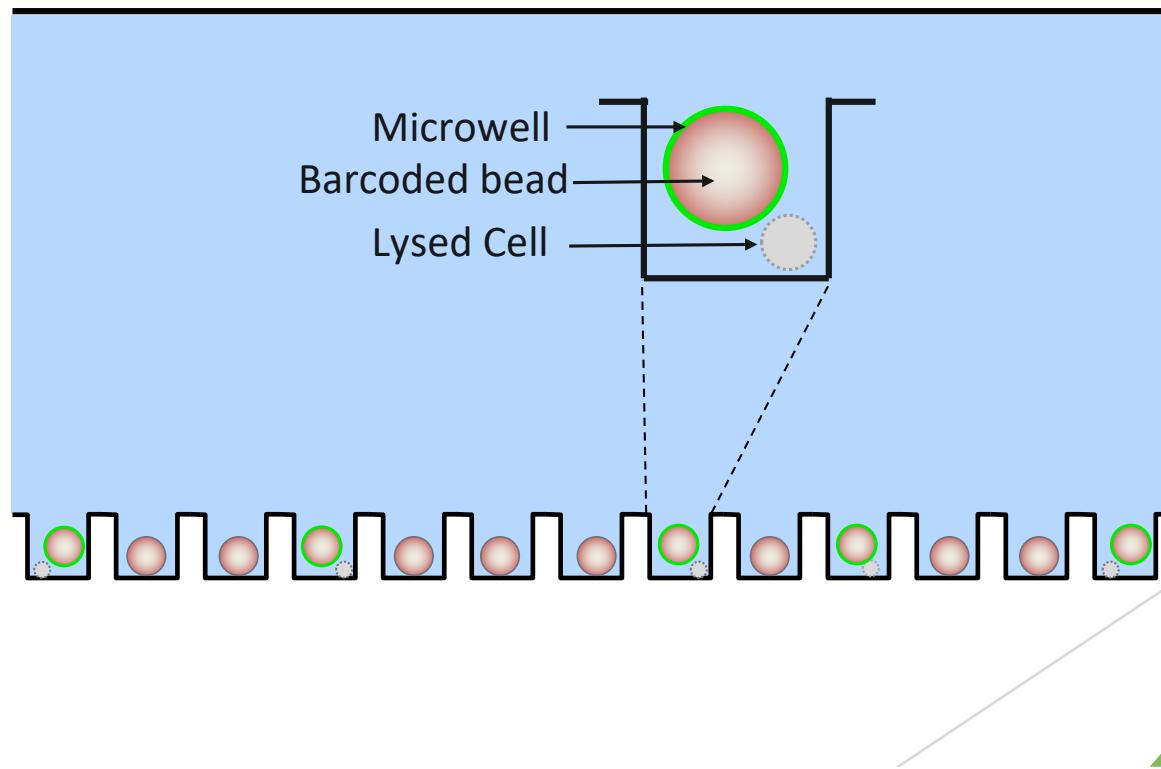
BD Rhapsody - microwell cartridge workflow

Bead loading



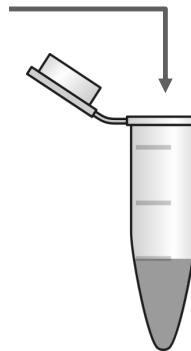
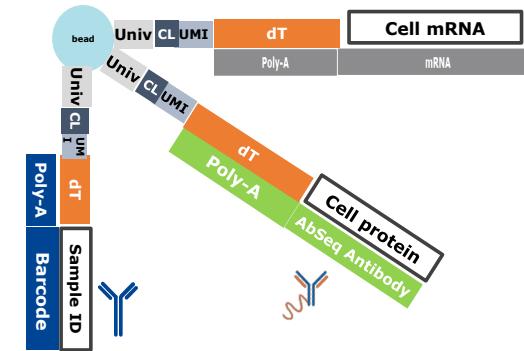
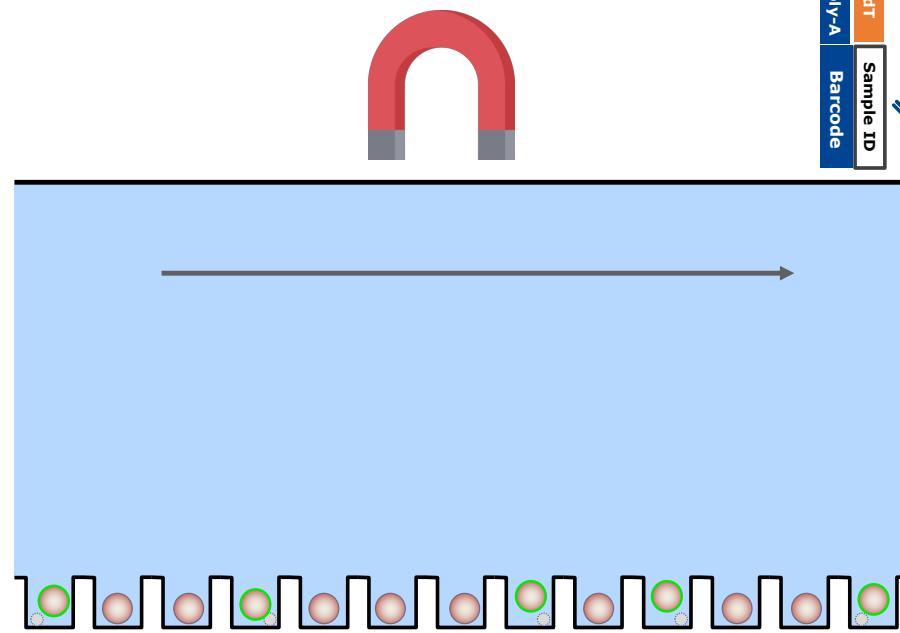
BD Rhapsody - microwell cartridge workflow

Lysis and mRNA hybridization

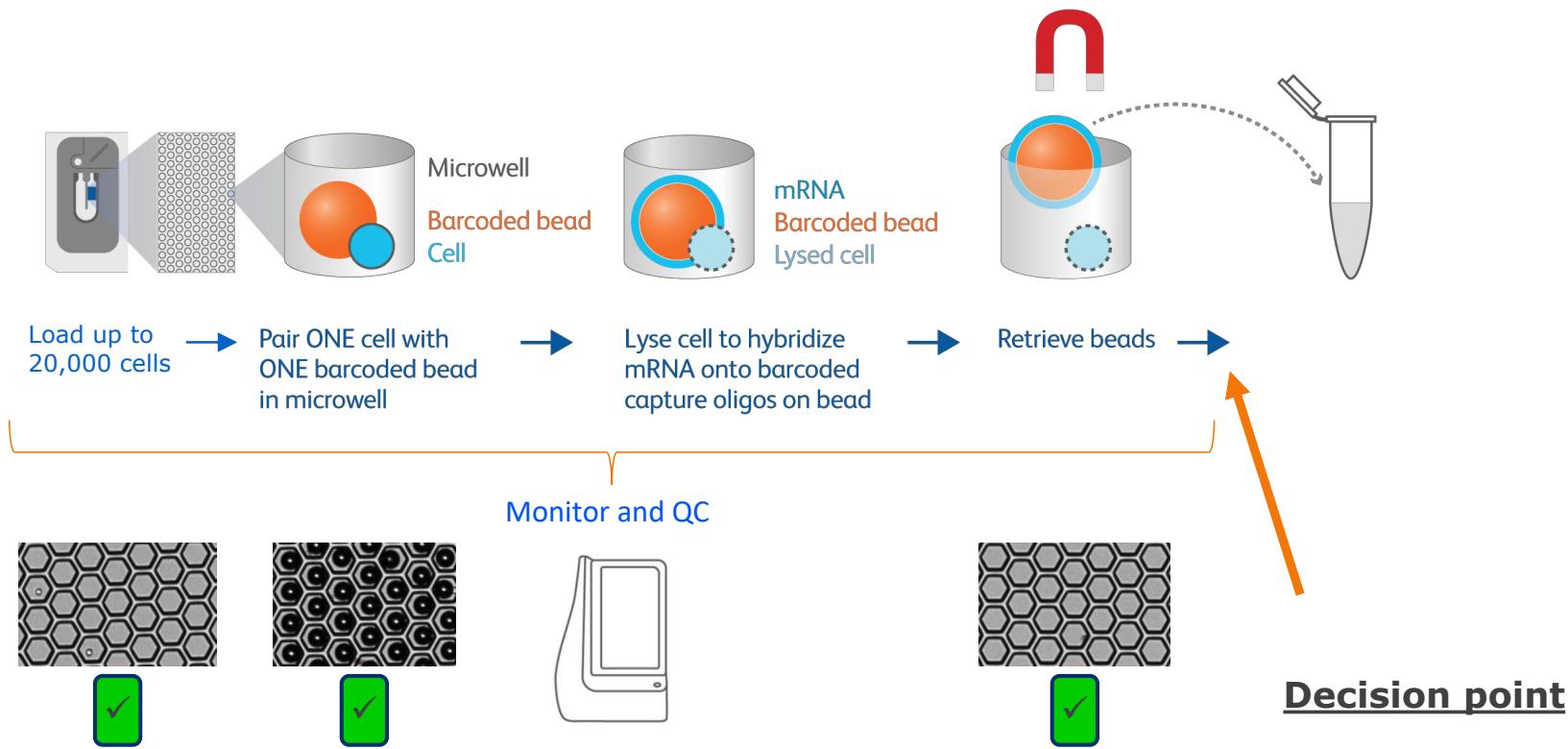


BD Rhapsody - microwell cartridge workflow

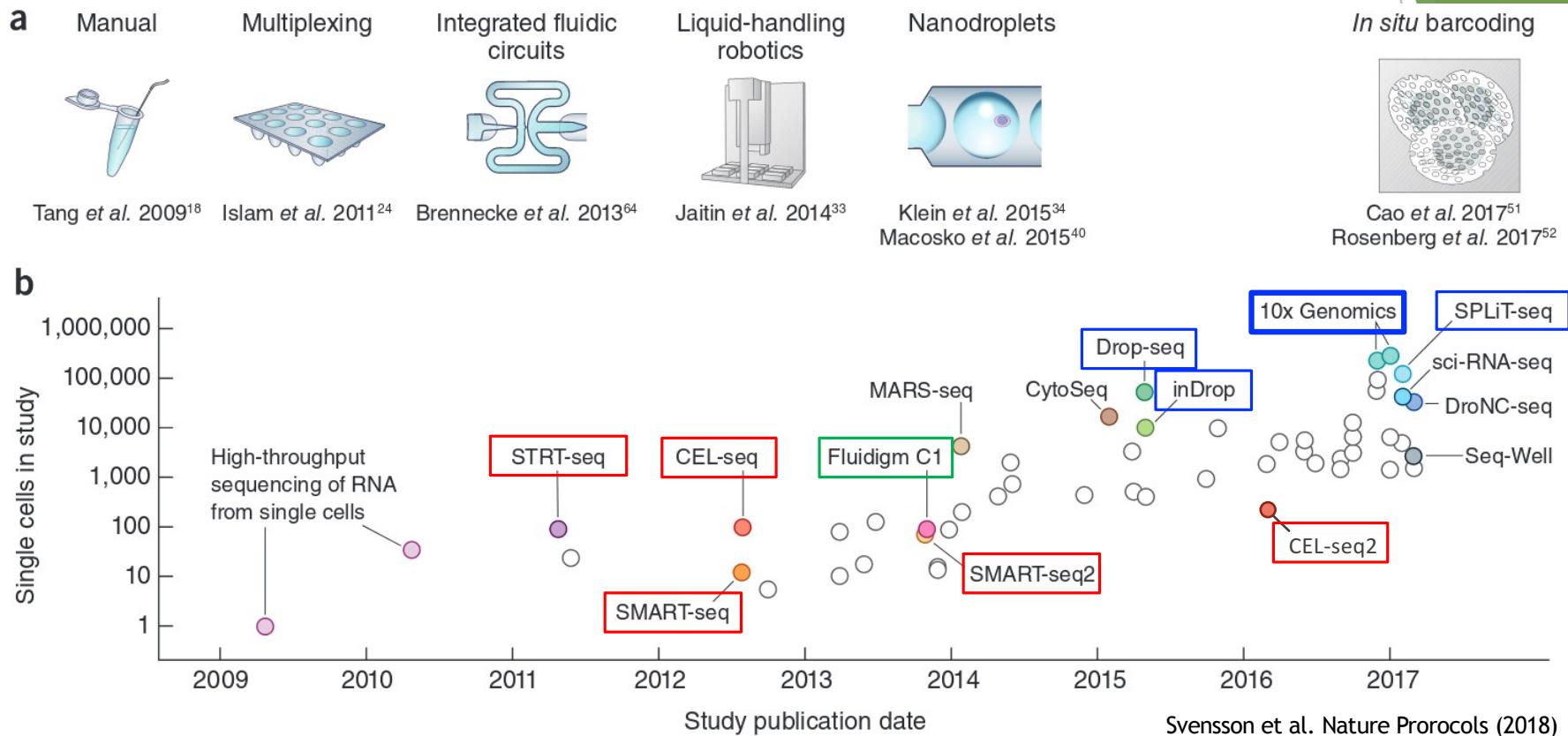
Retrieval of beads



BD Rhapsody Cartridge workflow



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Conclusion

- **General introduction about droplet-based scRNAseq**
 - Timeline of scRNAseq
- **Different types of droplet-based scRNAseq**
 - Drop-seq, InDrop, SPLiT-seq, BD Rhapsody, (10X Genomics)
- **Workflow of different droplet-based scRNAseq**
 - Single cell suspension, Primer synthesis (barcode, UMI, SP), Microfluidic setup, Cell lysis, RNA capture, cDNA amplification, Library preparation