

Droplet-based single cell RNA sequencing (scRNaseq)

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Erasmus MC, Group leader

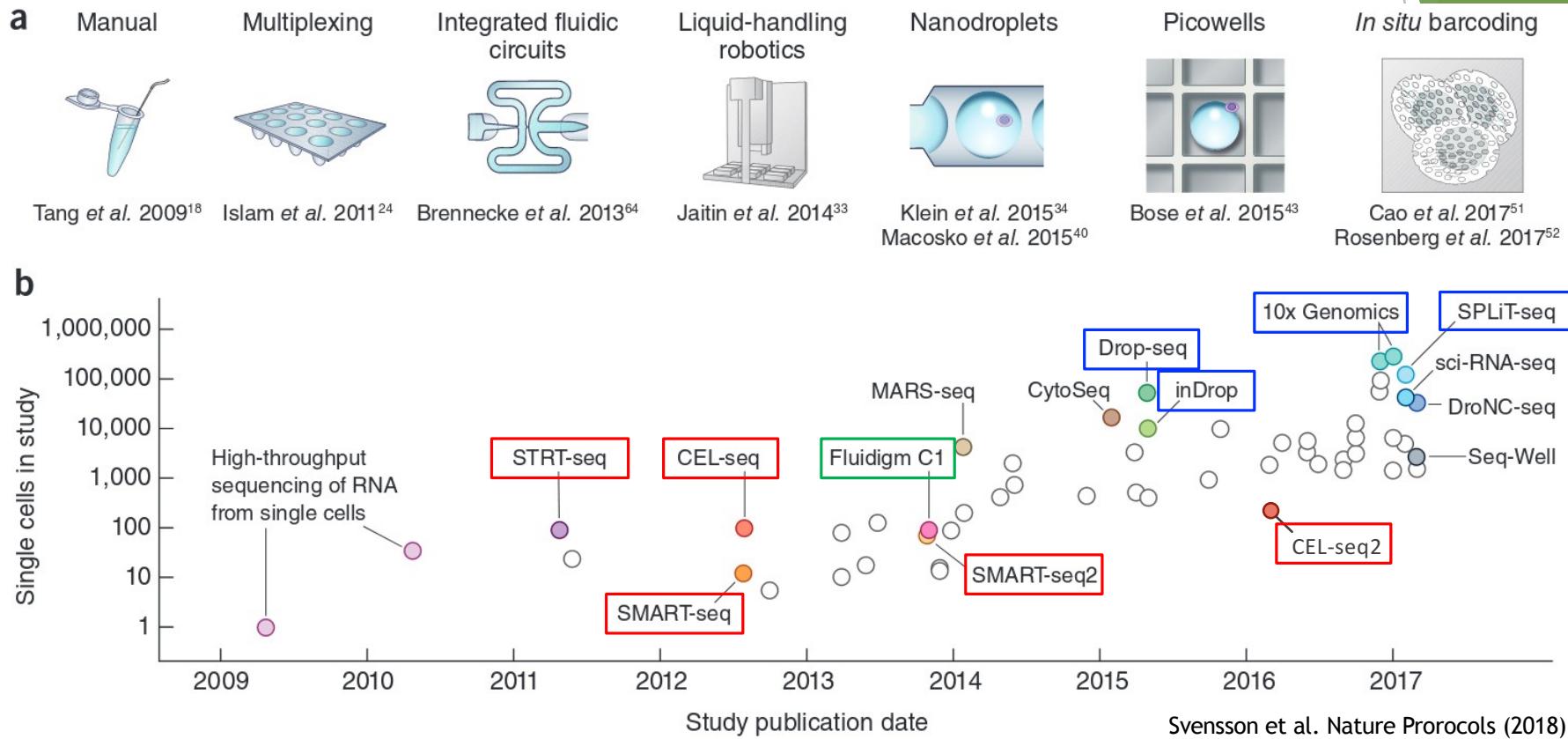
(Single cell technology for cancer biology)

2019 Single Cell Analysis Workshop, 2019/10/14

Outline

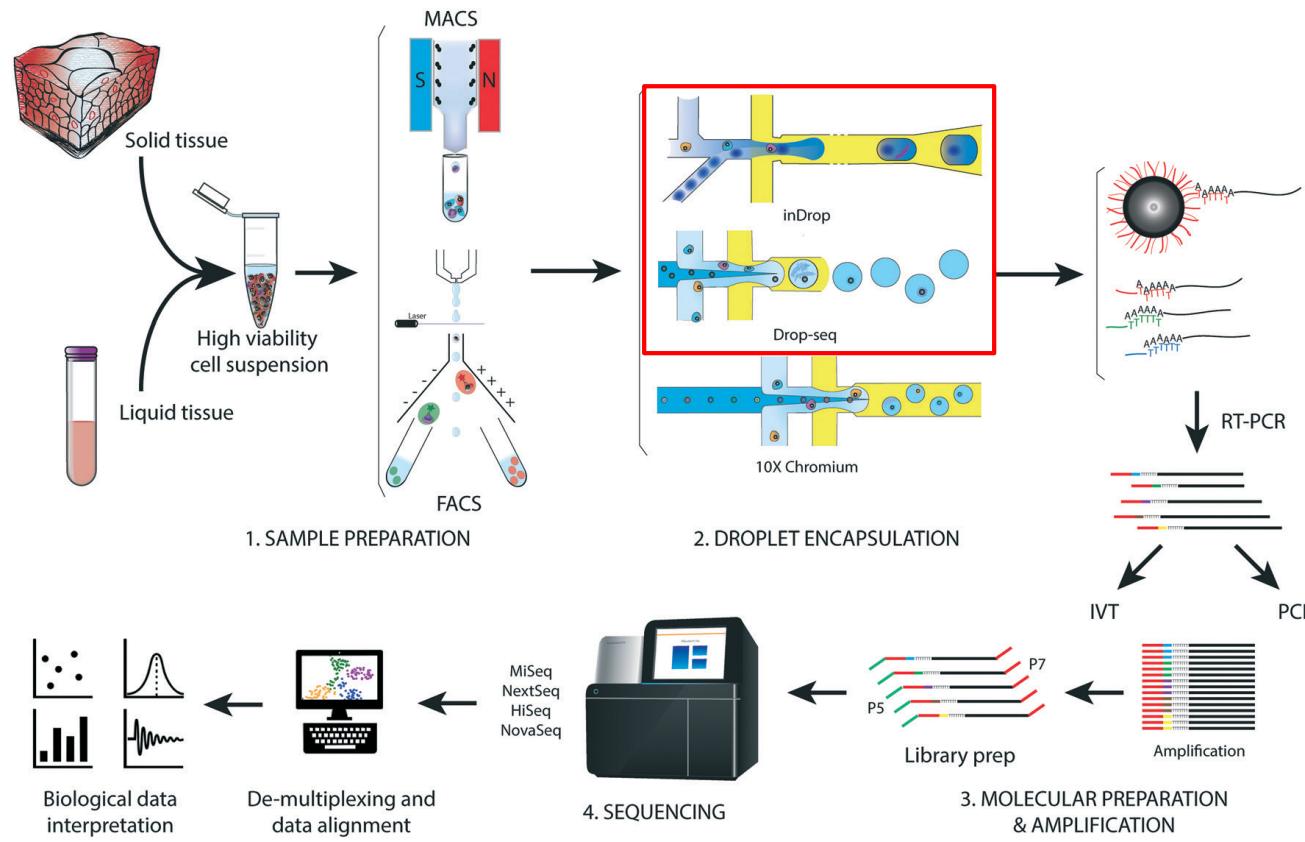
- General introduction about scRNAseq evolution
- Different types of droplet-based scRNAseq
- Workflow of different droplet-based scRNAseq

Evolution of scRNAseq techniques



- ~100s cells thanks to **multiplexing (barcode)**; ~1,000 cells thanks to **fluidics**
- ~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





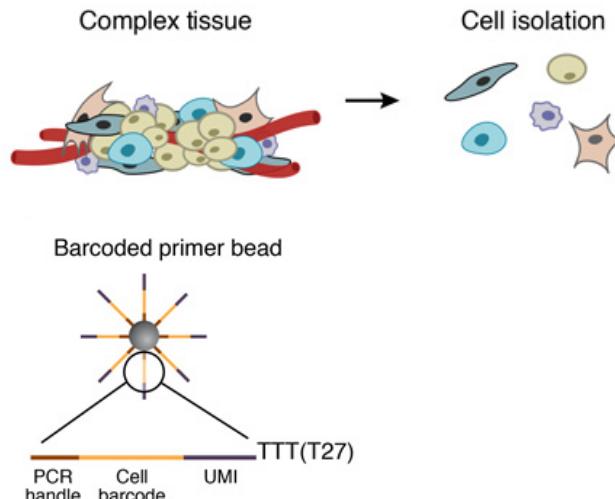
What's the difference between DropSeq and InDrop?

DropSeq workflow

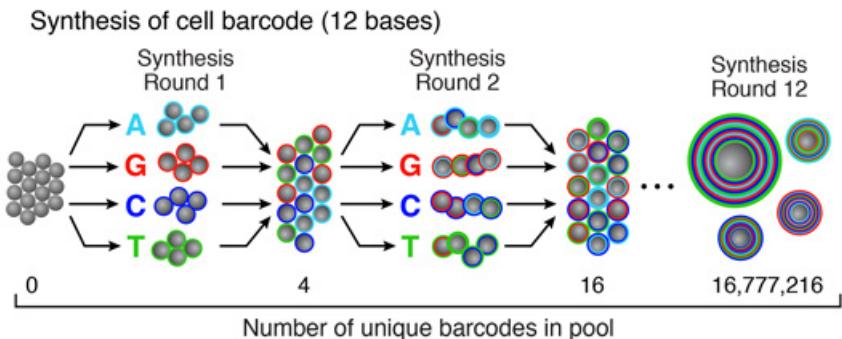
1. PREPARE A SINGLE-CELL SUSPENSION FROM A COMPLEX TISSUE

2. PRIMER SYNTHESIS

Sequence of primers on the microparticle

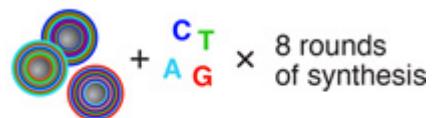


Split-and-pool synthesis of the cell barcode



Synthesis of a unique molecular identifier (UMI)

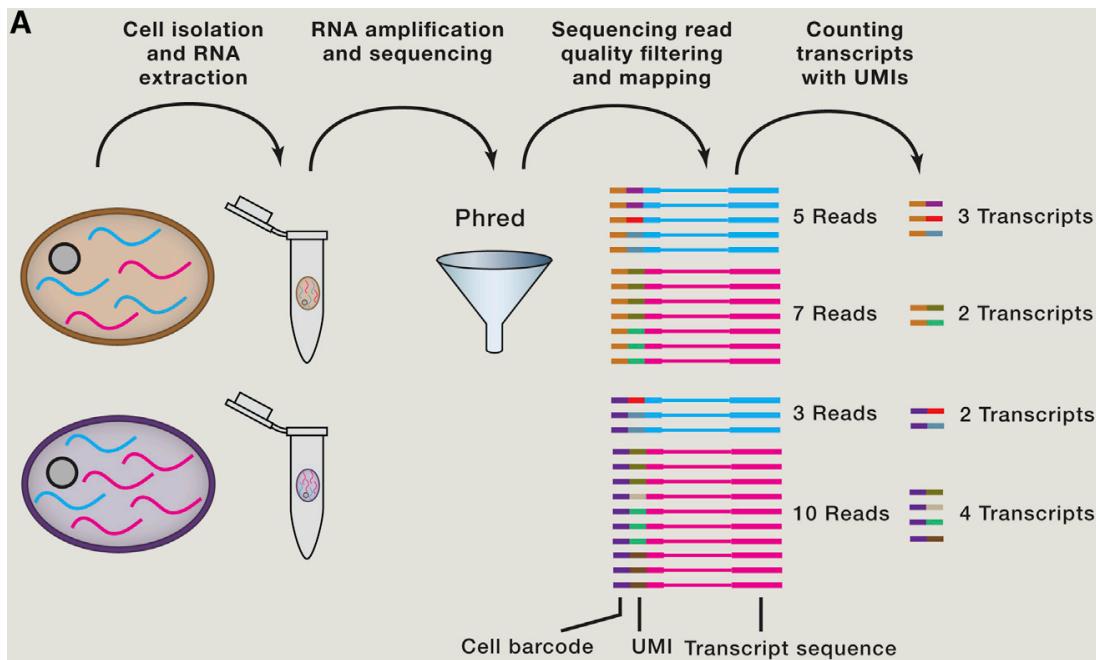
Synthesis of UMI (8 bases)



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

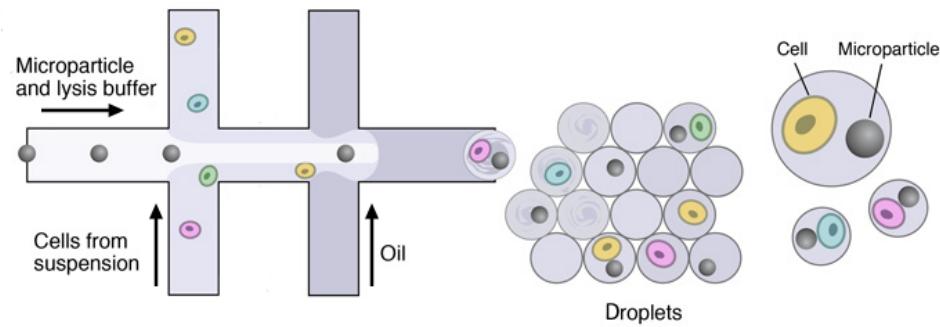
Cell, 2015, 161, 1202-1214

Quantification of mRNA Expression with unique molecular identifier UMIs

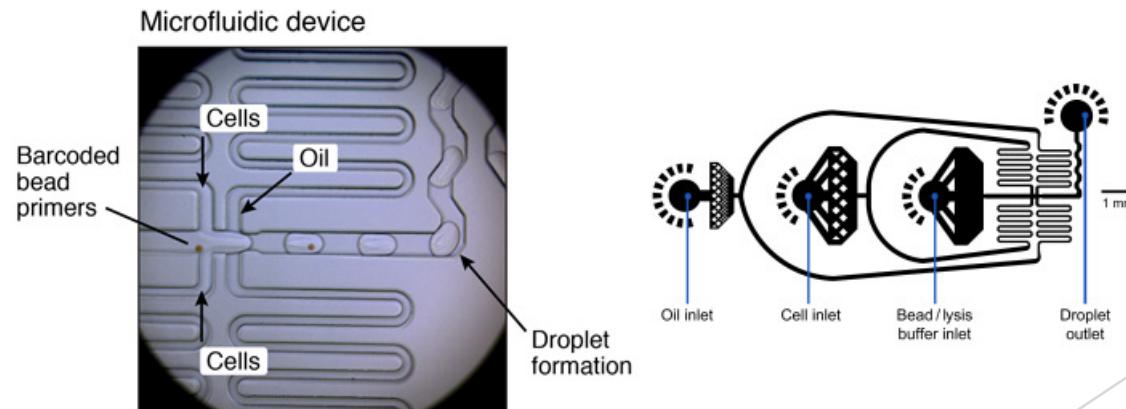


DropSeq workflow

3. MICROFLUIDIC DEVICE



Microfluidic setup

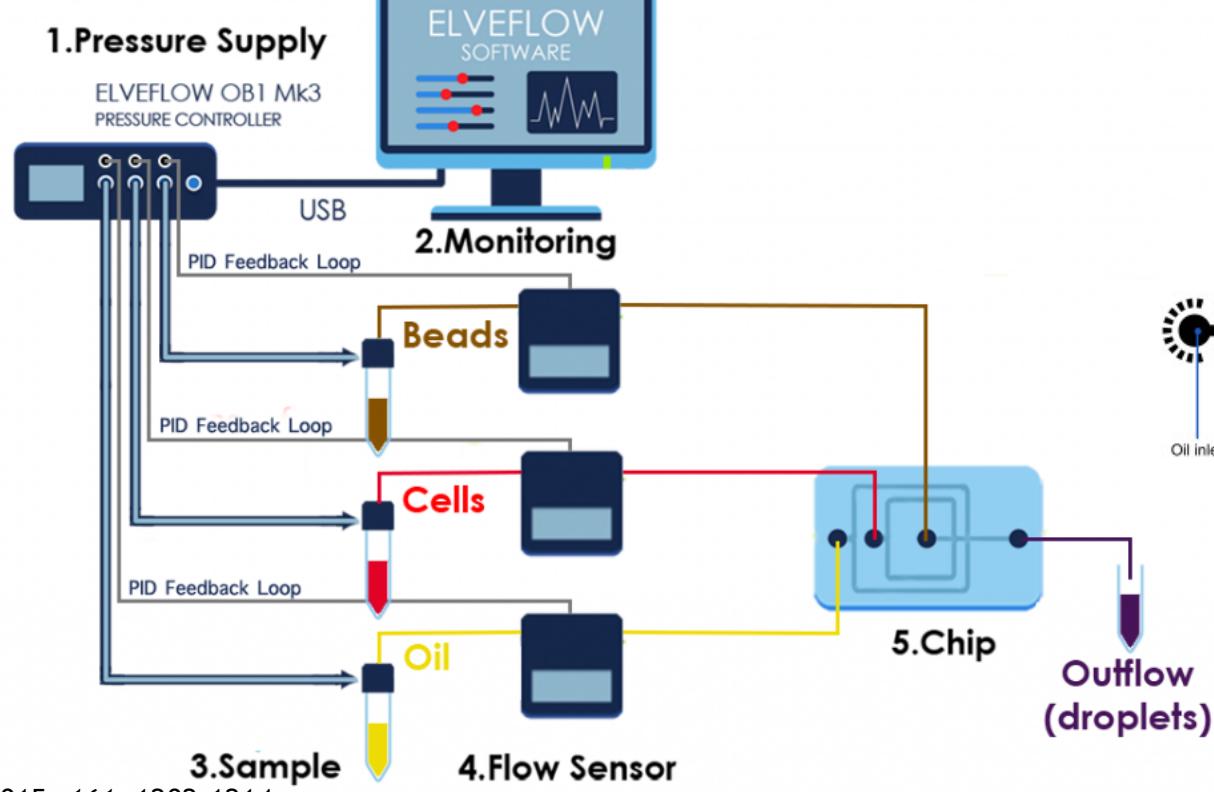


Cell, 2015, 161, 1202-1214

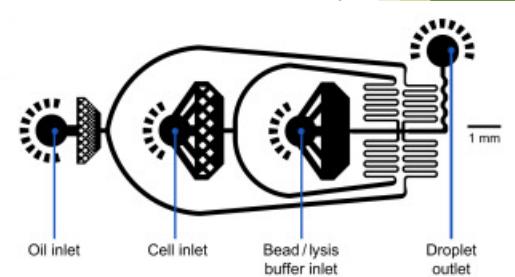
DropSeq workflow

3. MICROFLUIDIC DEVICE

Set-up Diagram

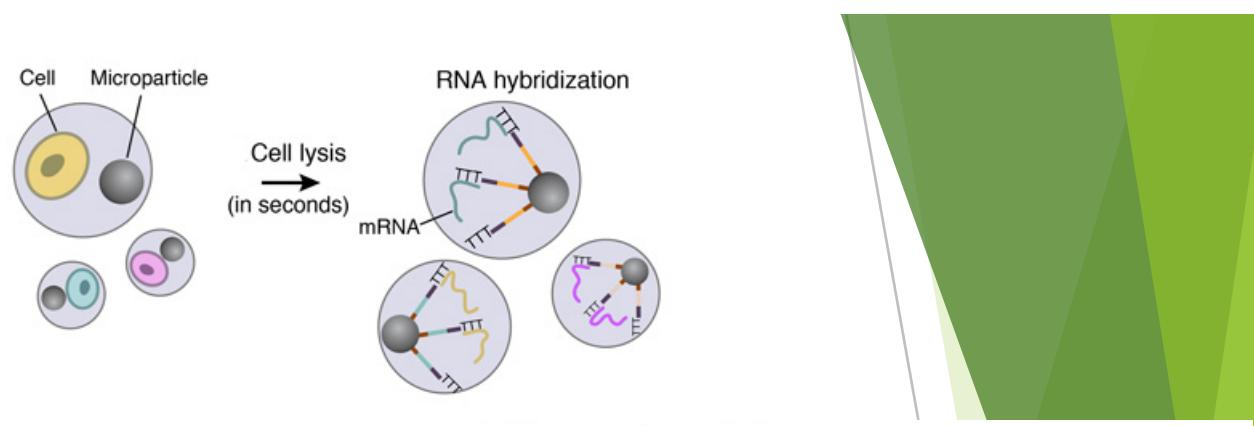


Cell, 2015, 161, 1202-1214



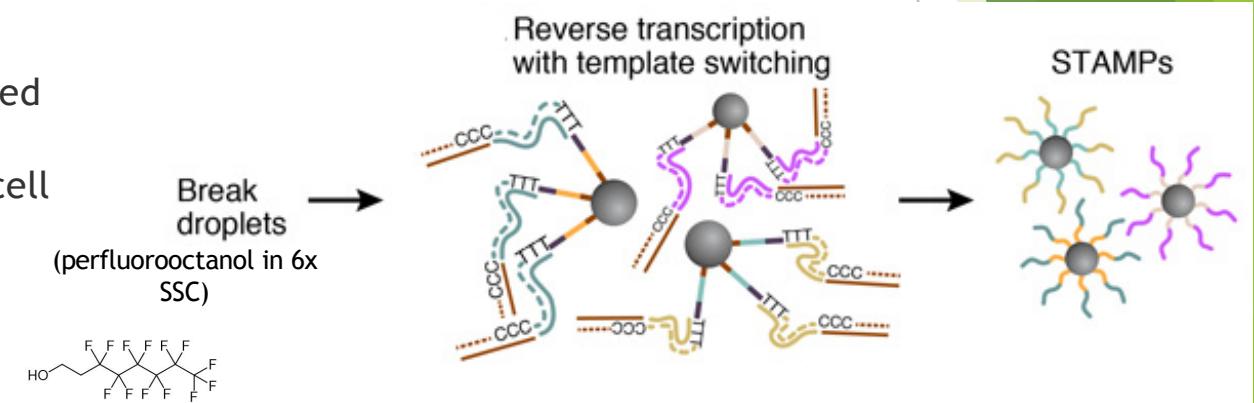
DropSeq workflow

4. CELL LYSIS AND RNA HYBRIDIZATION



5. STAMPS GENERATION

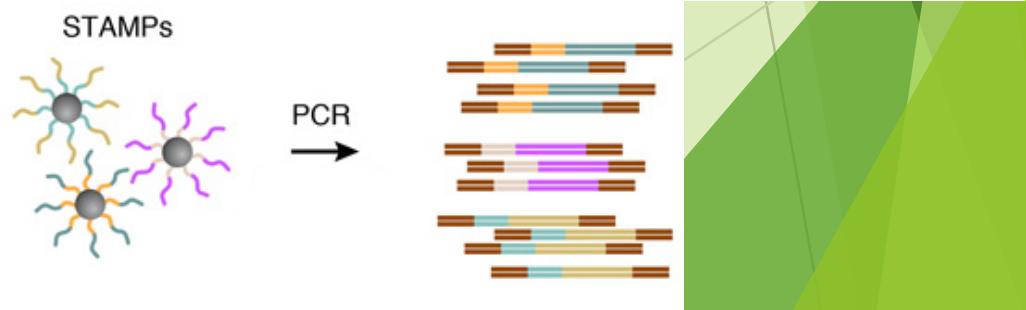
The mRNAs are then reverse-transcribed into cDNAs together in one reaction, forming a set of beads called “single-cell transcriptomes attached to microparticles” (STAMPs).



6. AMPLIFICATION OF STAMPS

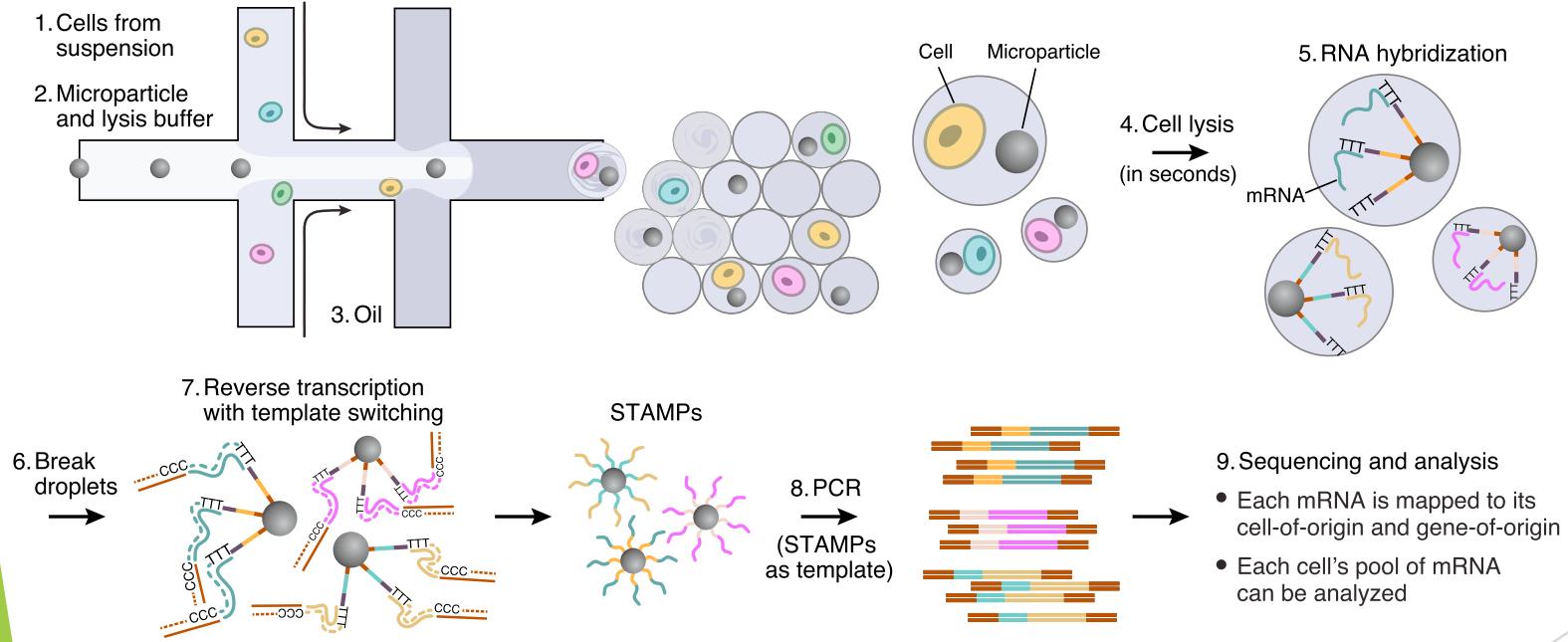
The barcoded STAMPs can then be amplified in pools by PCR reaction for high-throughput mRNA-sequencing, to analyze any desired number of individual cells.

Cell, 2015, 161, 1202-1214

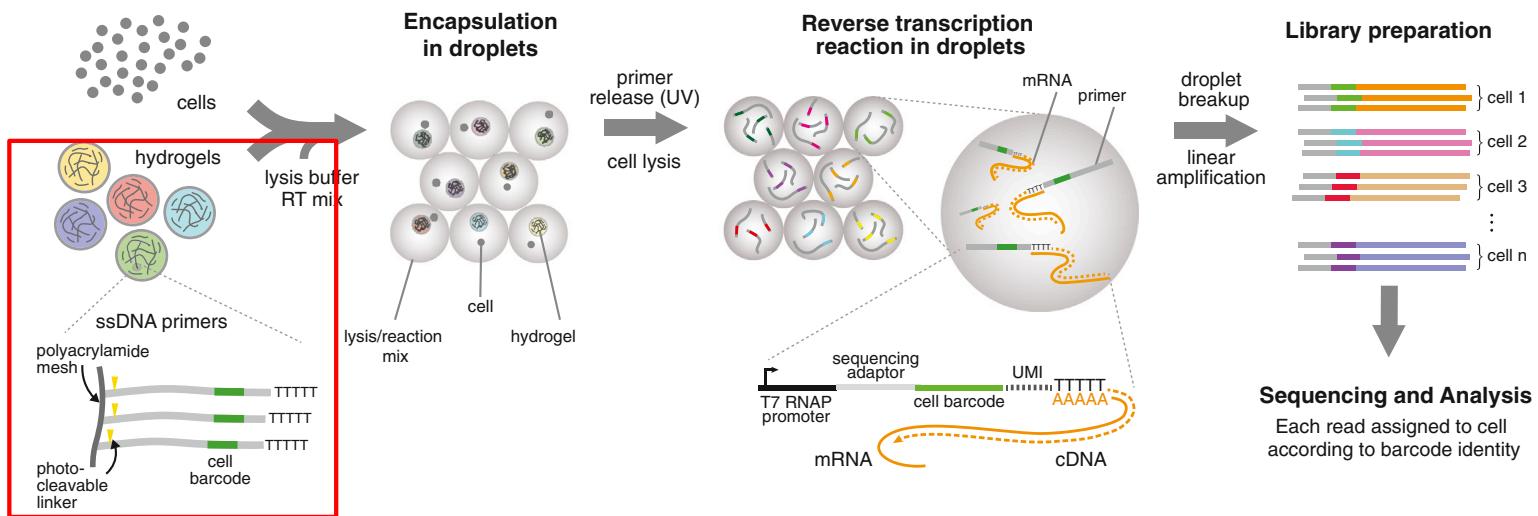


DropSeq overview

A



InDrop overview



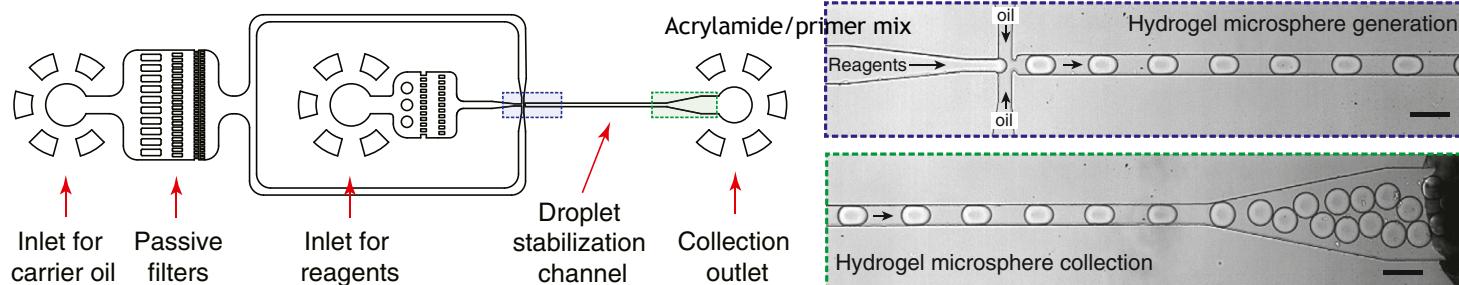
Cell, 2015, 161, 1187-1201

1CELLBIO

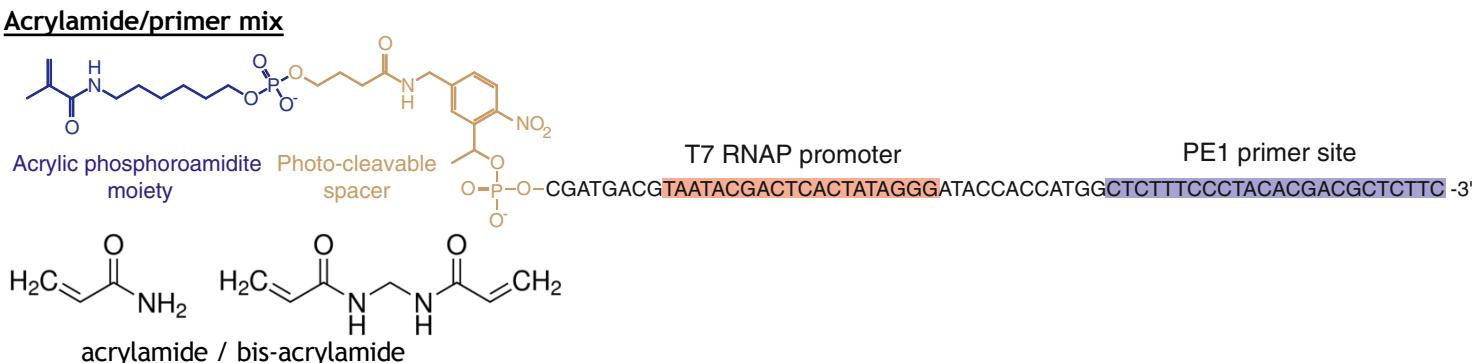
I) Synthesis of barcoding hydrogel beads



A



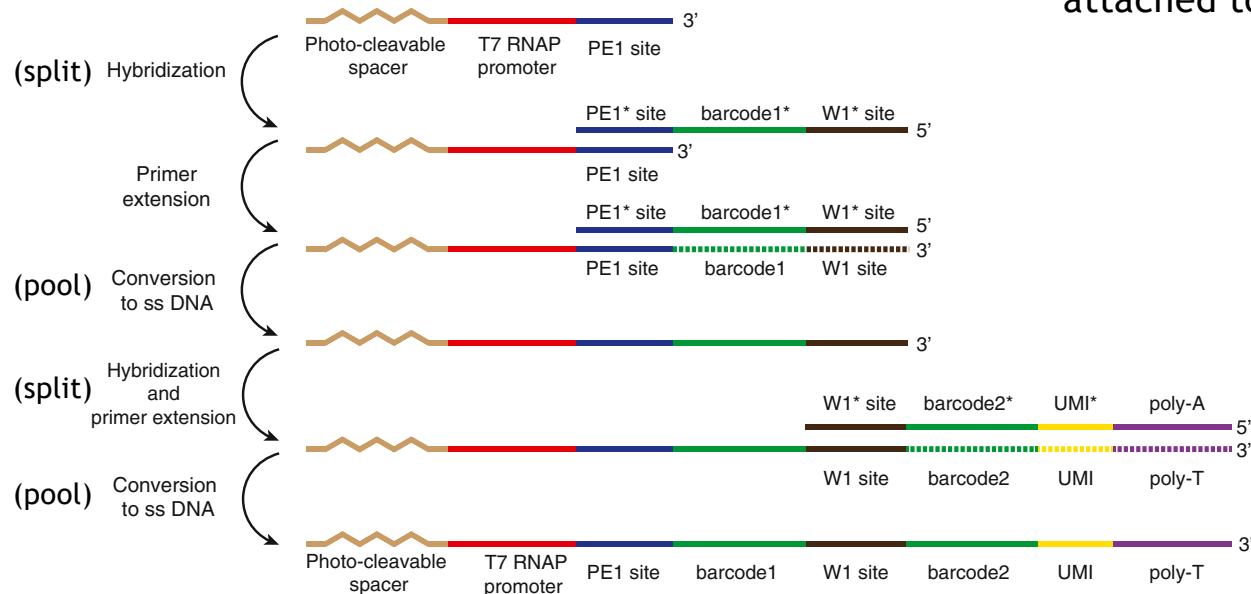
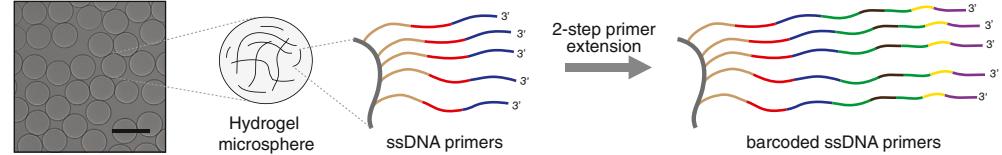
B



(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 µm.

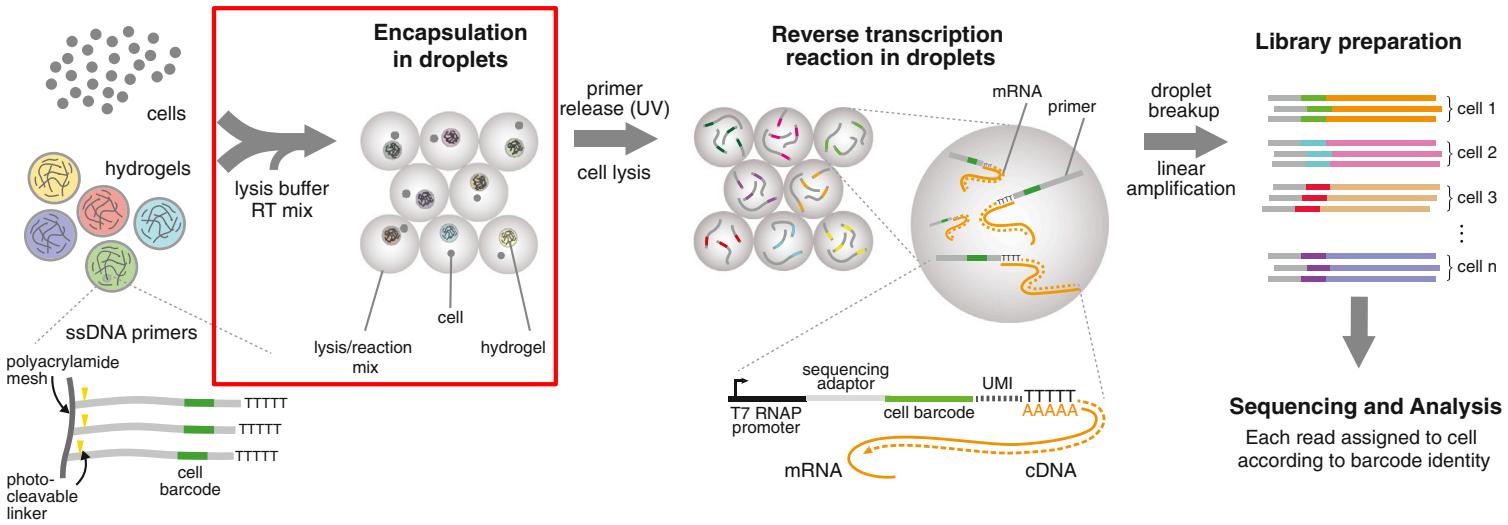
I) Synthesis of barcoding hydrogel beads

c)



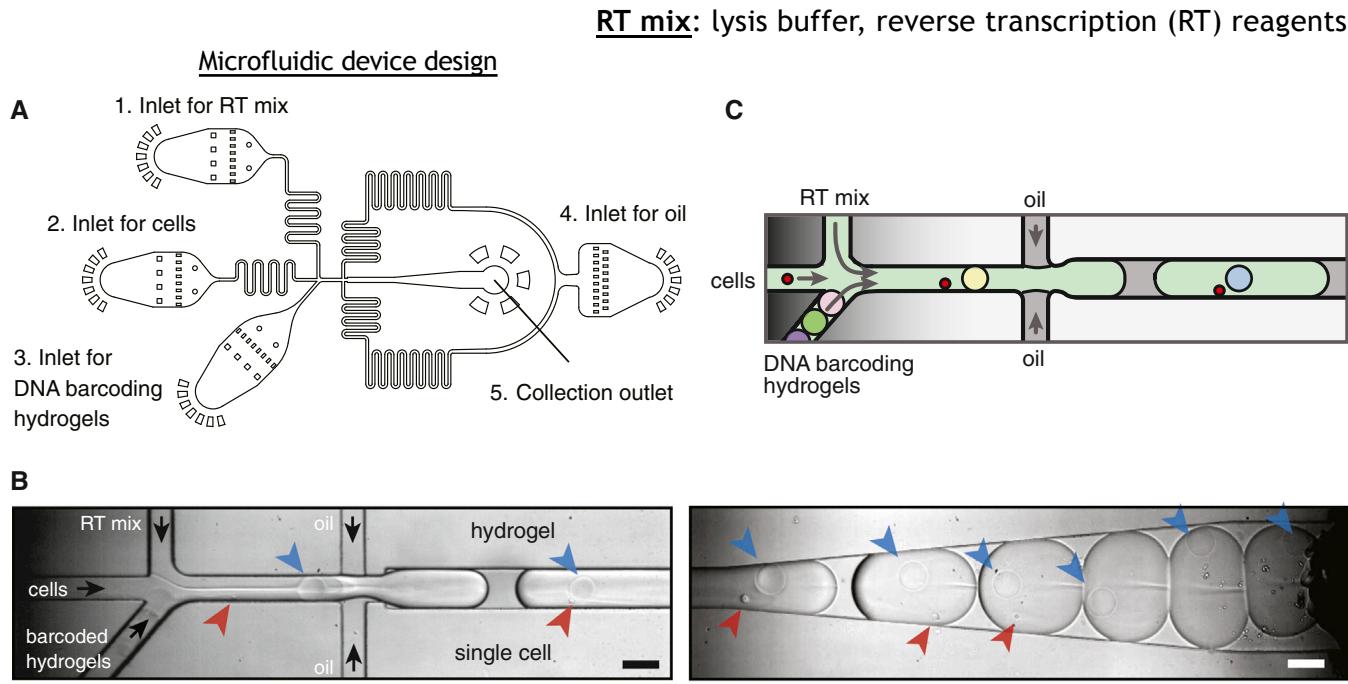
C) Primer extension reaction to incorporate barcode sequences into DNA oligonucleotides attached to hydrogel beads

InDrop overview



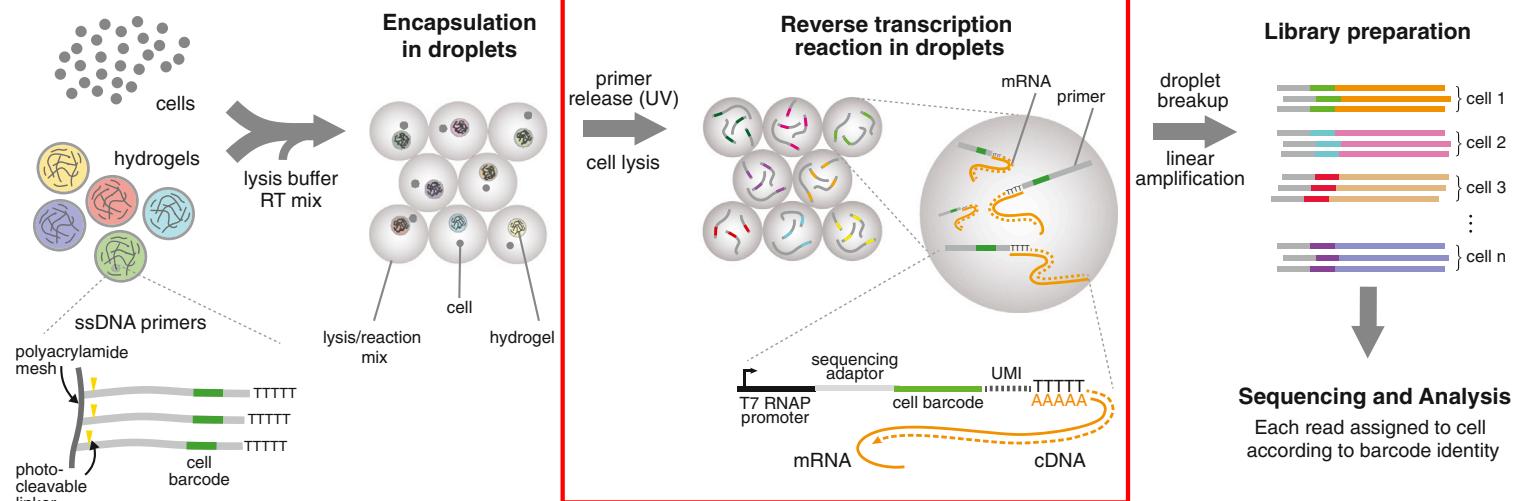
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II) Encapsulation in droplets



Cells (red), hydrogels (blue), and flow direction (black). Scale bars 100 um.

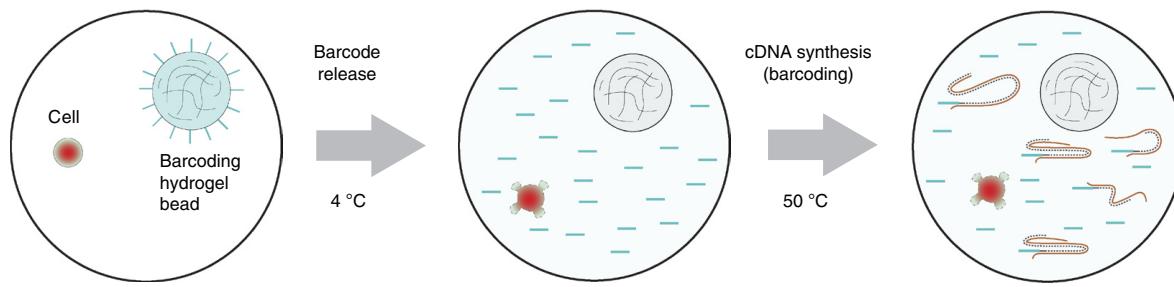
InDrop overview



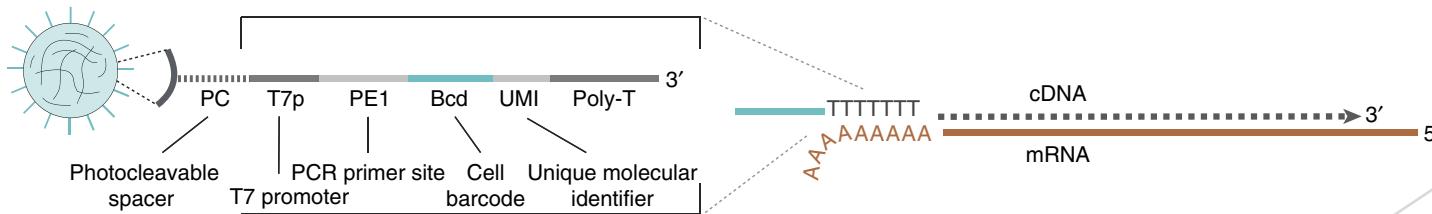
Cell, 2015, 161, 1187-1201

III) Reverse transcription in droplets

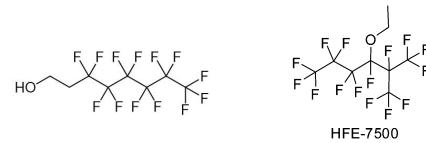
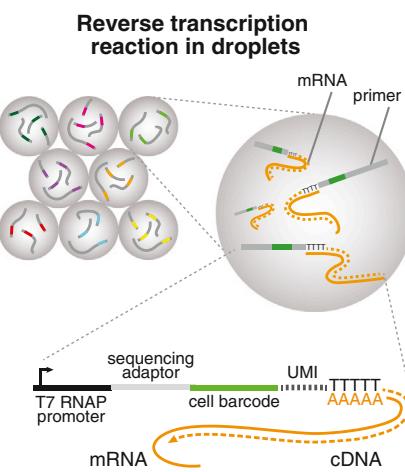
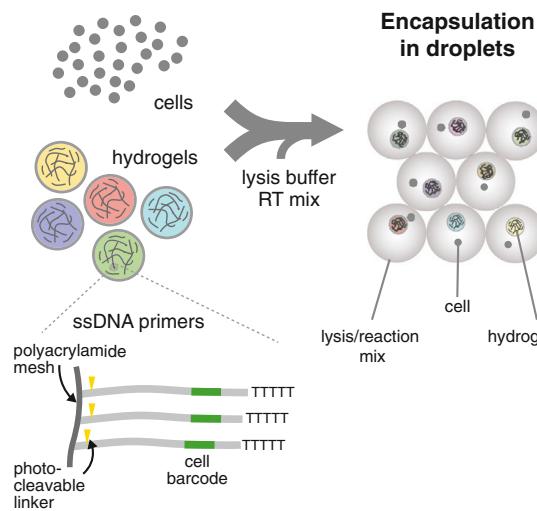
- After cell and hydrogel bead encapsulation, the barcoding cDNA primers are released from the beads using **365 nm UV light** (~10 mW/cm²; which is not damaging to DNA/RNA), followed by mRNA capture and reverse transcription



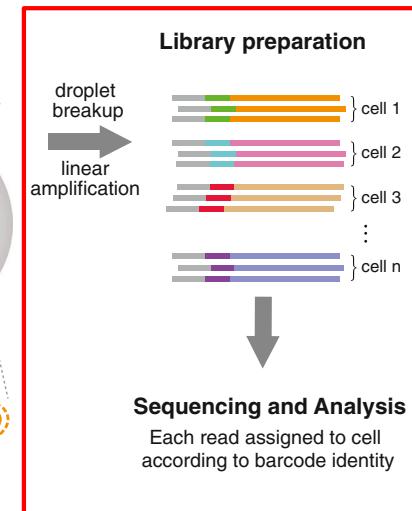
- mRNA capture and reverse transcription



InDrop overview

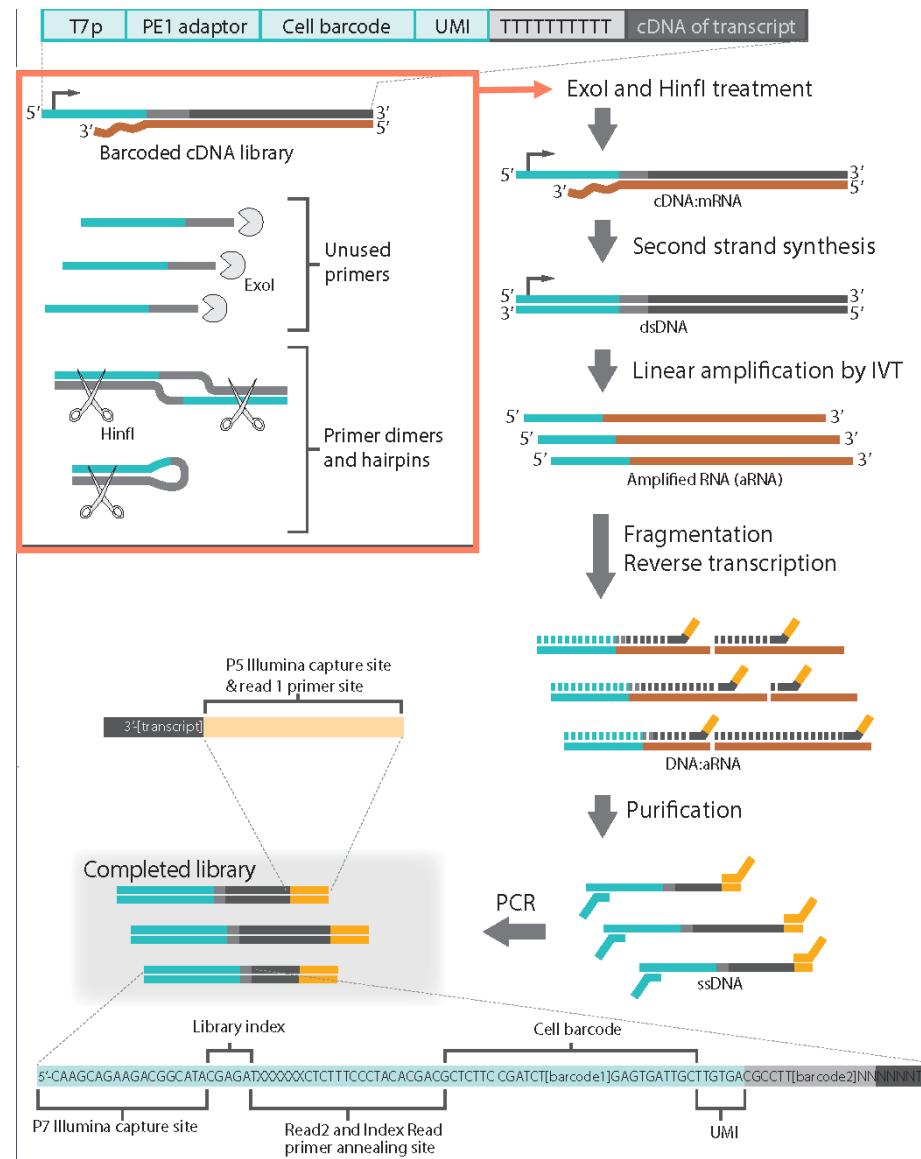


20% (v/v) perfluorooctanol + 80% (v/v) HFE-7500

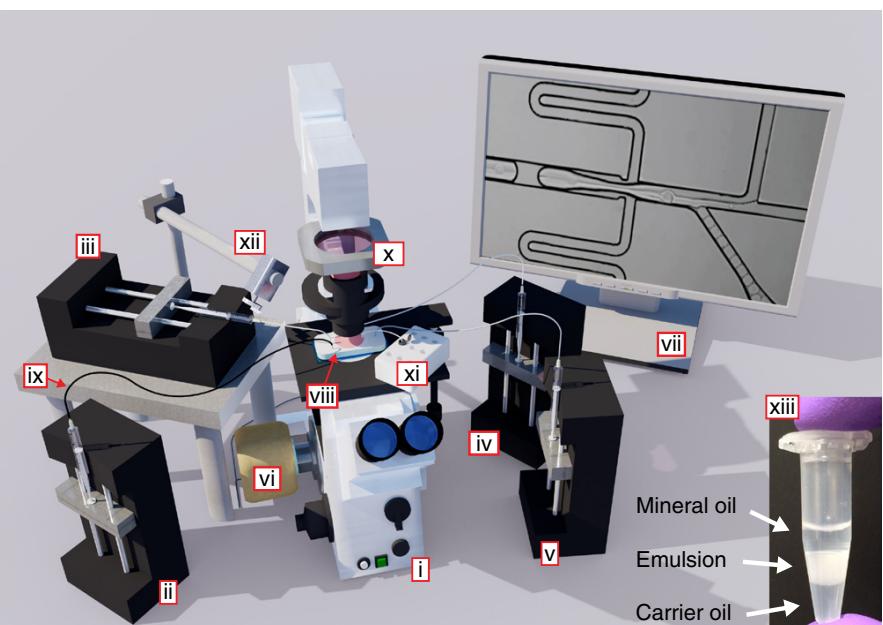


IV) InDrop library prep

- 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

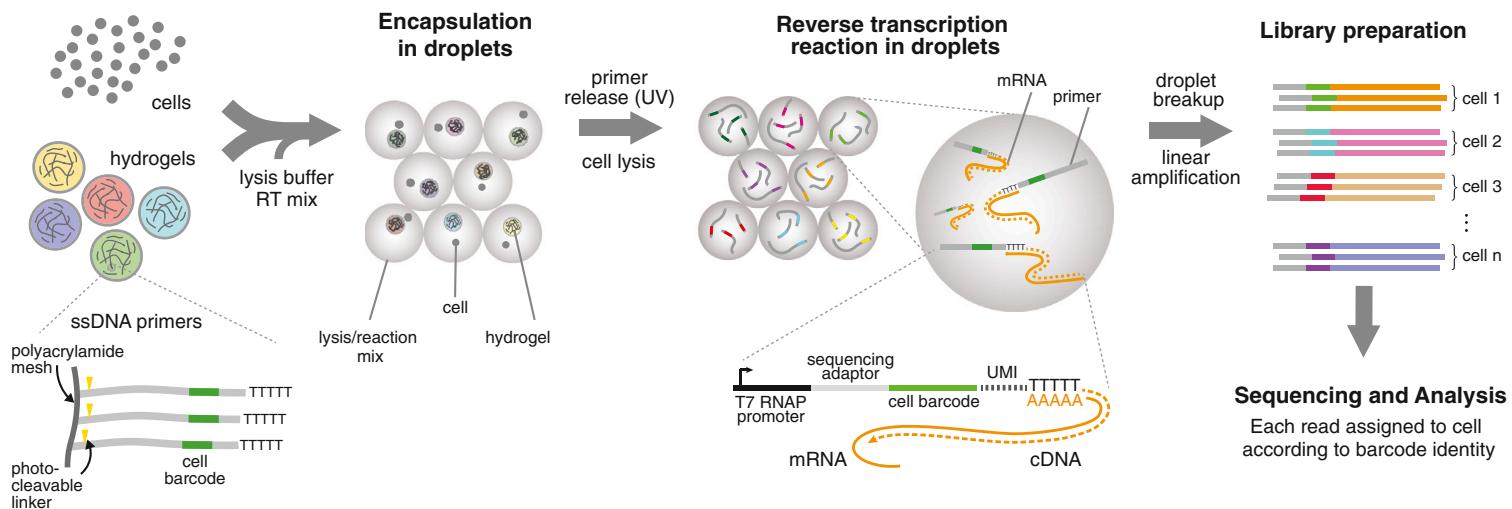


The inDrops platform



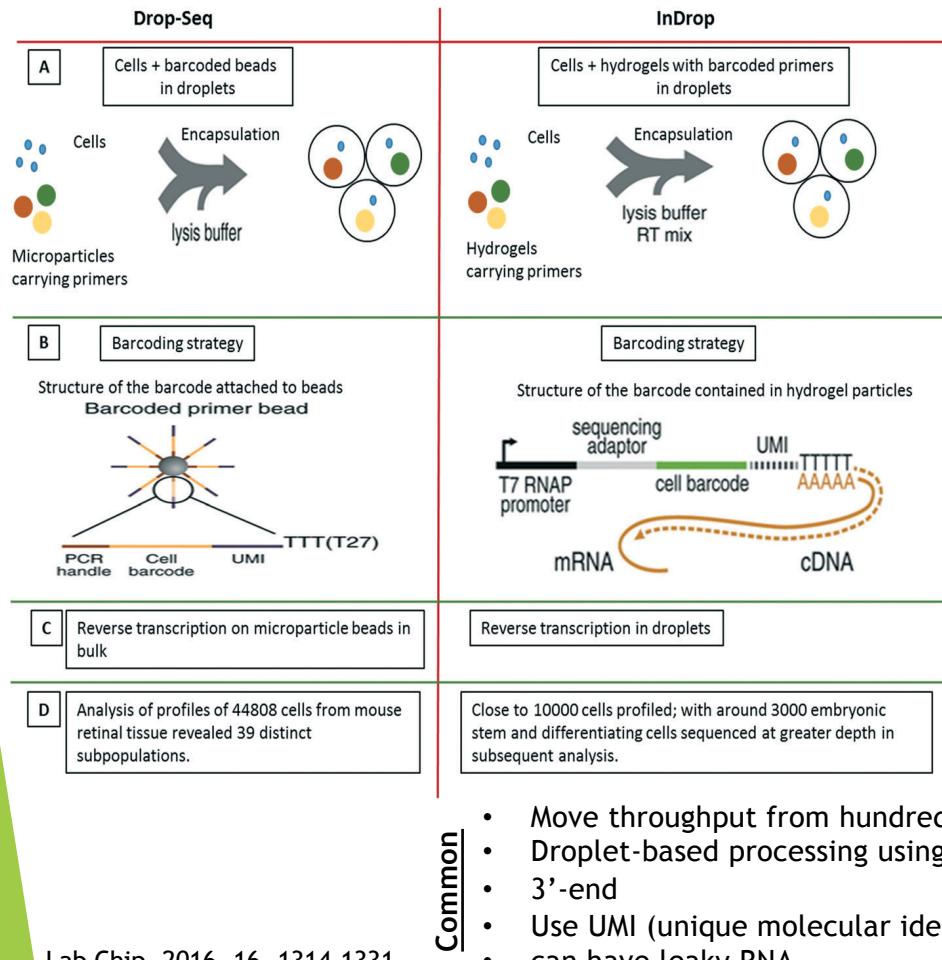
- (i) Inverted bright-field microscope;
- (ii-v) syringe pumps;
- (vi) fast speed camera;
- (vii) computer;
- (viii) microfluidic chip;
- (ix) barcoding hydrogel bead containing tubing, protected from ambient light by insertion into a second, opaque tubing, or alternatively, wrapped in aluminum foil;
- (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

InDrop single cell sequencing



- Lysis and reverse transcription occurs in the beads
- RT in droplets

DropSeq vs InDrop



Lab Chip, 2016, 16, 1314-1331

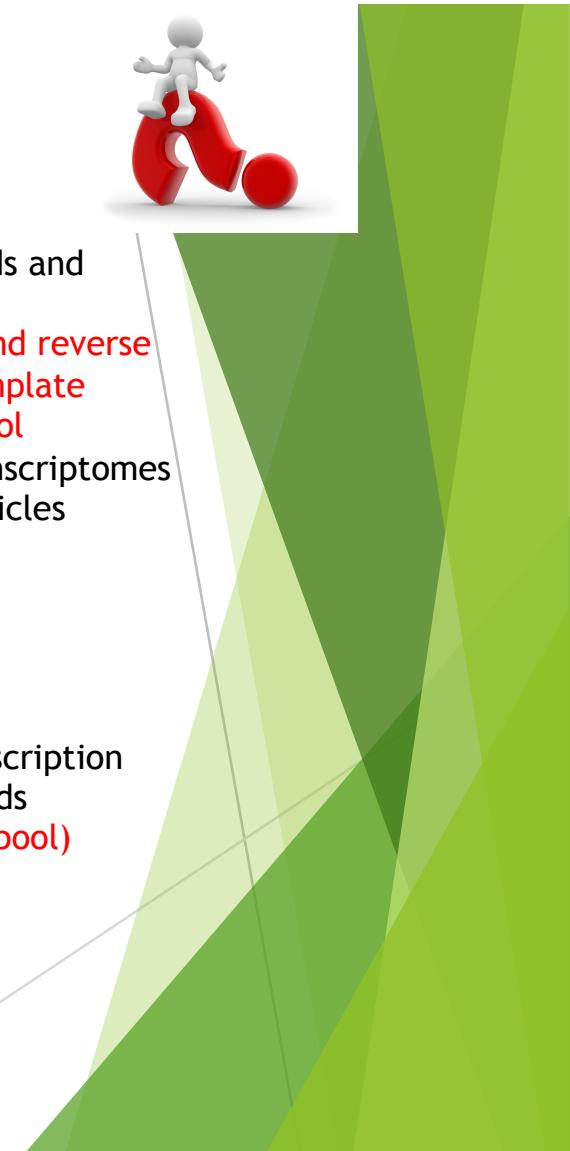
DropSeq

- Cells lysed in the beads and hybridize to primer
- Droplets are broken and reverse transcription (RT)/template switching occurs in pool
- STAMP:** single cell transcriptomes attached to microparticles

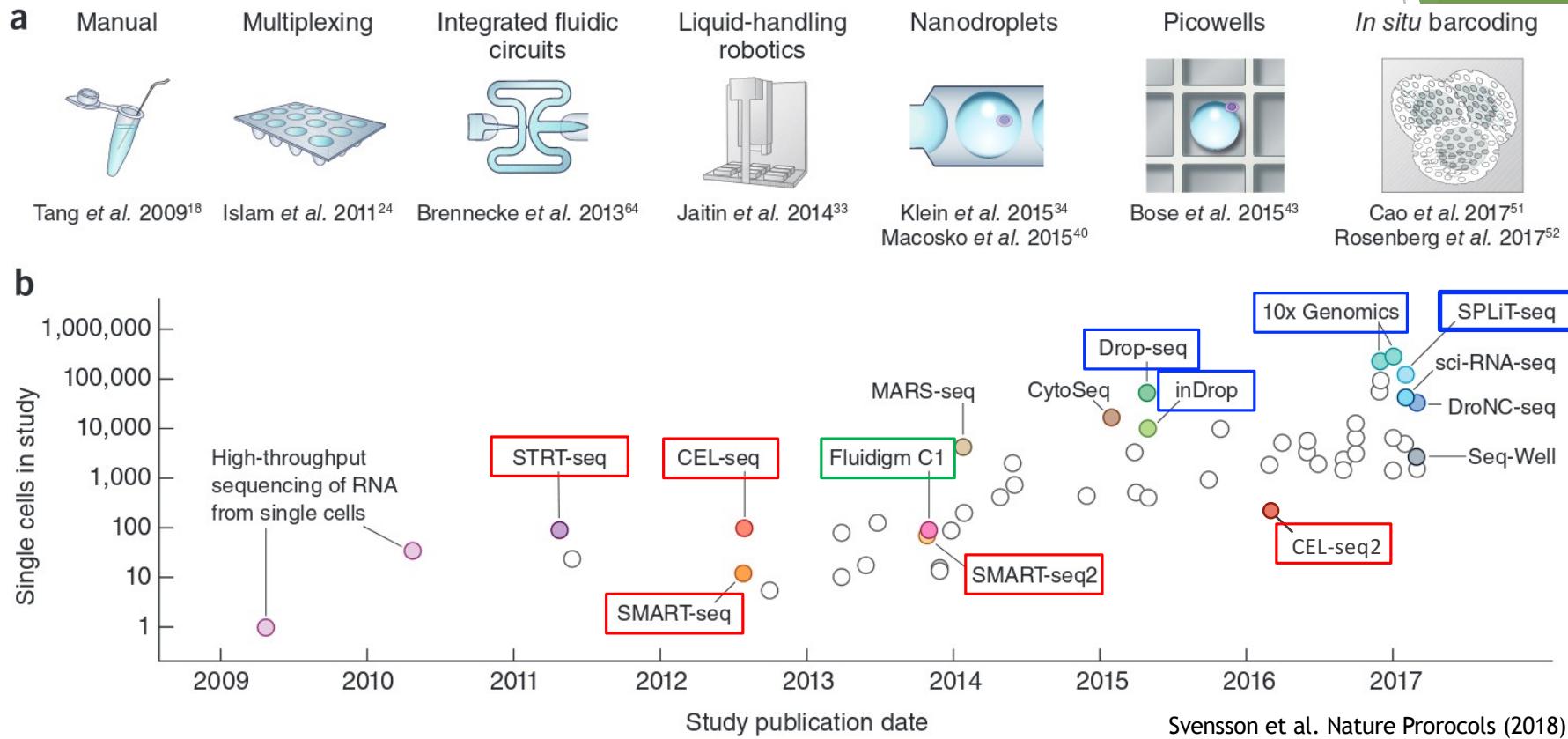


InDrop

- Lysis and reverse transcription (RT) occurs in the beads
- RT in droplets (not in pool)**



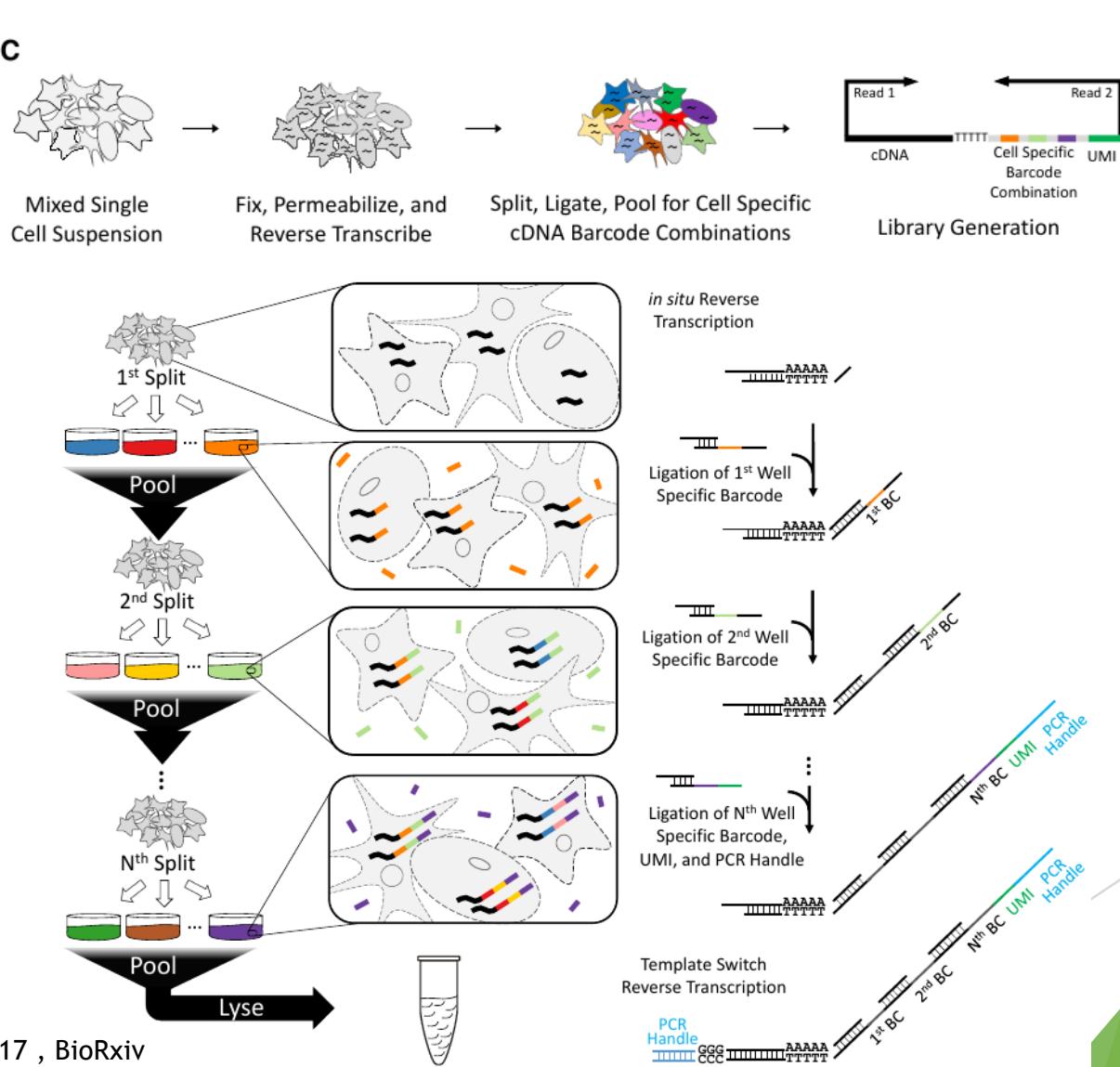
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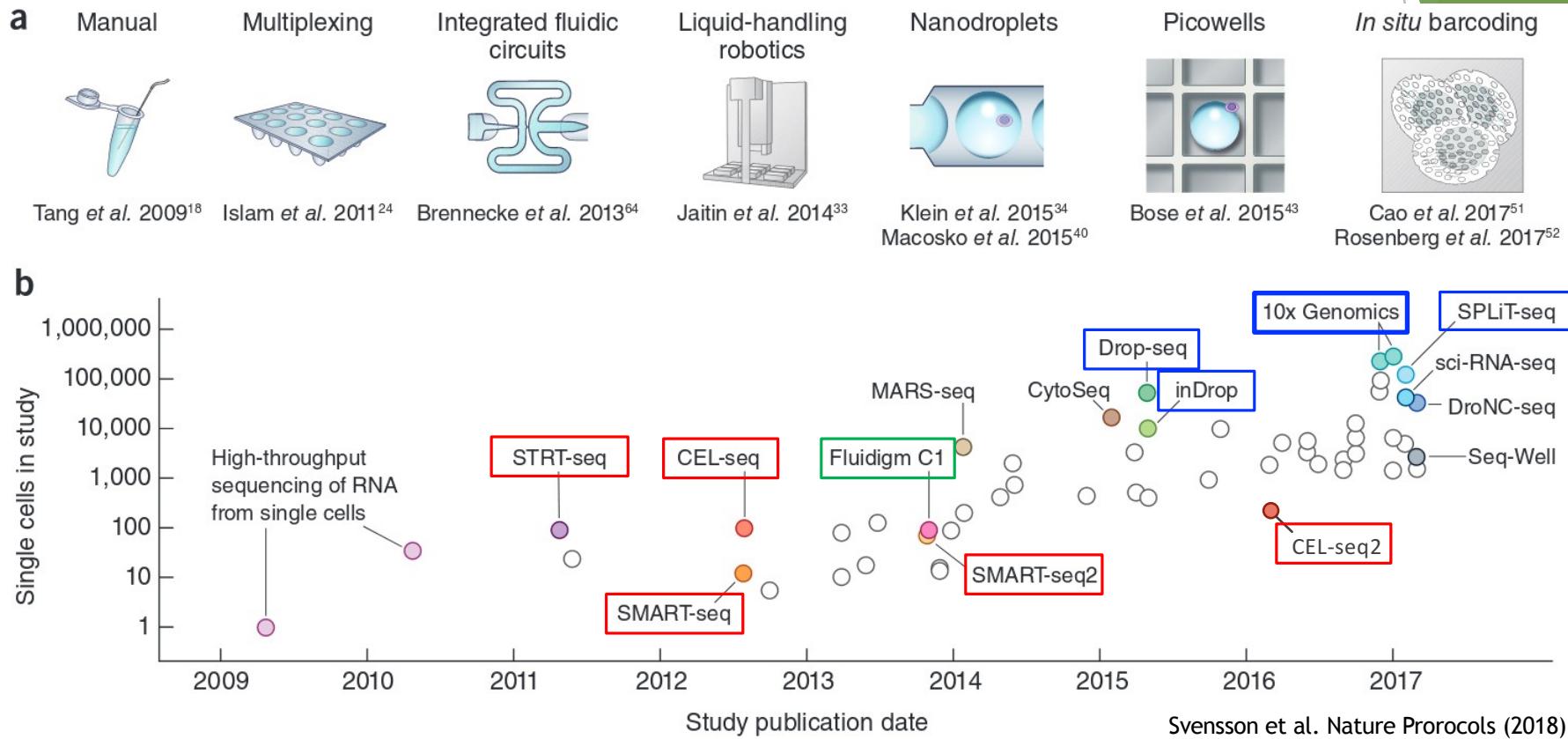
In situ barcoding

c



Rosenburg et al, 2017 , BioRxiv

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Conclusion

- **General introduction about scRNAseq evolution**
 - Timeline of scRNAseq
- **Different types of droplet-based scRNAseq**
 - Drop-seq, InDrop, SPLiT-seq, 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

