

# Single cell RNA sequencing (scRNASeq) platforms

**Miao-Ping Chien**

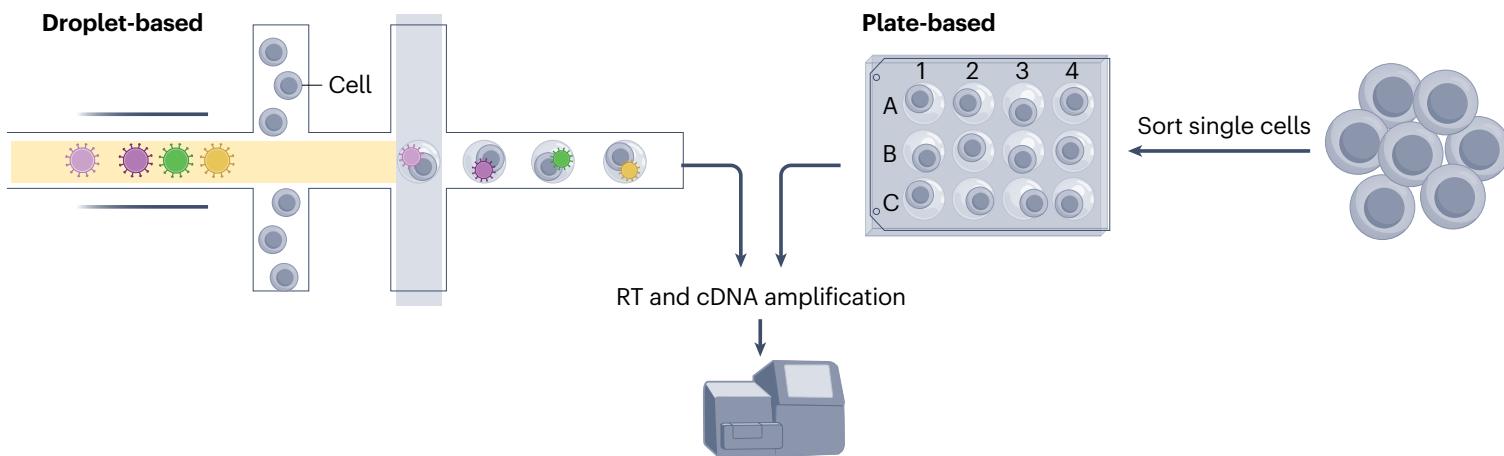
Erasmus MC, Associate professor

2024 Single Cell Analysis Workshop, 2024/10/29

# Single cell RNA sequencing (scRNASeq) platforms

Part I: Plate-based scRNASeq

Part II: Droplet-based scRNASeq



## **Outline of the Part I (Plate-based scRNASeq)**

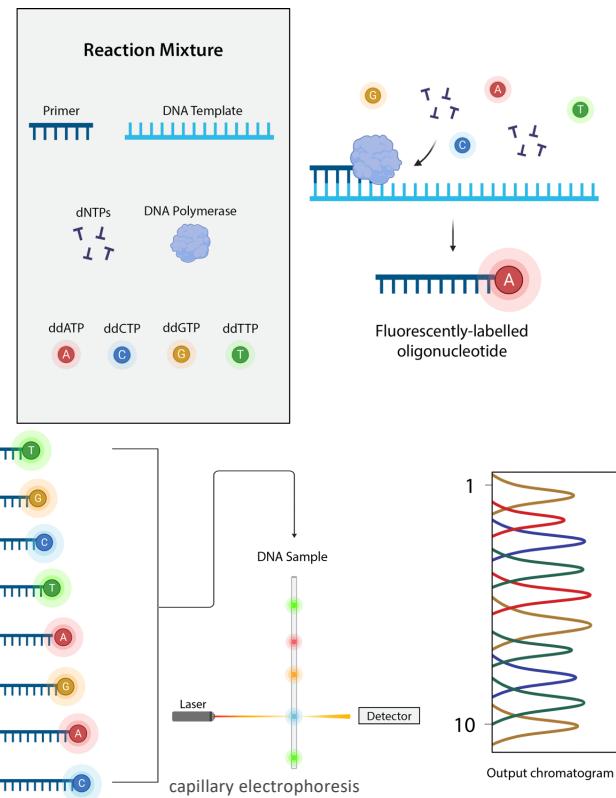
- General introduction about scRNASeq
- Different types of plate-based scRNASeq
- Workflow of different plate-based scRNASeq



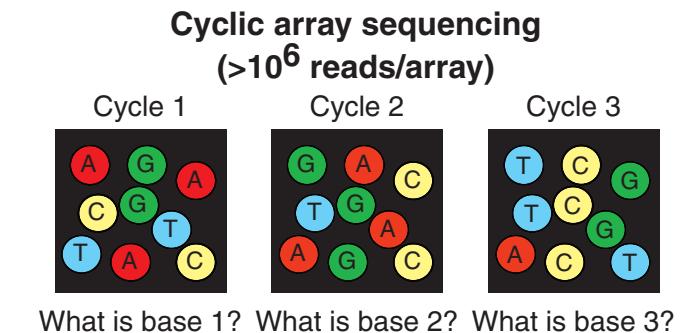
**single cell sequencing**      **?**      **=**      **next-generation sequencing**

# Traditional sequencing vs next-generation sequencing

- From sanger sequencing .....      ... to next-generation sequencing



A single DNA fragment at a time

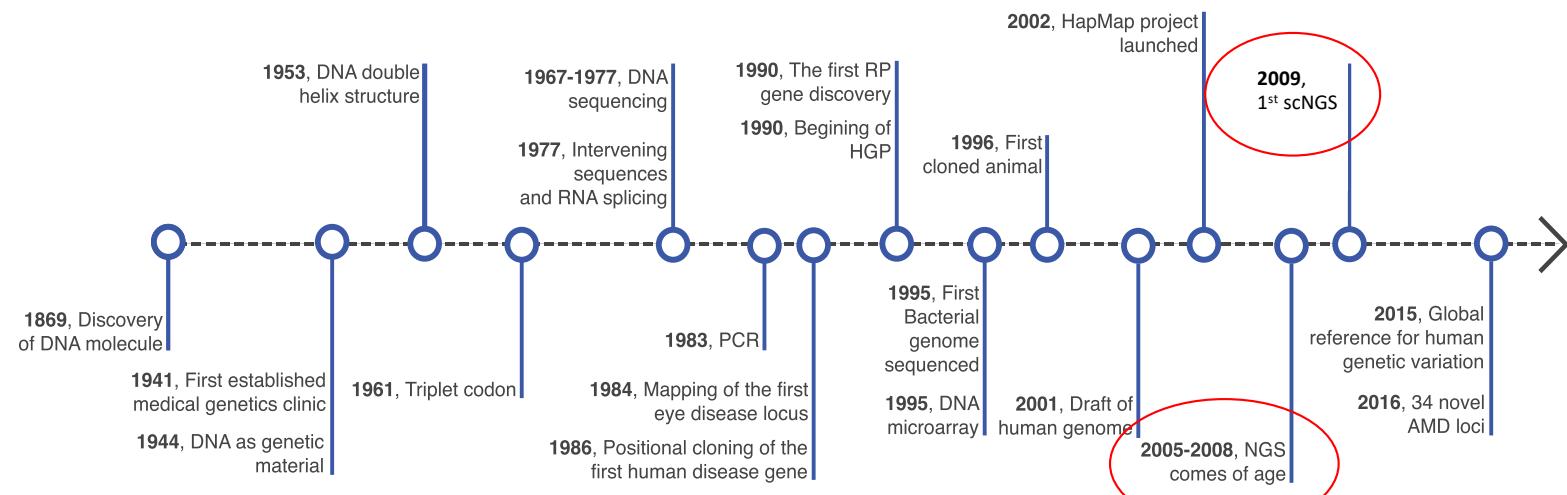


What is base 1? What is base 2? What is base 3?

- Illumina
- Roche 454
- ABI SOLiD
- ...

Millions of DNA fragments simultaneously per run

# Next-generation sequencing & single cell sequencing

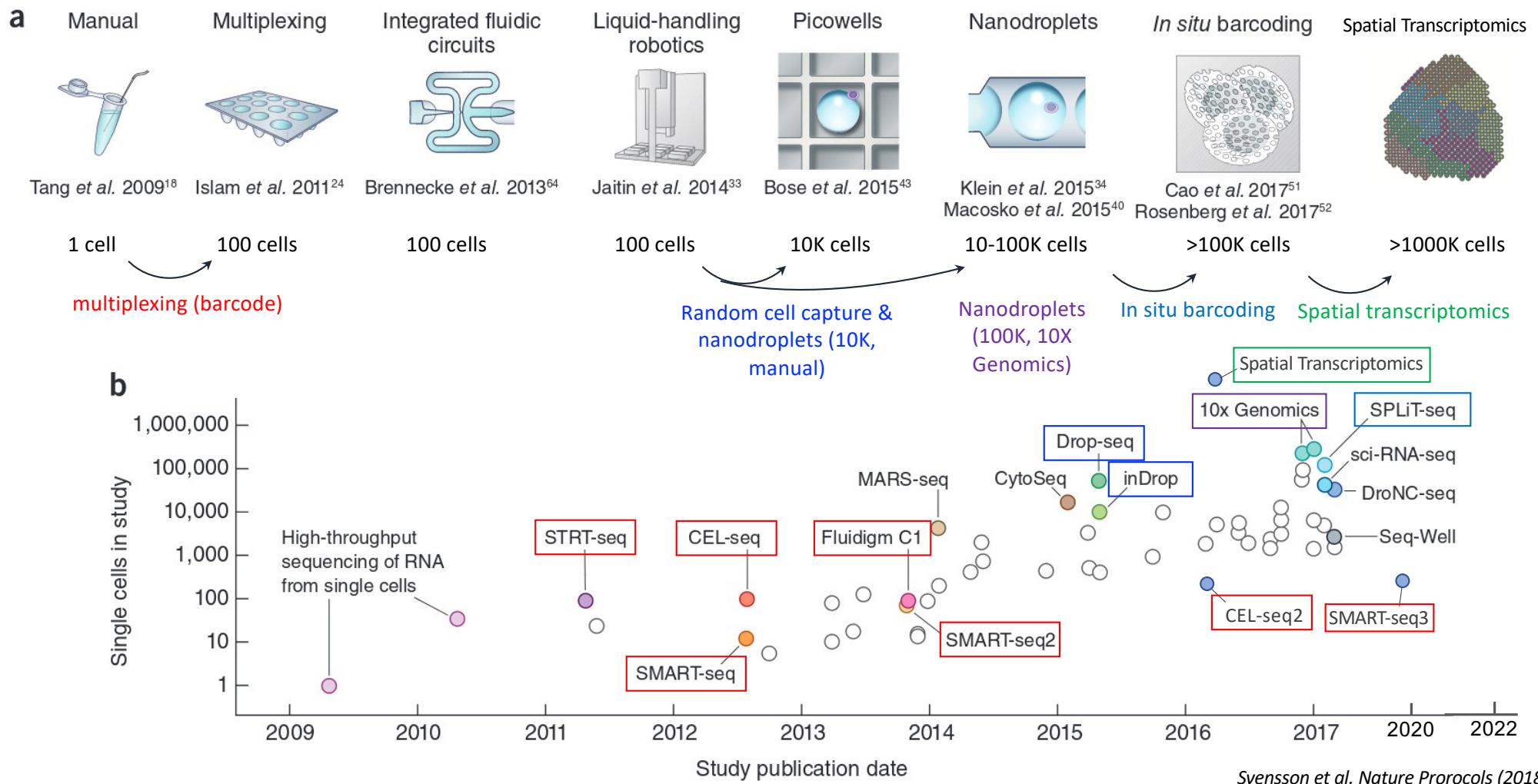


2005: 454 (Roche)

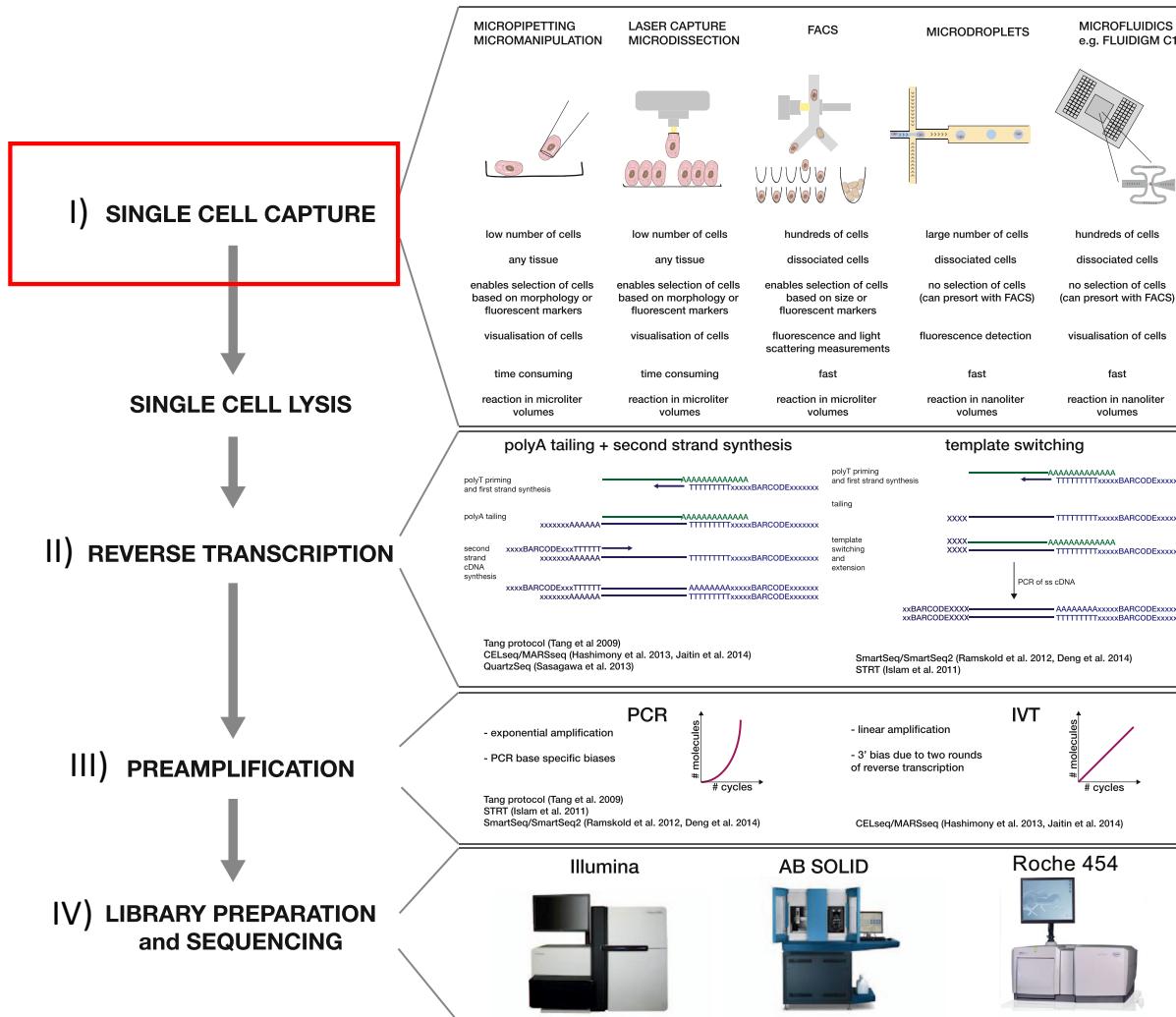
2007: Illumina

2008: SOLid (ABI)

# Evolution of scRNASeq techniques



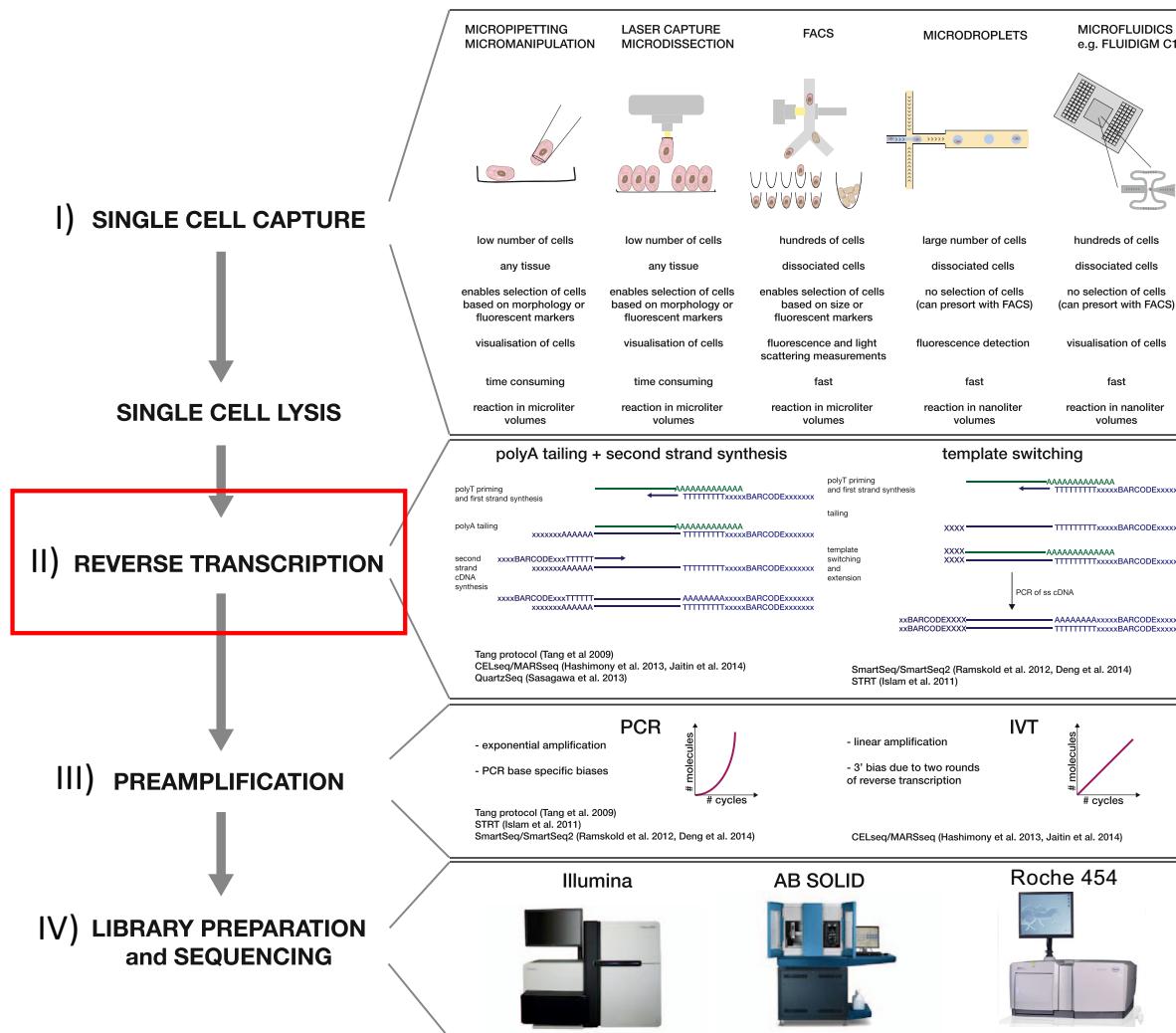
# Single-cell RNA sequencing experiment workflow



# I) Single cell capture

	Plate-based methods (SMART-seq2/3, CEL-seq2)	10X Genomics, Drop-seq, InDrop	Fluidigm (C1)	Takara Bio (iCell8), Rhapsody	
MICROPIPETTING MICROMANIPULATION					
low number of cells	low number of cells	hundreds of cells	large number of cells	hundreds of cells	medium-Large number of cells
any tissue	any tissue	dissociated cells	dissociated cells	dissociated cells	some selection criteria
enables selection of cells based on morphology or fluorescent markers	enables selection of cells based on morphology or fluorescent markers	enables selection of cells based on size or fluorescent markers	no selection of cells (can presort with FACS)	no selection of cells (can presort with FACS)	some selection criteria
visualisation of cells	visualisation of cells	fluorescence and light scattering measurements	fluorescence detection	visualisation of cells	
time consuming	time consuming	fast	fast	fast	fast
reaction in microliter volumes	reaction in microliter volumes	reaction in microliter volumes	reaction in nanoliter volumes	reaction in nanoliter volumes	

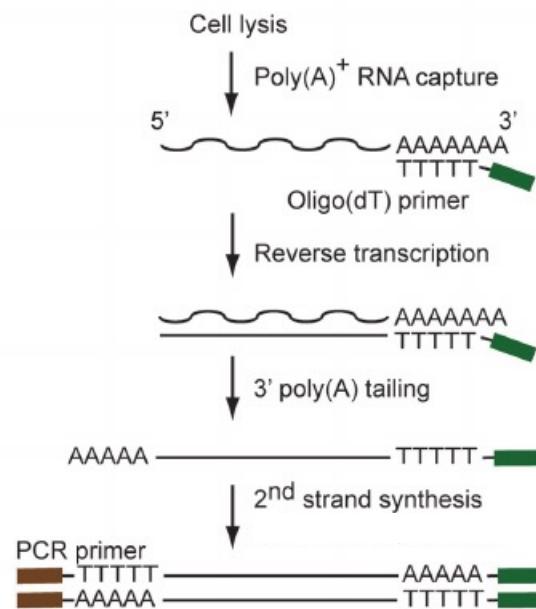
# Single-cell RNA sequencing experiment workflow



## II) Reverse transcription

Cel-Seq(2), InDrop

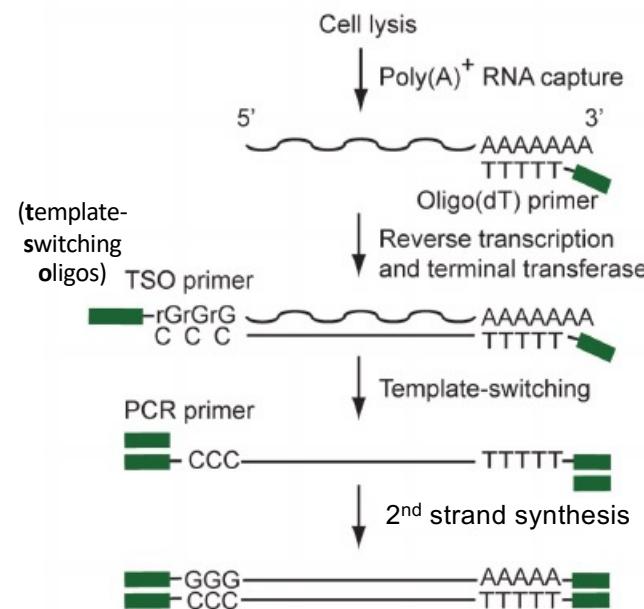
### 1) PolyA tailing + 2<sup>nd</sup> strand synthesis



PolyA tailing: added by template-free terminal transferase (in the presence of dATP)

Smart-Seq(2/3), Drop-seq, 10X

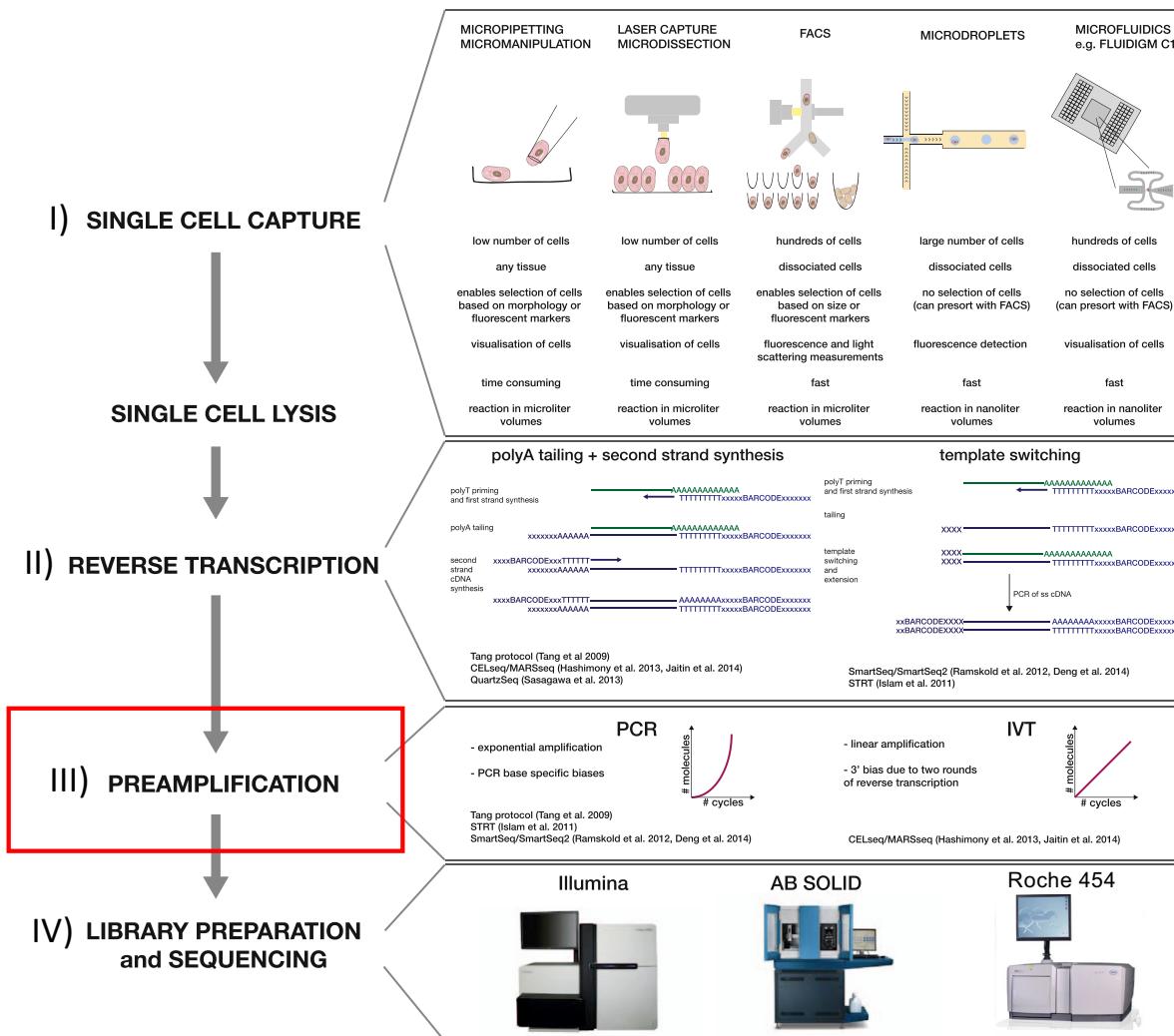
### 2) Template switching + 2<sup>nd</sup> strand synthesis



Template switching: added a few nucleotides in the 3'-end (usually "C") by MMLV reverse transcriptase

\*In the template switching step, the polymerase switches from the mRNA as a template to the TSO as template and continue with the synthesis of the first cDNA strand using TSO as template

# Single-cell RNA sequencing experiment workflow

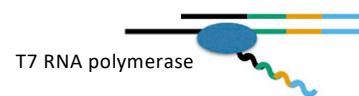


### III) Preamplification

Cel-Seq(2), InDrop

(In vitro transcription)  
**IVT**

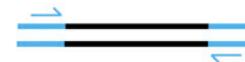
- linear amplification (slow)
- less error ( $<1/10^6$ )



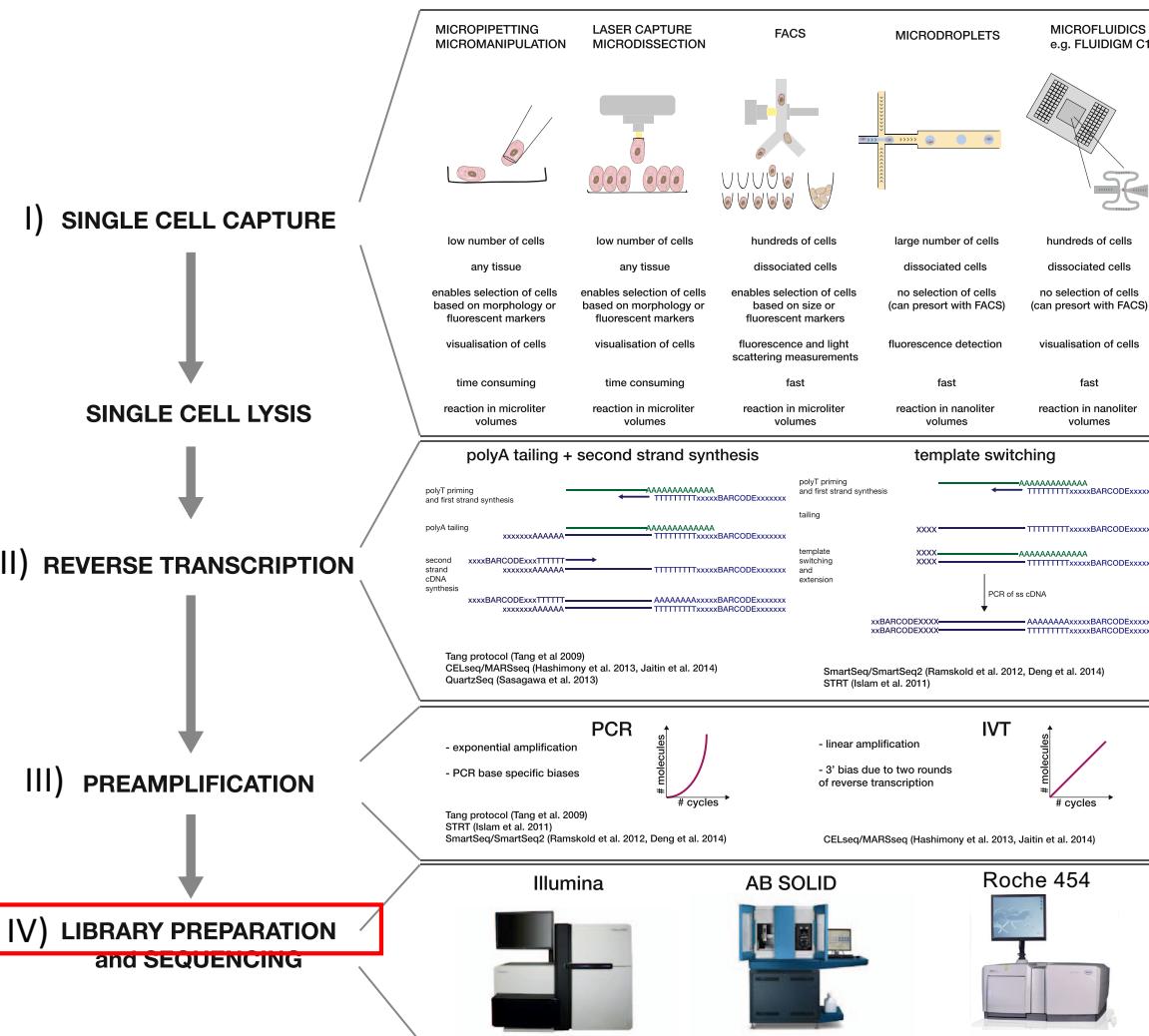
Smart-Seq(2/3), Drop-seq, 10X

**PCR**

- exponential amplification (fast)
- error prone ( $1/10^5$ )



# Single-cell RNA sequencing experiment workflow



# IV) Library preparation

## Cel-Seq(2), InDrop

### RNA fragmentation & reverse transcription (cDNA)

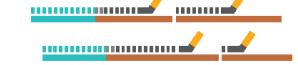
RNA fragmentation:  
heat & mild-base



5'                          3'  
5'                          3'  
5'                          3'

Amplified RNA (aRNA)

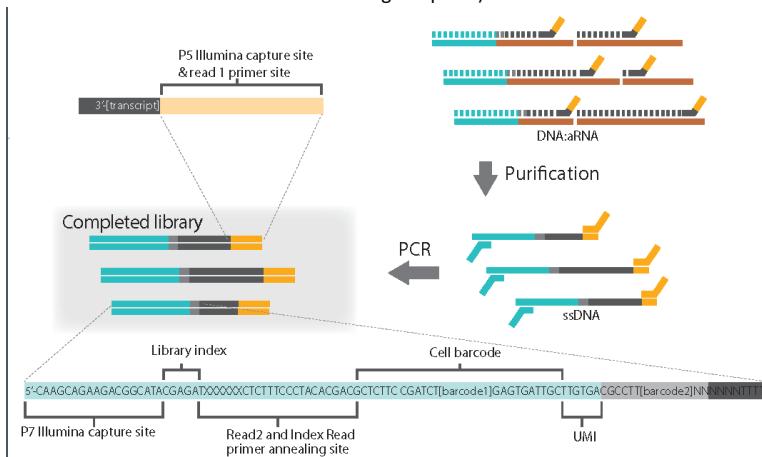
(RT with random hexamer primers containing adaptors)



Purification

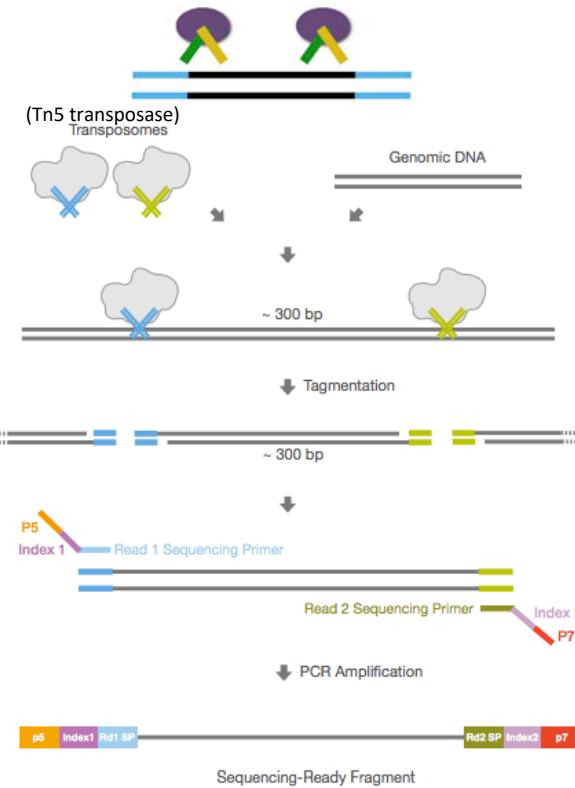


PCR

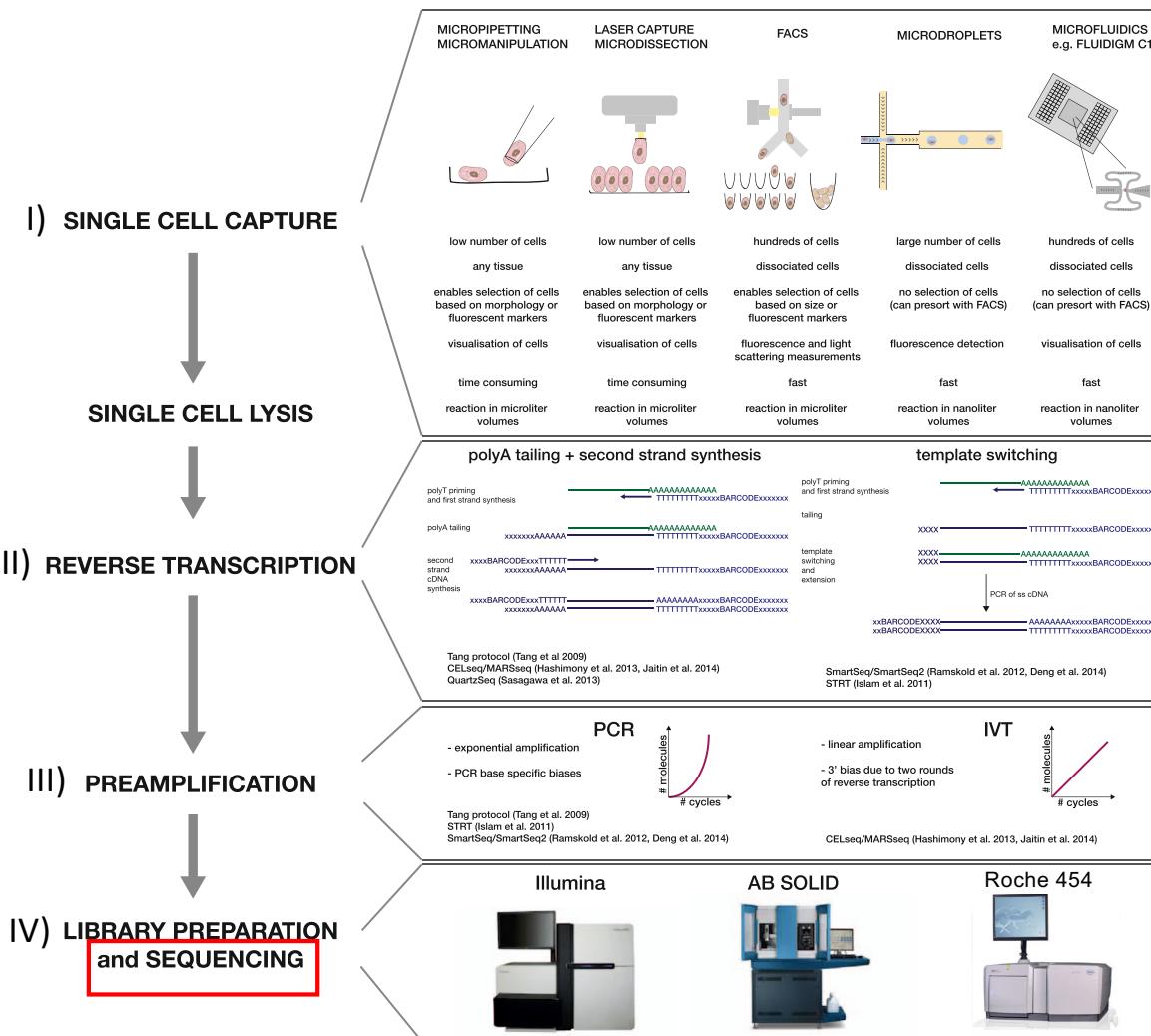


## Smart-Seq(2/3), Drop-seq, 10X

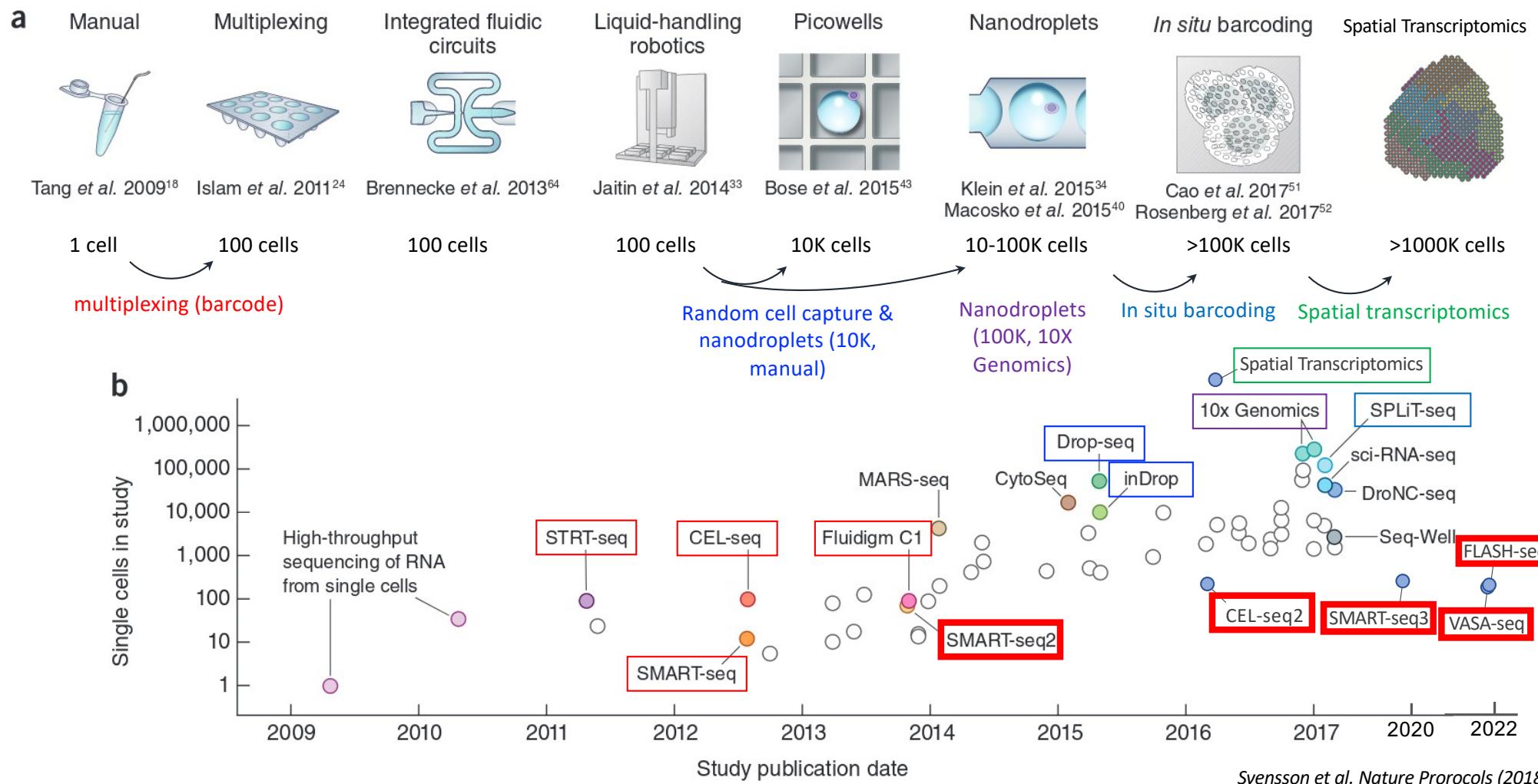
### Tagmentation



# Single-cell RNA sequencing experiment workflow



# Evolution of scRNASeq techniques



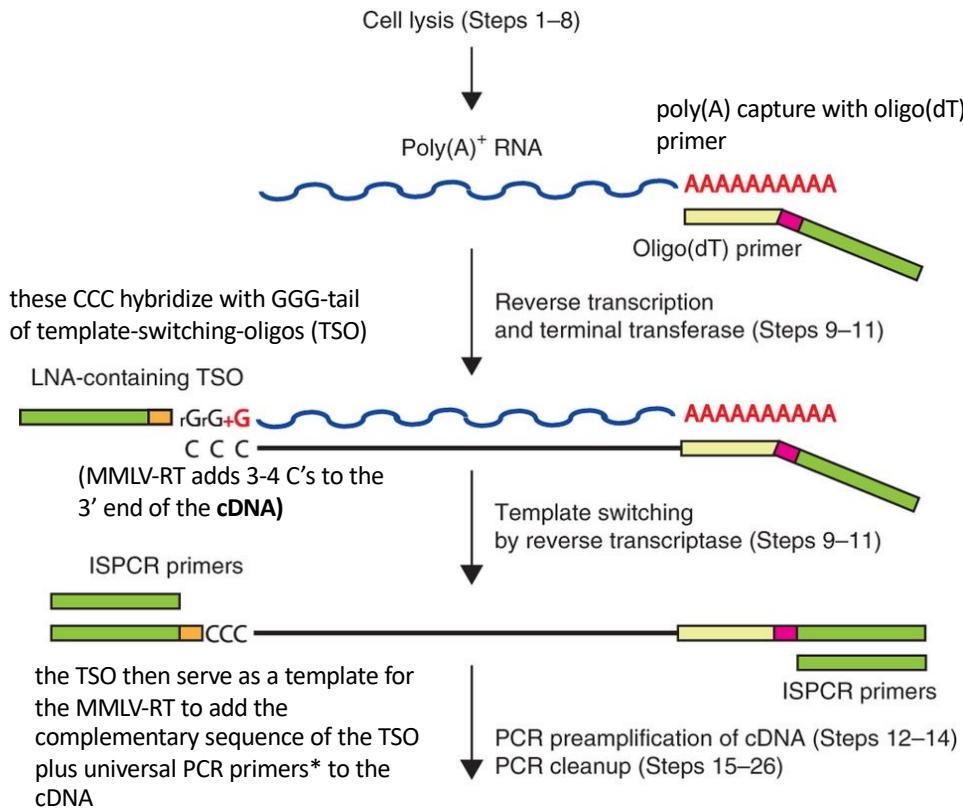
## **(The most popular) plate-based scRNAseq**

- SMART-seq2/3
- CEL-seq2
- VASA-seq
- FLASH-seq

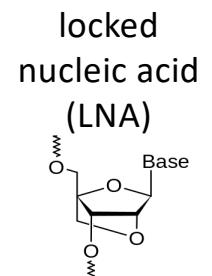
# SMART-seq2

**“SMART”:** Switching Mechanism At the 5' end of the RNA Transcript

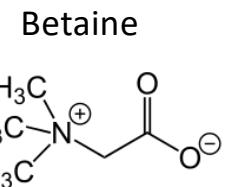
## RNA capture and cDNA synthesis



- A modified guanosine (a locked nucleic acid, LNA) is incorporated in TSO: enhance thermal stability & anneal strongly to the untemplated 3' extension of the cDNA



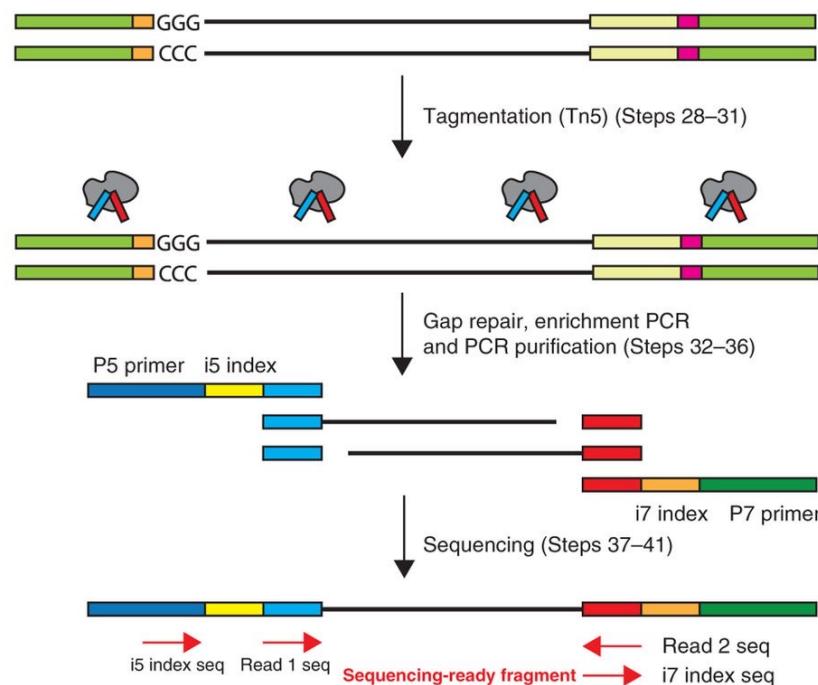
- Betaine, a methyl group donor, can remove the steric hindrance of secondary structures of RNAs (such as hairpins or loops) so that early termination of chain elongation can be blocked.



# SMART-seq2

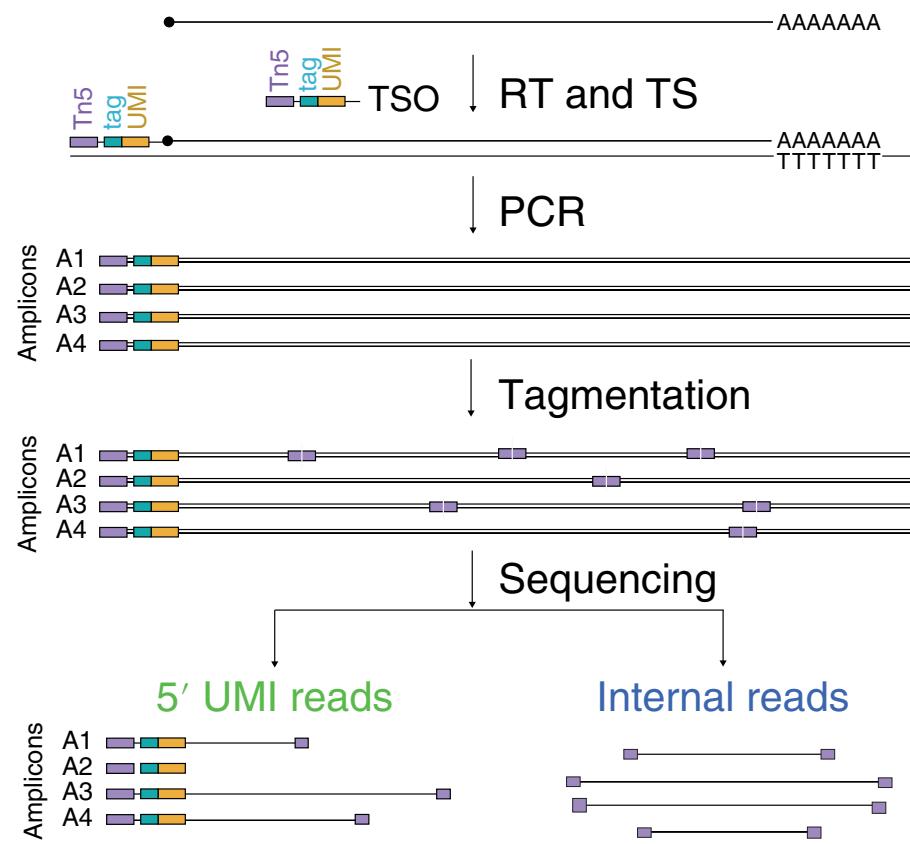
“SMART”: Switching Mechanism At the 5' end of the RNA Transcript

## Library preparation

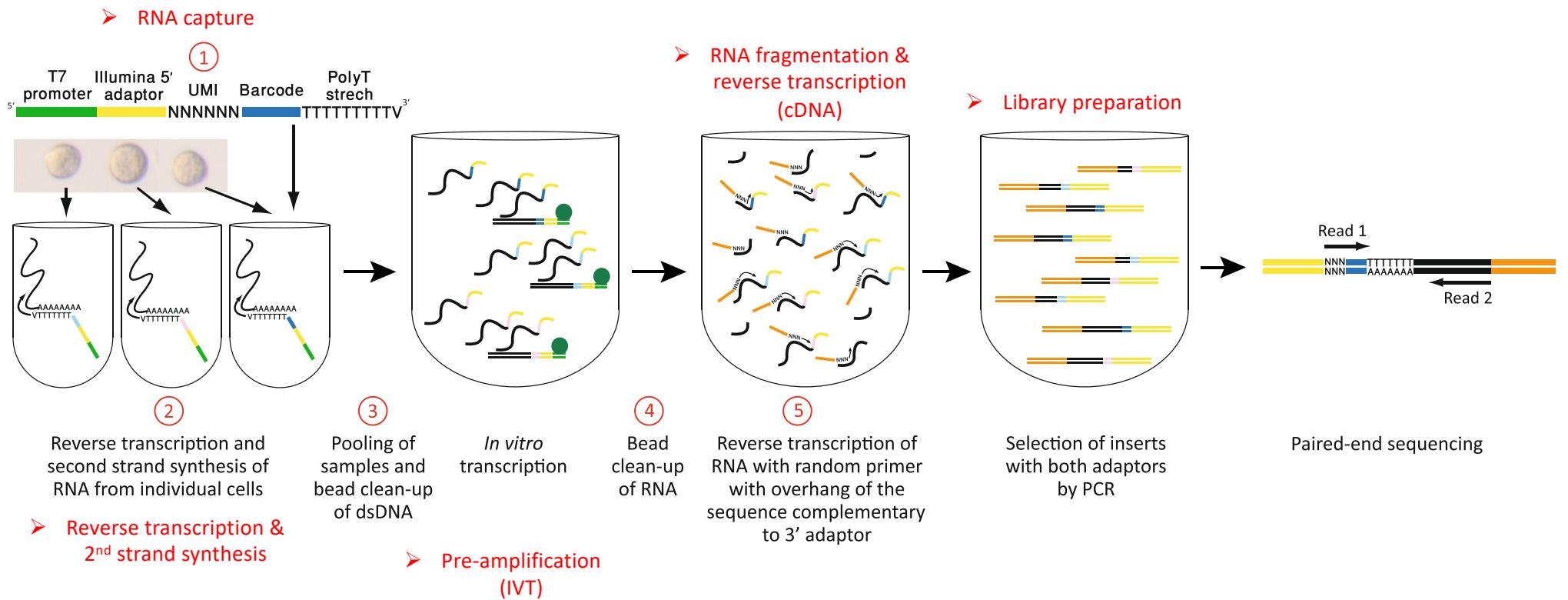


- amplification with few PCR cycles
- **tagmentation:** combining fragmentation and sequencing adapter integration
  - hyperactive derivative of the Tn5 transposase **cuts** the cDNA and **ligates** sequencing adapters

# SMART-seq3

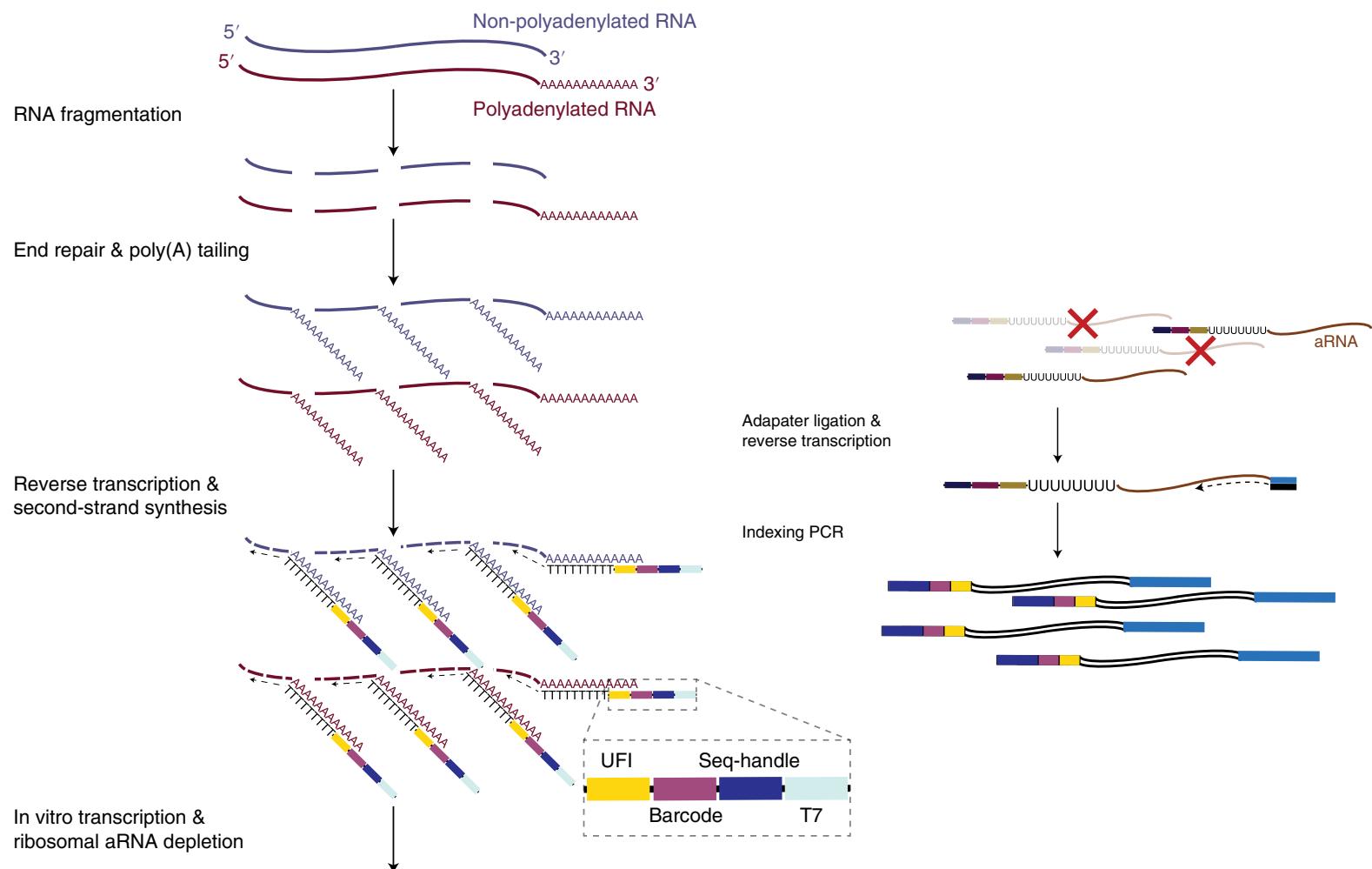


# CEL-seq2

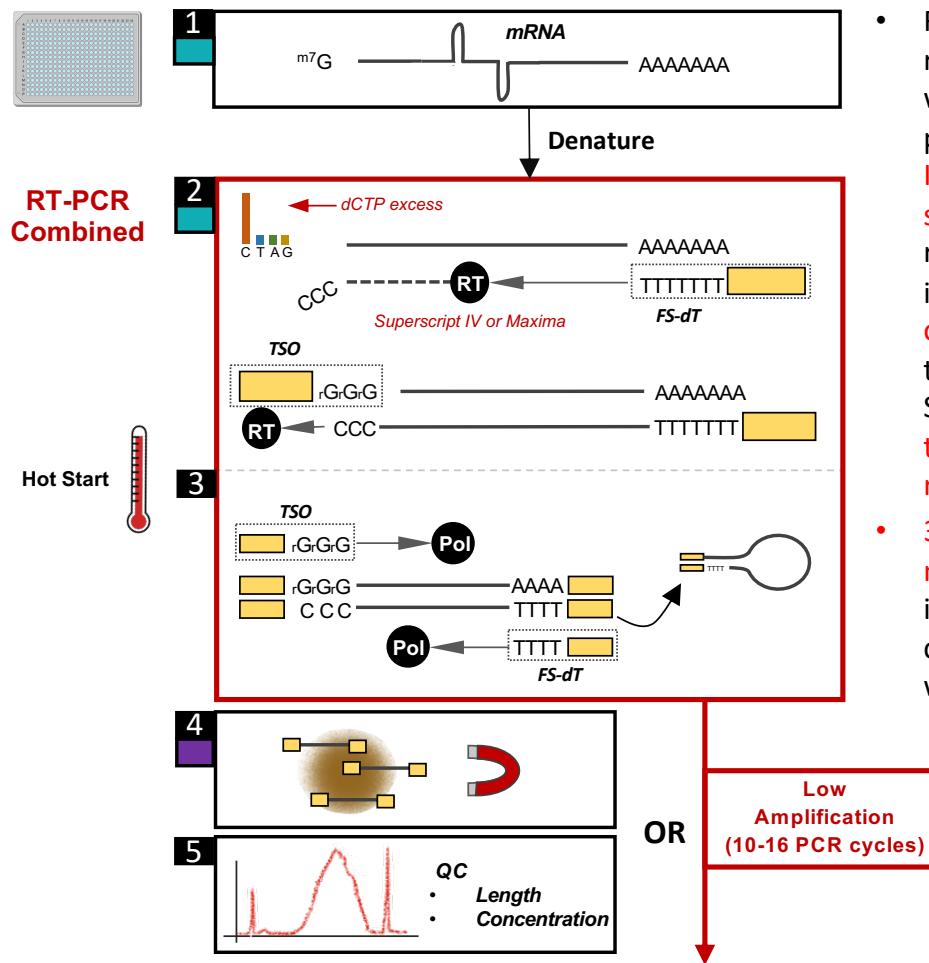


# VASA-seq: vast transcriptome analysis of single cells by dA-tailing

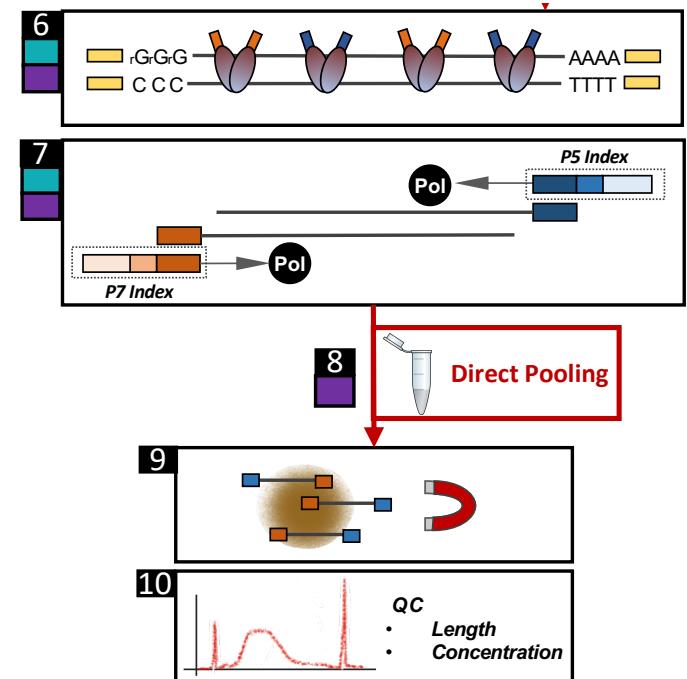
→ Profile full-length RNAs and both polyadenylated & non-polyadenylated RNAs



# FLASH-seq: a full-length scRNA-seq protocol capable of generating sequencing-ready libraries in less than 4.5 hours (modified protocol from SMART-seq3)



- Replace Superscript II reverse transcriptase with the more processive **Superscript IV (SSRTIV)** and shortened the RT reaction time, increased the **amount of dCTP** to favor the C-tailing activity of SSRTIV and boost template-switching reaction.
- 3'-terminal locked nucleic acid guanidine in the TSO (prone to cause strand invasion) with **riboguanosine**.

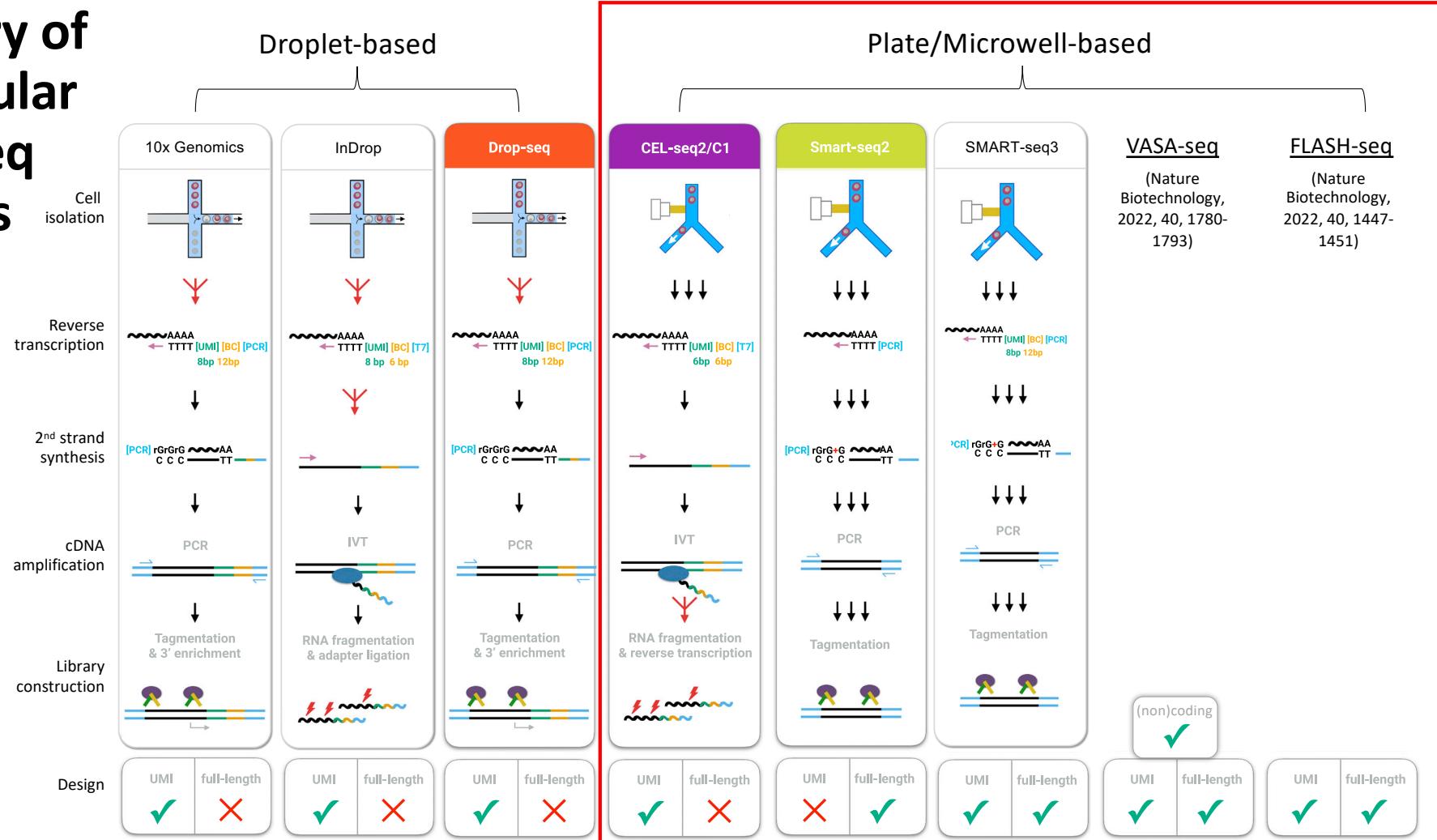


Liquid Handling Robot (i.e., Fluent® Automatic Workstation, Tecan)

Nanodispenser (i.e., I.DOT, Cellink)

**FLASH-seq Specific Steps**

# Summary of the popular scRNASeq methods



Molecular Cell, 2017 65, 631–643; Nature Biotechnology, 2022

# **Conclusion for Part I (plate-based scRNASeq)**

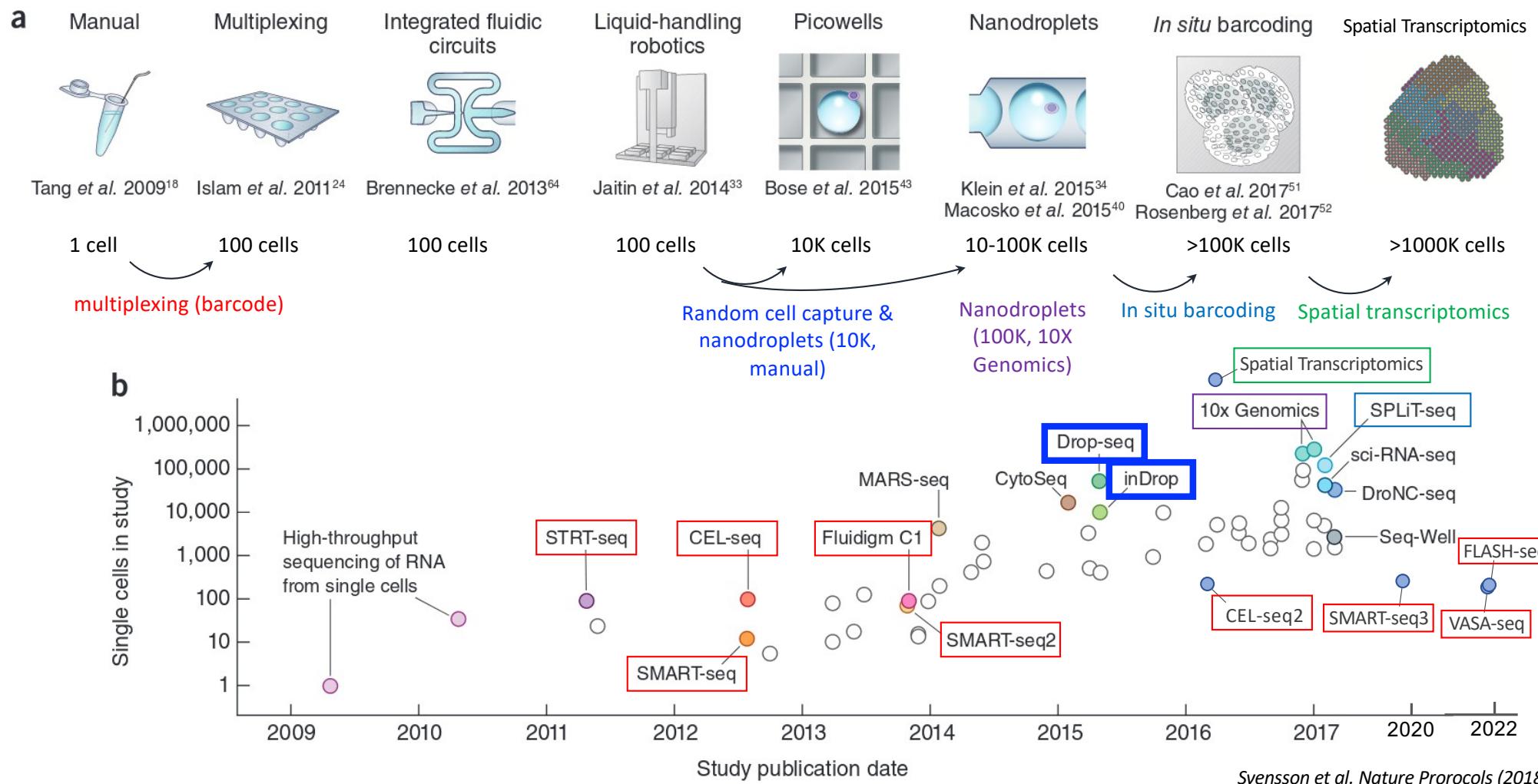
- **General introduction about scRNASeq**
  - scSeq vs NGS
- **Workflow of different plate-based scRNASeq**
  - Single cell capture, cell lysis, reverse transcription, pre-amplification, library preparation, sequencing
- **Different types of plate-based scRNASeq**
  - SMART-seq2/3, CEL-seq2, VASA-seq, FLASH-seq

# Part II: Droplet-based scRNAseq

## **Outline of the Part II (Droplet-based scRNAseq)**

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

# Evolution of scRNASeq techniques

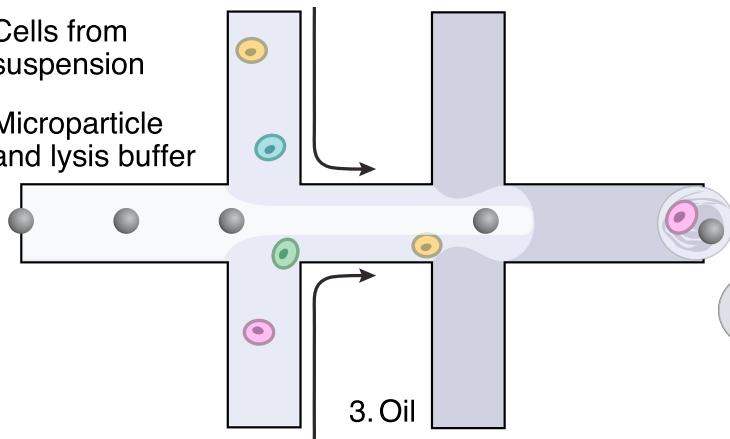




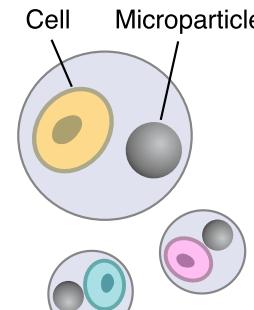
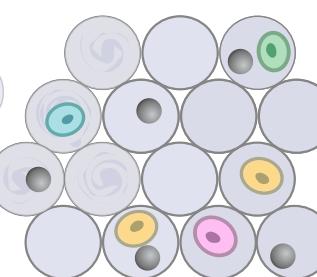
# DropSeq overview

1. Cells from suspension

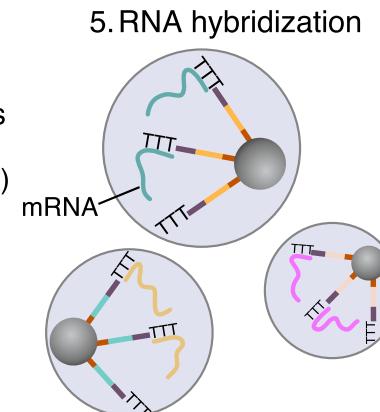
2. Microparticle and lysis buffer



3. Oil

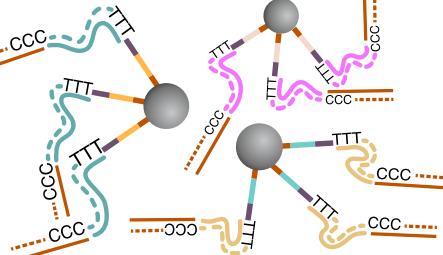


4. Cell lysis  
(in seconds)

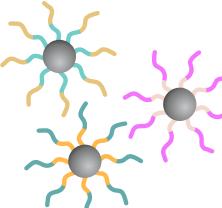


5. RNA hybridization

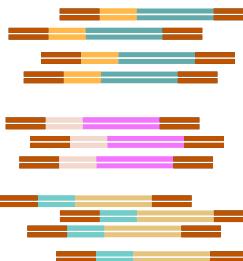
6. Break droplets



STAMPs



7. Reverse transcription with template switching  
8. PCR  
(STAMPs as template)



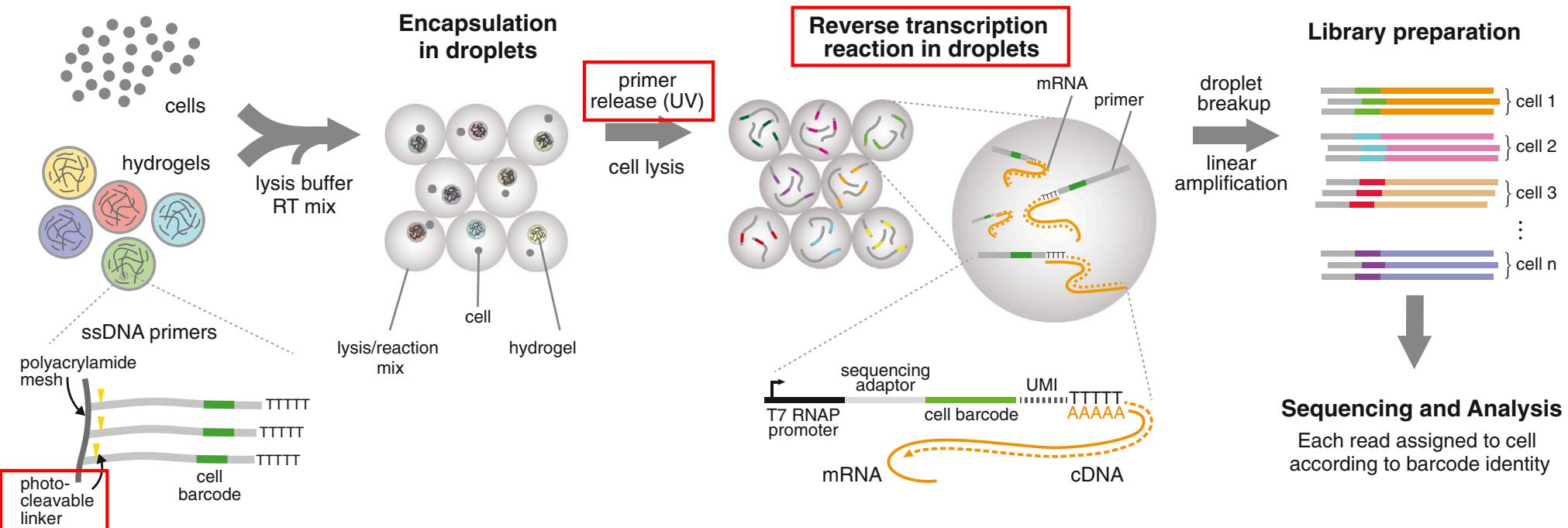
9. Sequencing and analysis

- Each mRNA is mapped to its cell-of-origin and gene-of-origin
- Each cell's pool of mRNA can be analyzed

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)

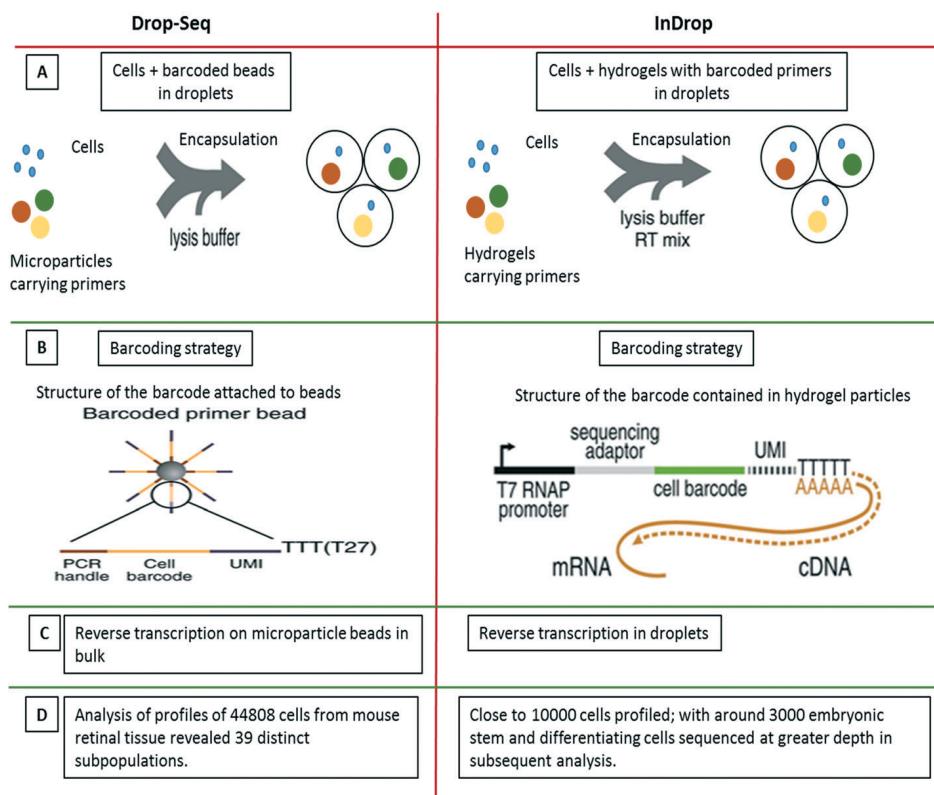
# InDrop overview

1CELLBIO



Cell, 2015, 161, 1187–1201

# DropSeq vs InDrop



Common

- Move throughput from hundreds (plate-based) to thousands of cells
- Droplet-based processing using microfluidics
- 3'-end
- Use UMI (unique molecular identifier)

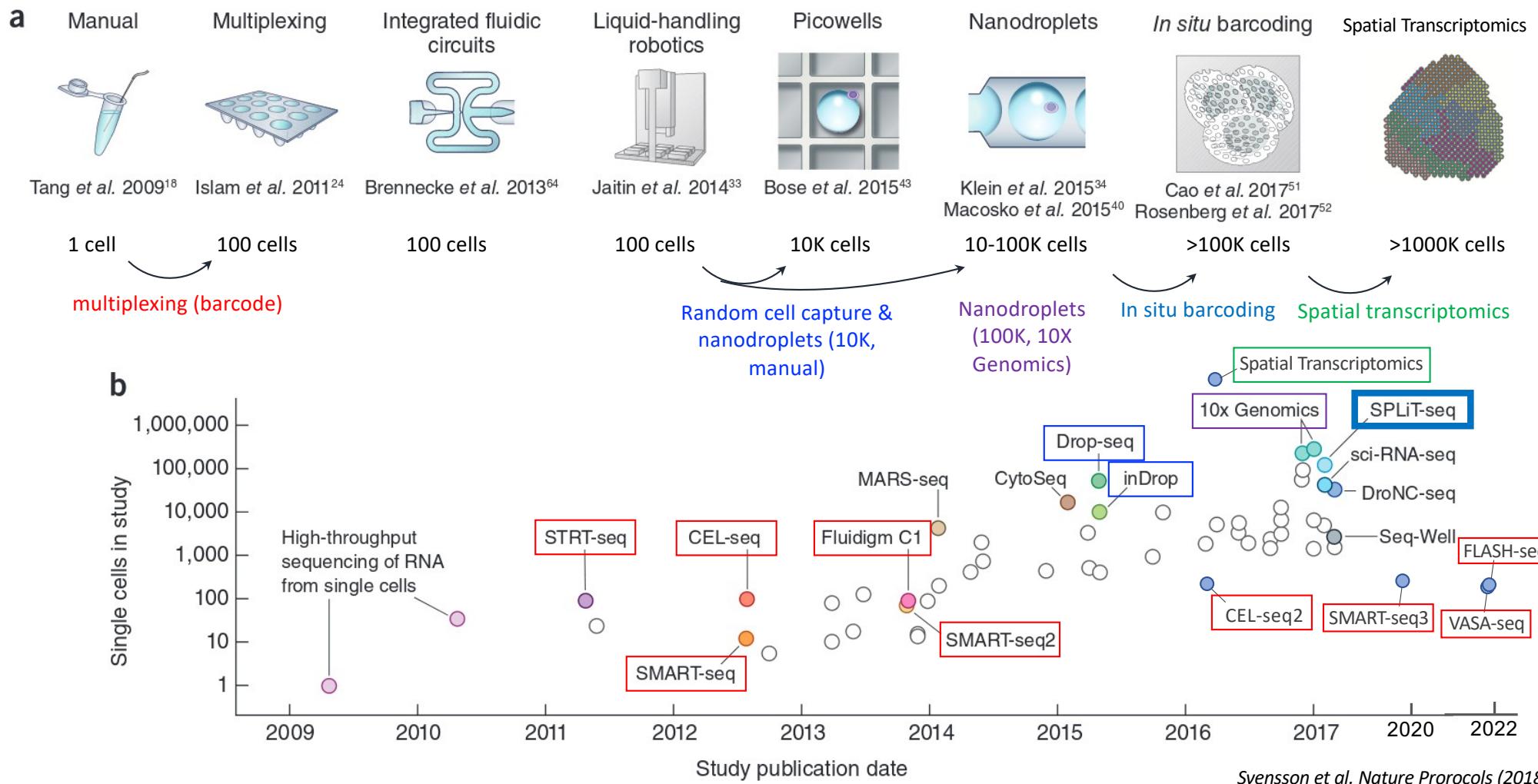
## DropSeq

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

## InDrop

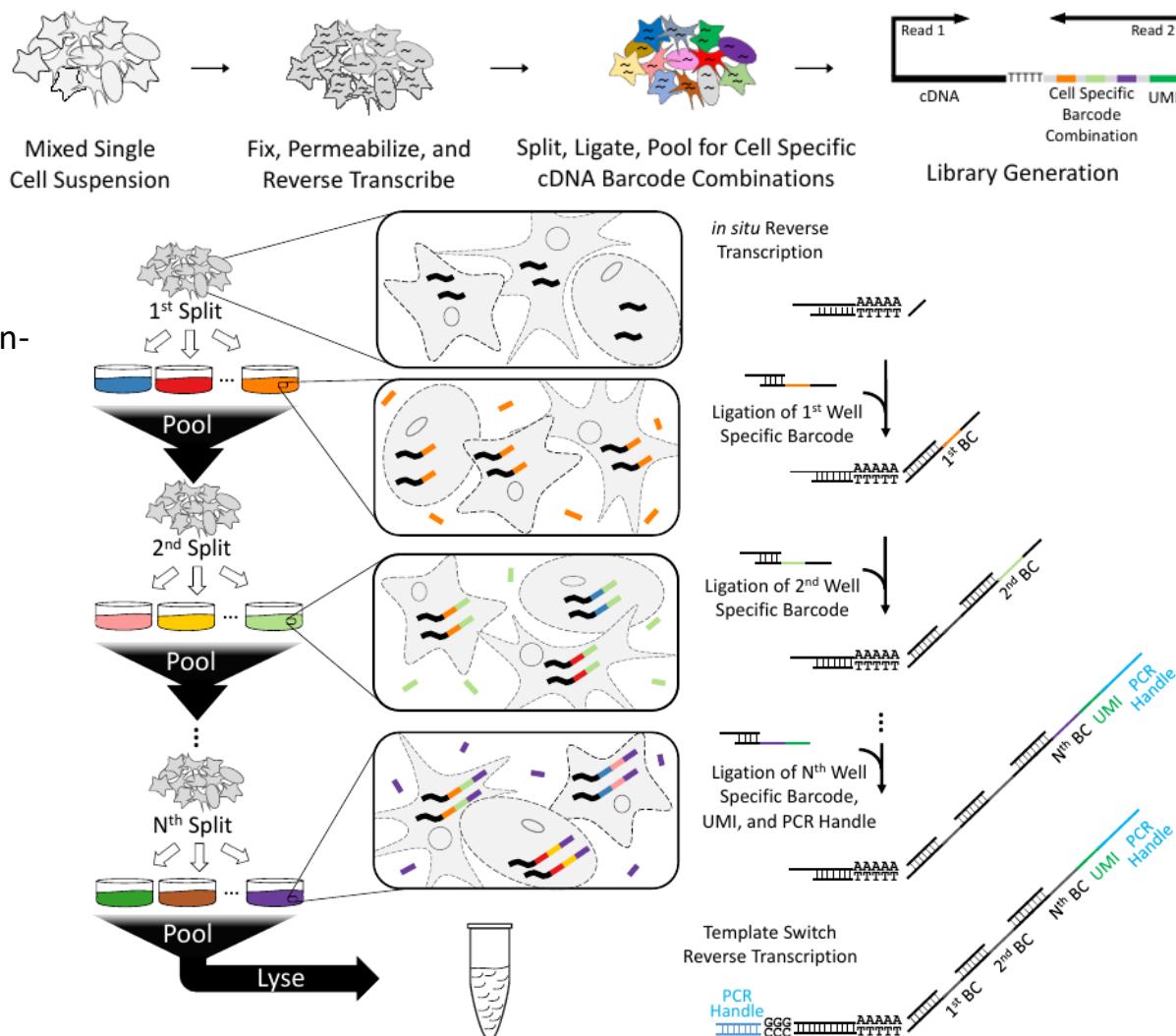
- Reverse transcription (RT) in droplets (not in pool)

# Evolution of scRNASeq techniques



# In situ barcoding

**SPLIT-seq:**  
Split Pool Ligation-based  
Transcriptome sequencing

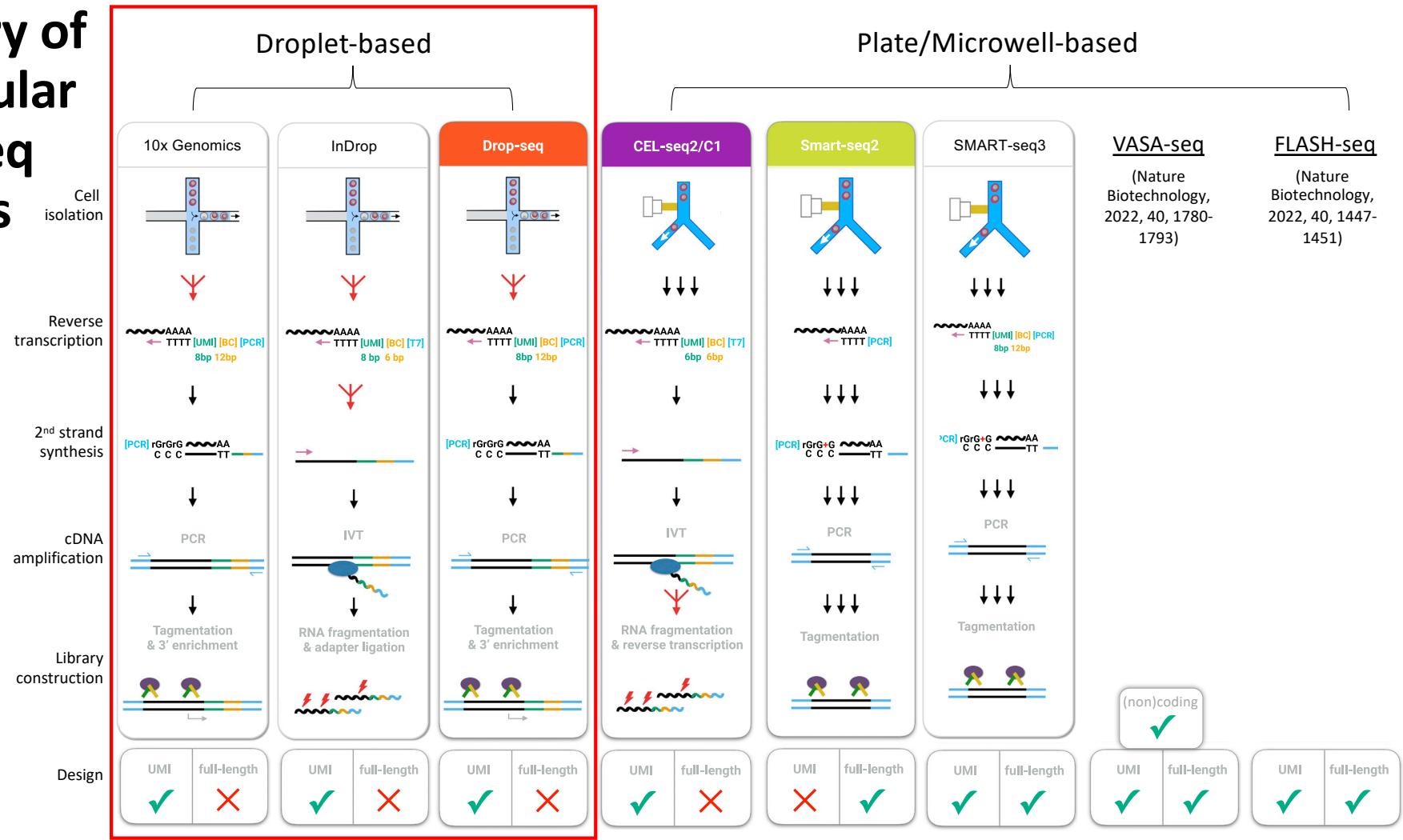


 **Parse**  
BIOSCIENCES

 **SCALE**  
biosciences  
CELL TO INSIGHT

Rosenburg et al, 2018, Science

# Summary of the popular scRNASeq methods



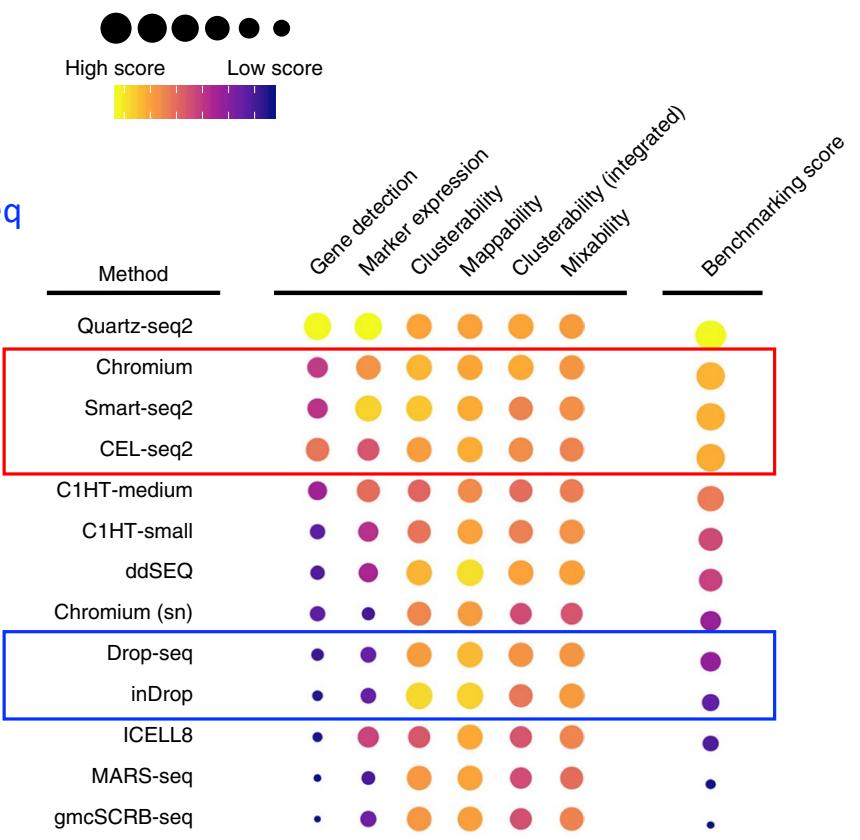
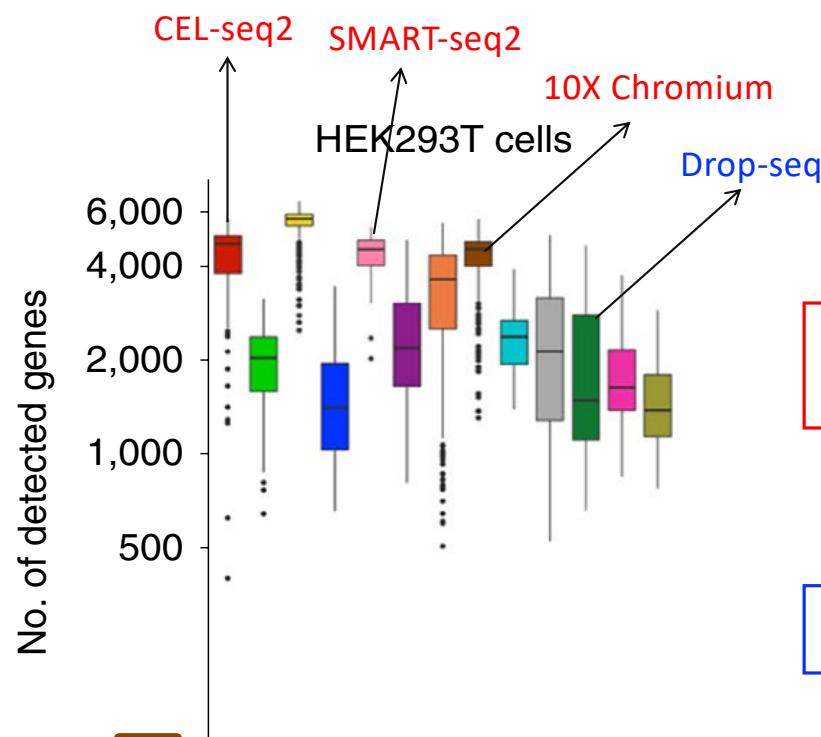
\* Modified library prep protocols can also generate full-length libraries using the cDNA products from 10X Genomics

Molecular Cell, 2017 65, 631–643; Nature Biotechnology, 2022

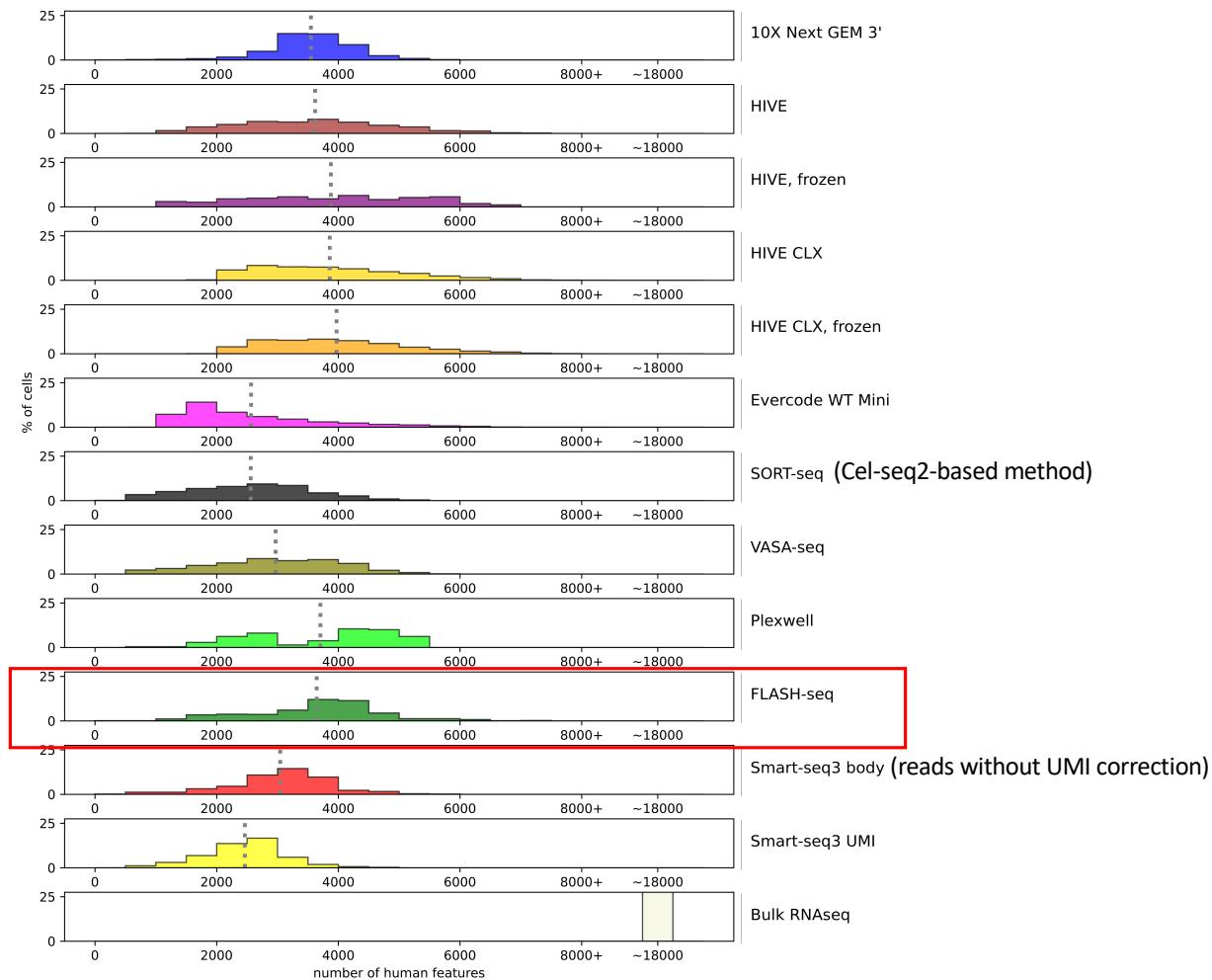
## **Conclusion for Part II (Droplet-based methods)**

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

# Benchmarking scRNAseq methods



# Benchmarking scRNAseq methods



# Which method should I use?



- If you want to study overall variability in transcription of cells within or across different tissues? Many cells (droplet-based methods)
- If you want to look at a few genes associated with a specific process, such as cell death (of a small amount of cells)? Plate-based methods (deeper sequencing depth); panel-based methods
- To capture low-abundance gene? Plate-based methods (deeper sequencing depth)
- To get a full-length coverage of transcriptomes from single cells (study splice variant)? SMART-seq2/3, VASA-seq, FLASH-seq (PacBio, Nanopore)
- To have fewer errors or identify SNV from RNA-inferred DNA data? IVT-based amplification method (CEL-seq2, InDrop, VASA-seq, FLASH-seq)
- To study transcription start sites? SMART-seq2/3, 10X Genomics 5' method, VASA-seq, FLASH-seq
- To capture poly(A)- and non-poly(A)-RNAs & study non-coding RNAs? VASA-seq

