

Plate-based single cell RNA sequencing (scRNaseq)

Miao-Ping Chien

Erasmus MC, Group leader

2020 Single Cell Analysis Workshop, 2020/10/19

Outline

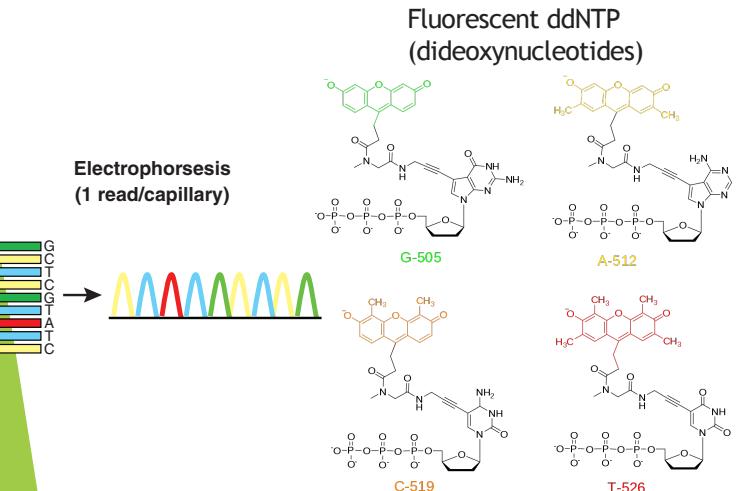
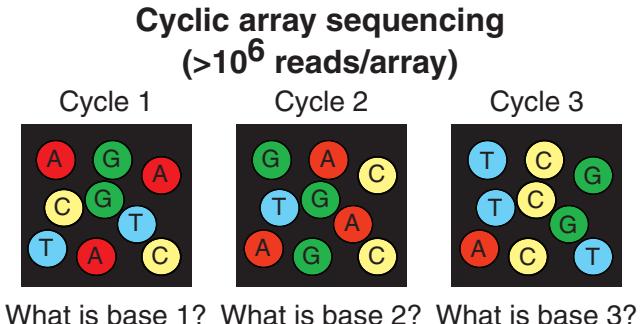
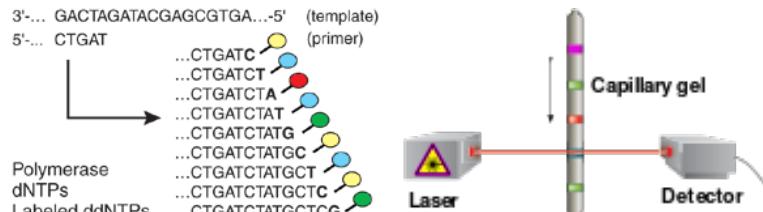
- General introduction about scRNAseq
- Different types of plate-based scRNAseq
- Workflow of different plate-based scRNAseq
- Which method to choose?



single cell sequencing $\stackrel{?}{=}$ next-generation sequencing

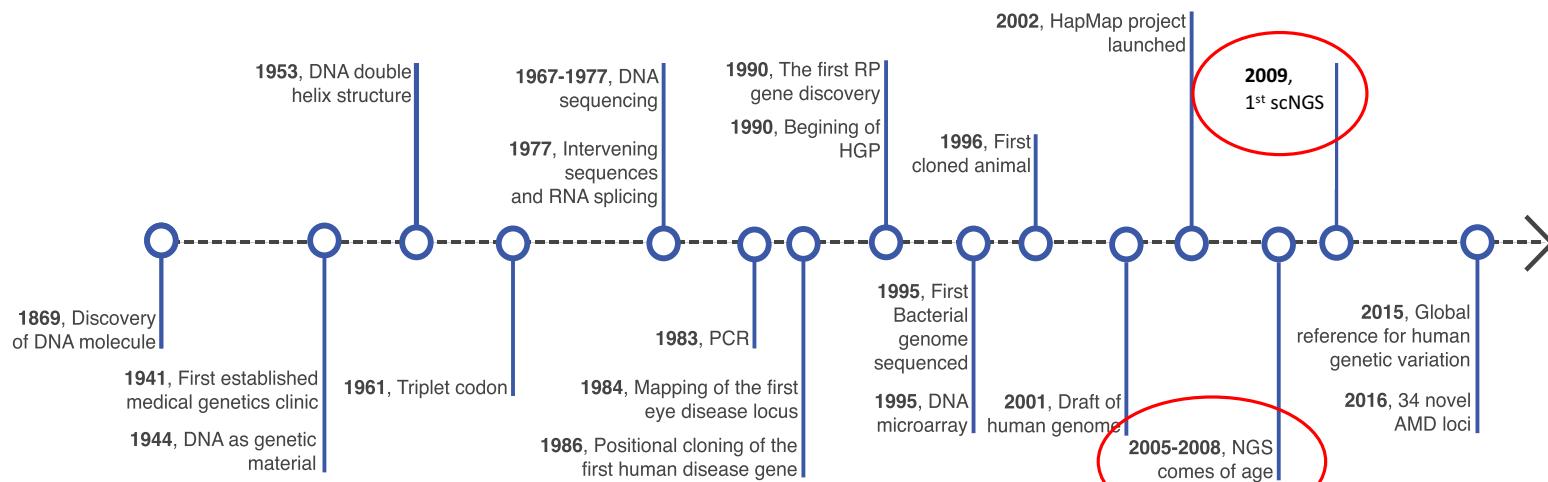
Traditional sequencing vs next-generation sequencing?

- From sanger sequencing to next-generation sequencing



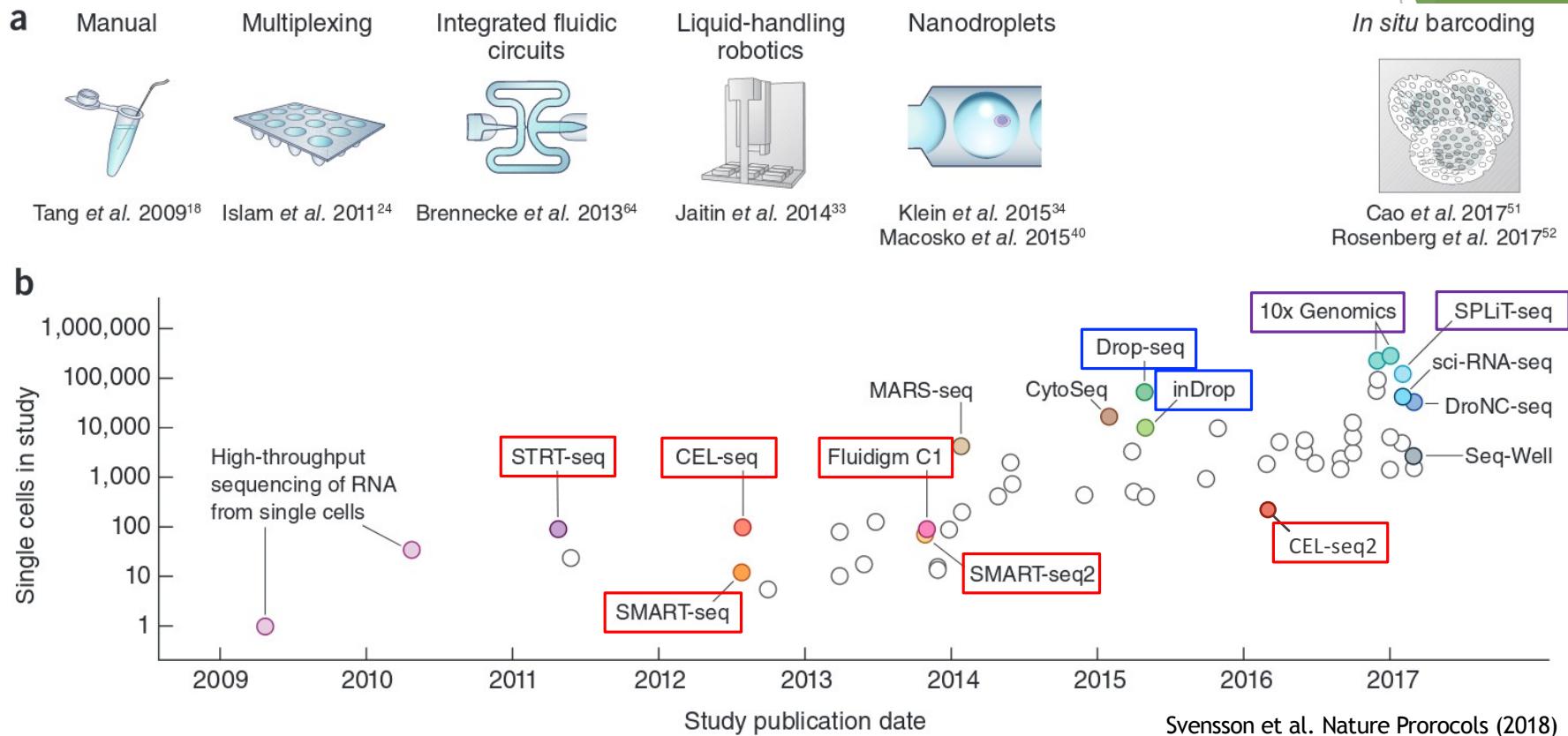
- Illumina/Solexa
- Roche 454
- ABI SOLiD
- ...

Next-generation sequencing & single cell sequencing



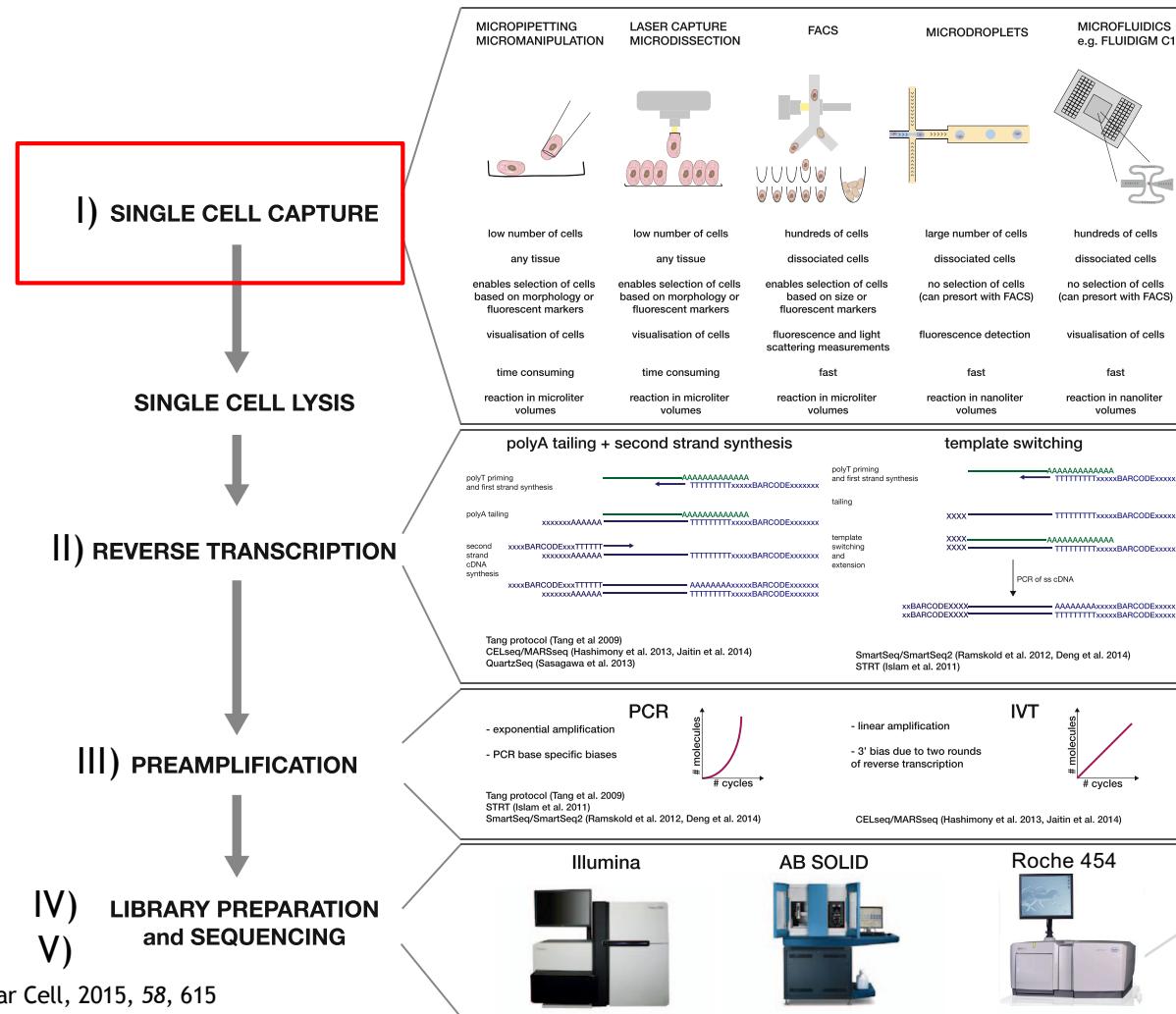
2005: 454 (Roche)
2007: Illumina
2008: SOLid (ABI)

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**

Single-cell RNA sequencing experiment workflow



I) Single cell capture

10X Genomics
/Drop-seq/InDrop

Fluidigm (C1),
Wafergen (iCell8),
CellenOne

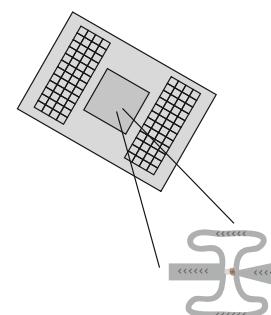
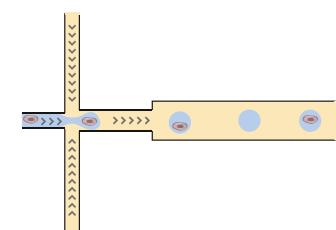
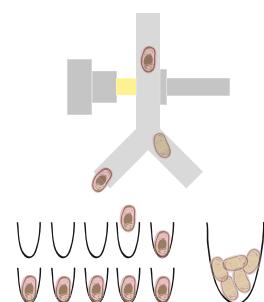
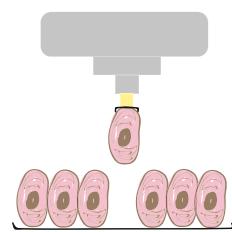
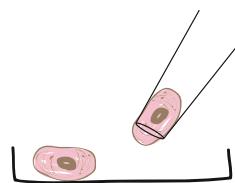
MICROPIPETTING
MICROMANIPULATION

LASER CAPTURE
MICRODISSECTION

FACS

MICRODROPLETS

MICROFLUIDICS
e.g. FLUIDIGM C1



low number of cells

low number of cells

hundreds of cells

large number of cells

hundreds of cells

any tissue

any tissue

dissociated cells

dissociated cells

dissociated cells

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)

visualisation of cells

visualisation of cells

fluorescence and light
scattering measurements

fluorescence detection

visualisation of cells

time consuming

time consuming

fast

fast

fast

reaction in microliter
volumes

reaction in microliter
volumes

reaction in microliter
volumes

reaction in nanoliter
volumes

reaction in nanoliter
volumes

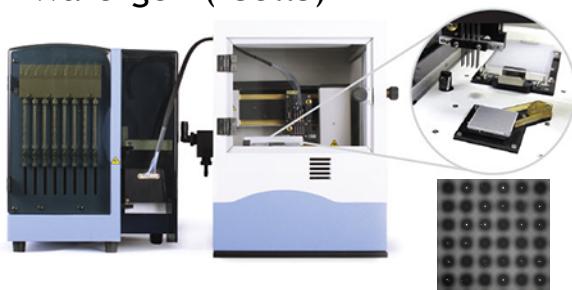
I) Single cell capture

Fluidigm (C1):



<https://www.youtube.com/watch?v=TF4NJRE4Xg4>

Wafergen (iCell8)



<https://www.youtube.com/watch?v=gyh6Sto0GfU>

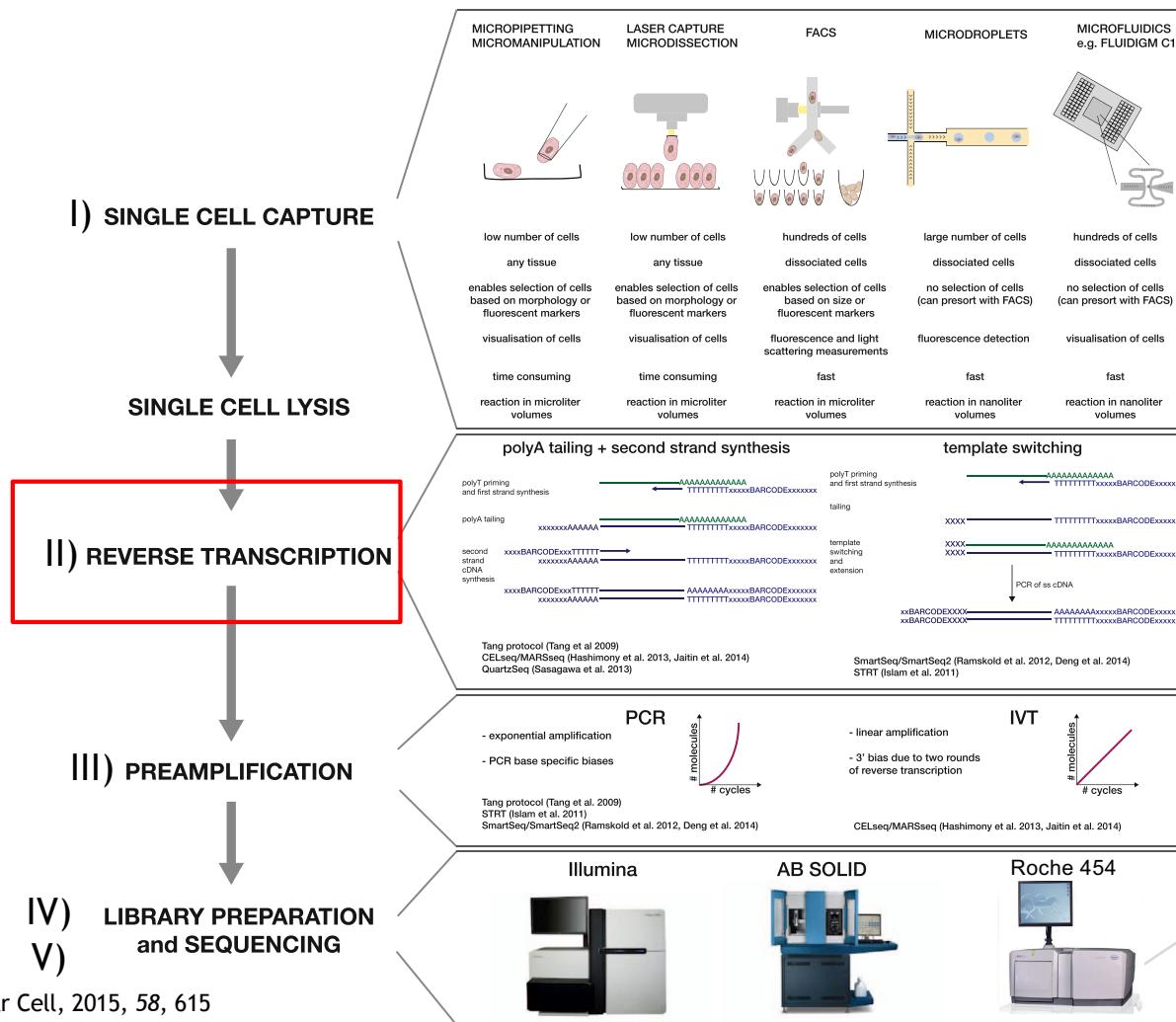


Cellenion (CellenONE)



<https://www.cellenion.com/products/cellenone-x1/>

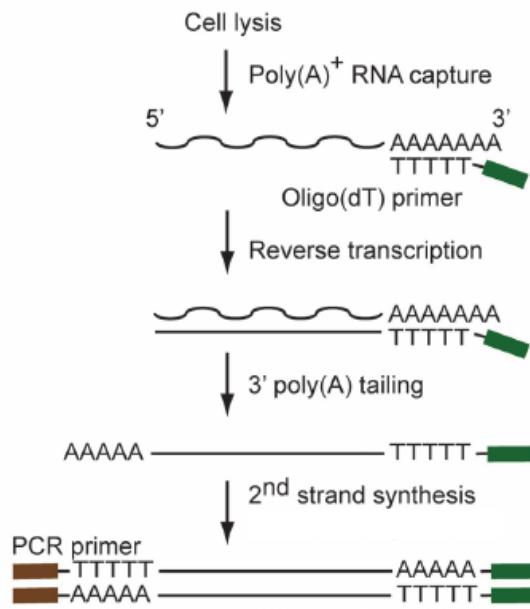
Single-cell RNA sequencing experiment workflow



II) Reverse transcription

Cel-Seq(2), (InDrop)

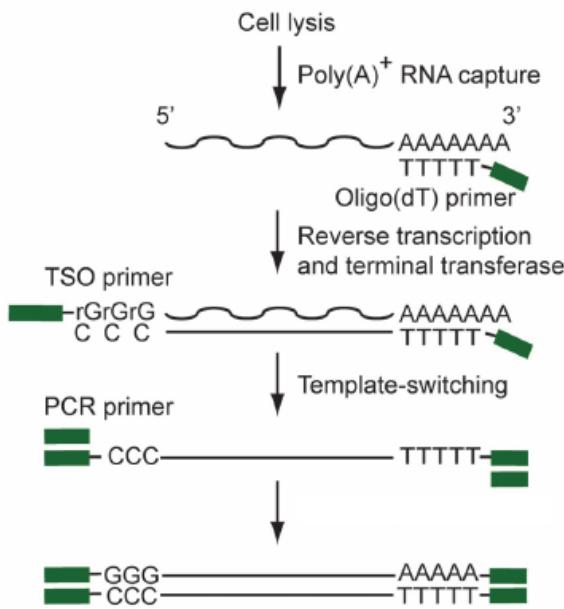
1) PolyA tailing + 2nd strand synthesis



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

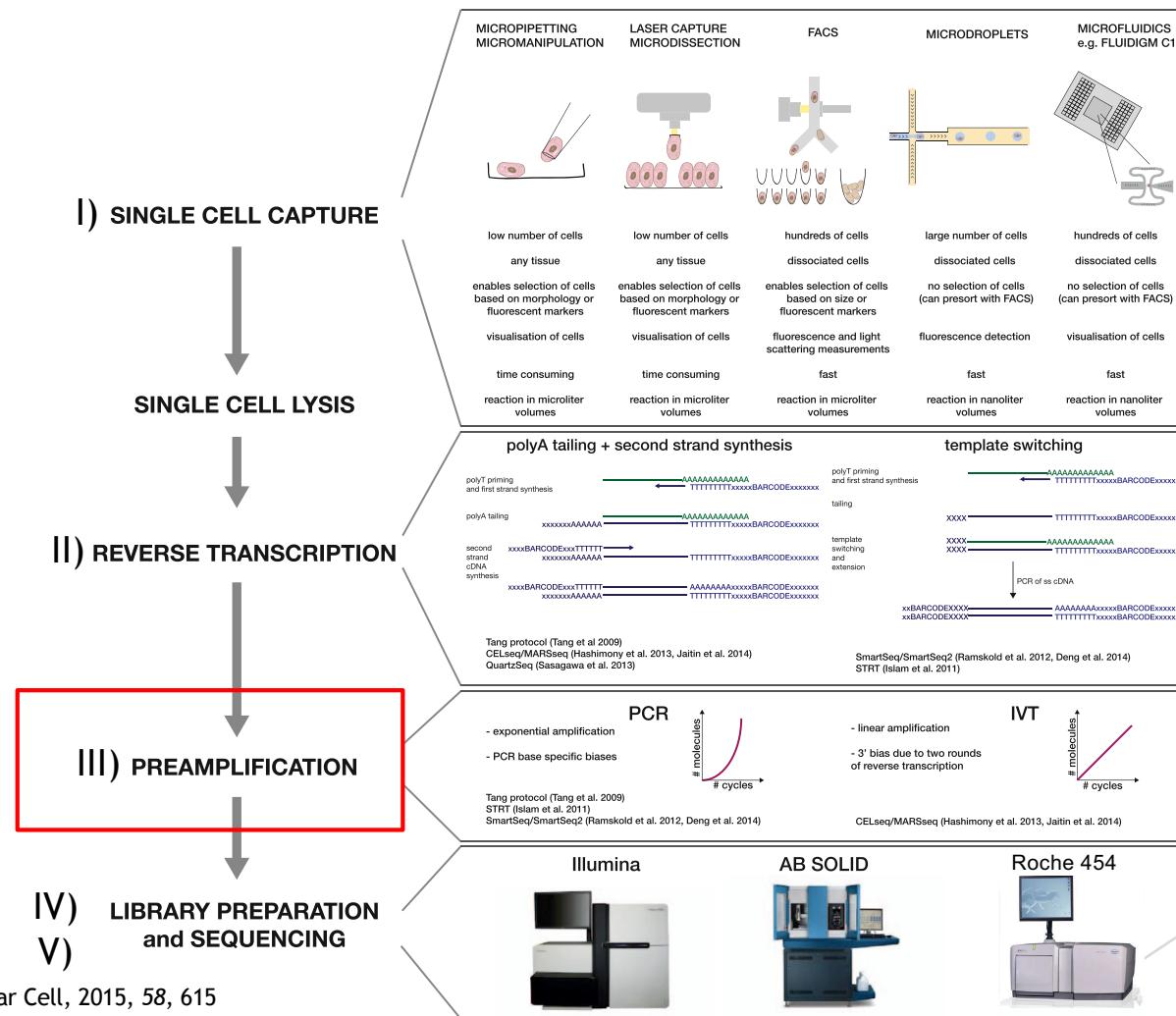
Smart-Seq(2), STRT-Seq, (Drop-seq, 10X)

2) Template switching



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

Single-cell RNA sequencing experiment workflow



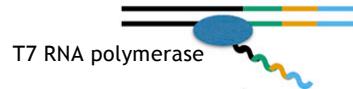
III) Preamplification

Cel-Seq(2), (InDrop)

(In vitro transcription)

IVT

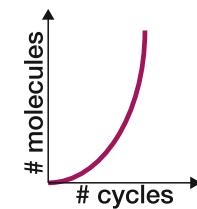
- linear amplification (slow)
- less error



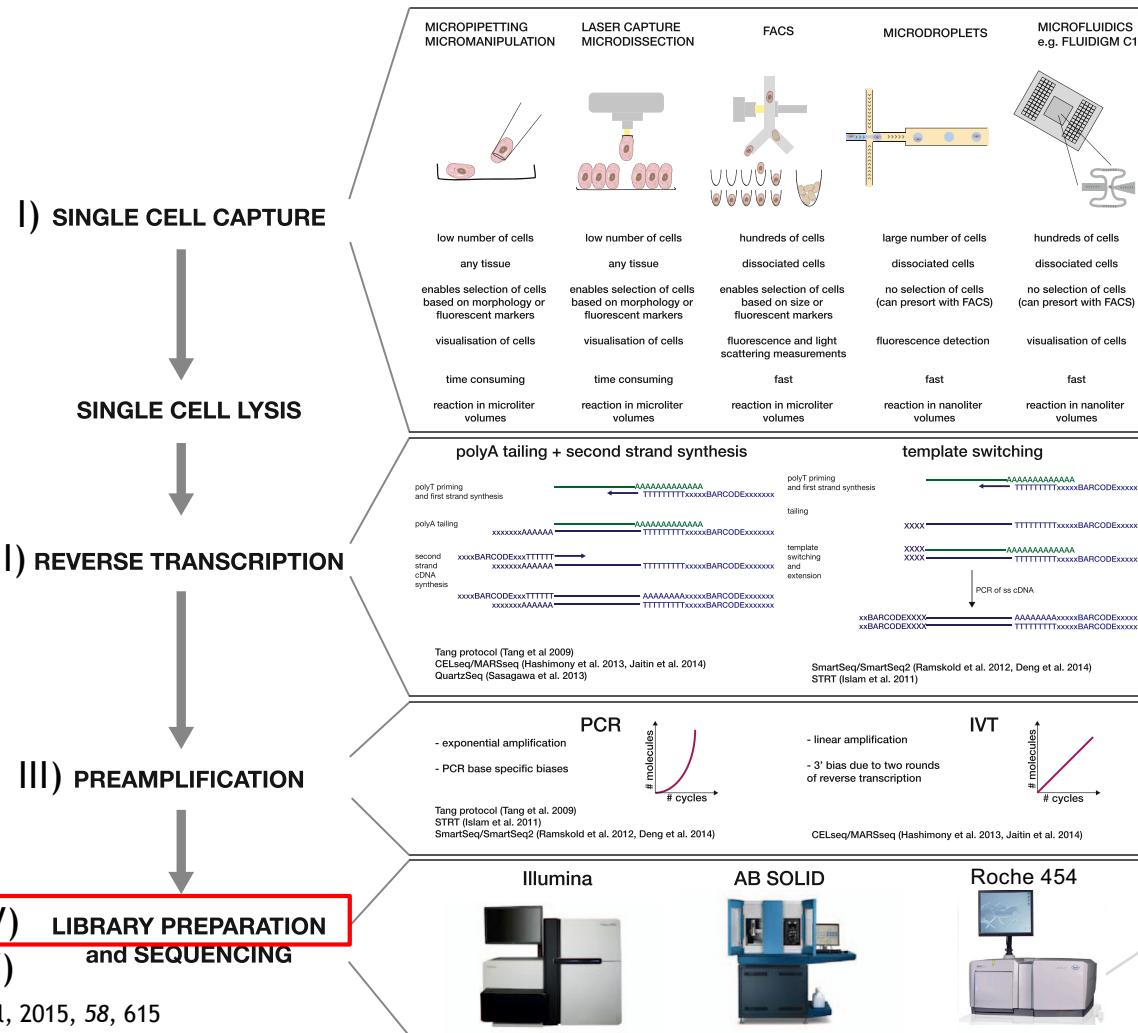
Smart-Seq(2), STRT-Seq, (Drop-seq, 10X)

PCR

- exponential amplification (fast)
- error prone



Single-cell RNA sequencing experiment workflow



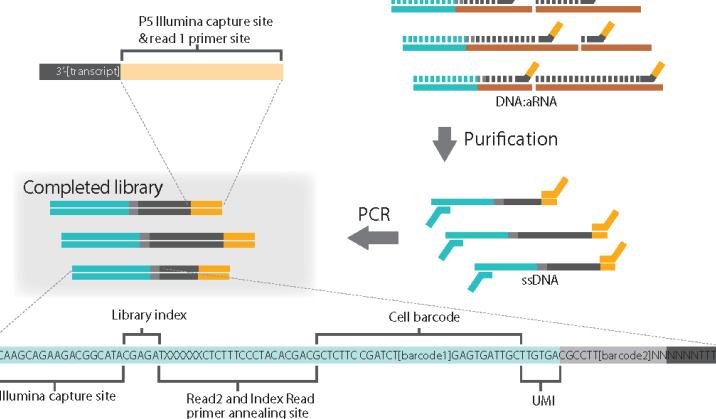
IV) Library preparation

Cel-Seq(2), (InDrop)

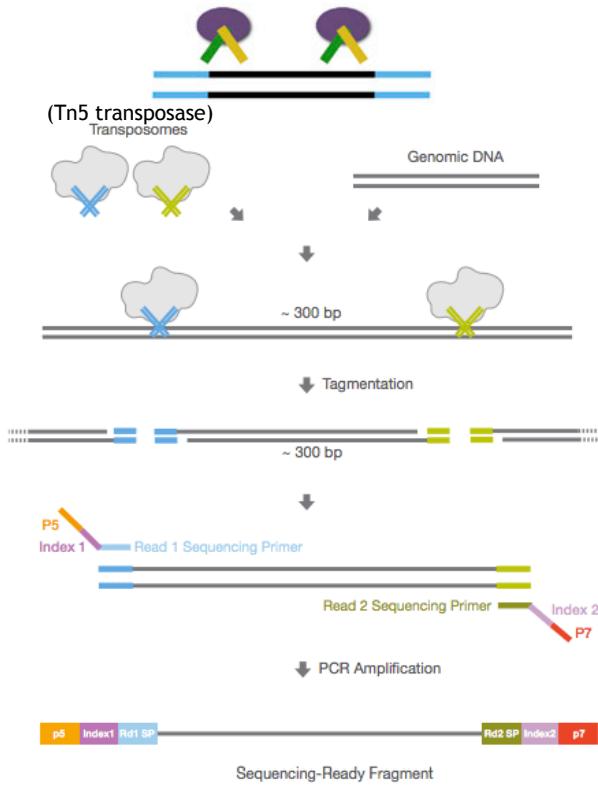
RNA fragmentation & reverse transcription (cDNA)



RNA Fragmentation:
heat & mild-base



Smart-Seq(2),
(Drop-seq, 10X)
Tagmentation



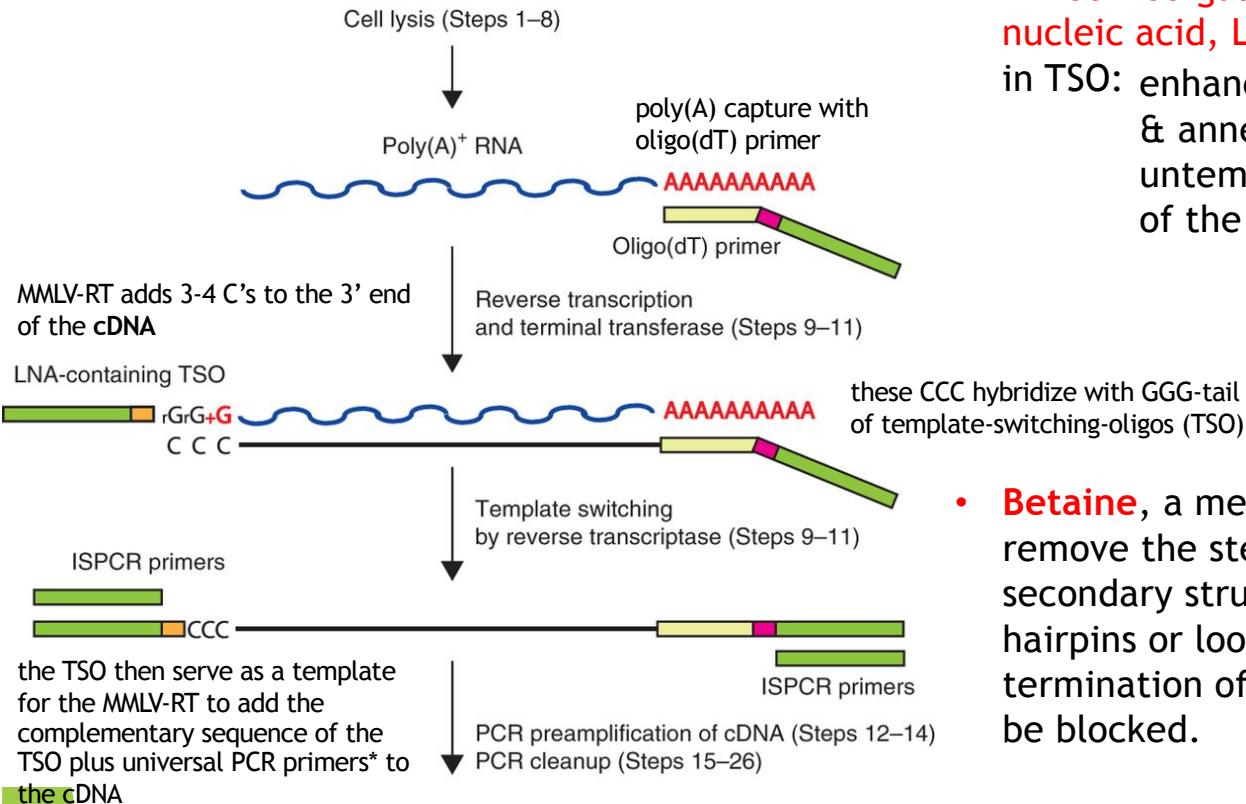
Three most popular plate-based scRNAseq

- SMART-seq2
- CEL-seq2
- STRT-seq

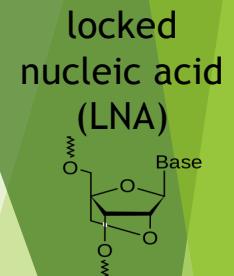
SMART-seq2

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

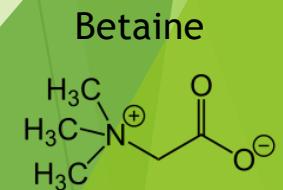
RNA capture and cDNA synthesis



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

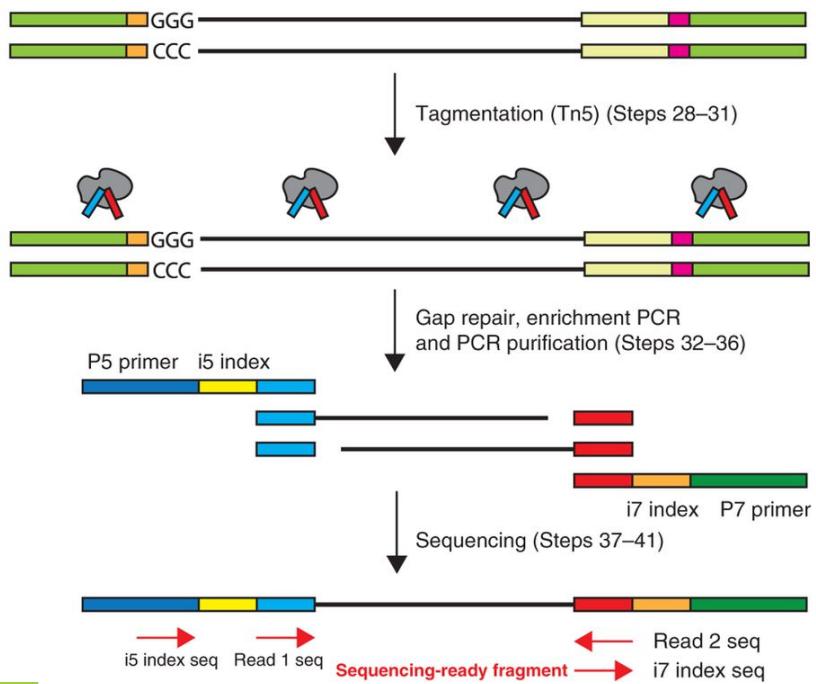


- **Betaine**, a methyl group donor, can remove the steric hindrance of secondary structures of RNAs (such as hairpins or loops) so that early termination of chain elongation can be blocked.



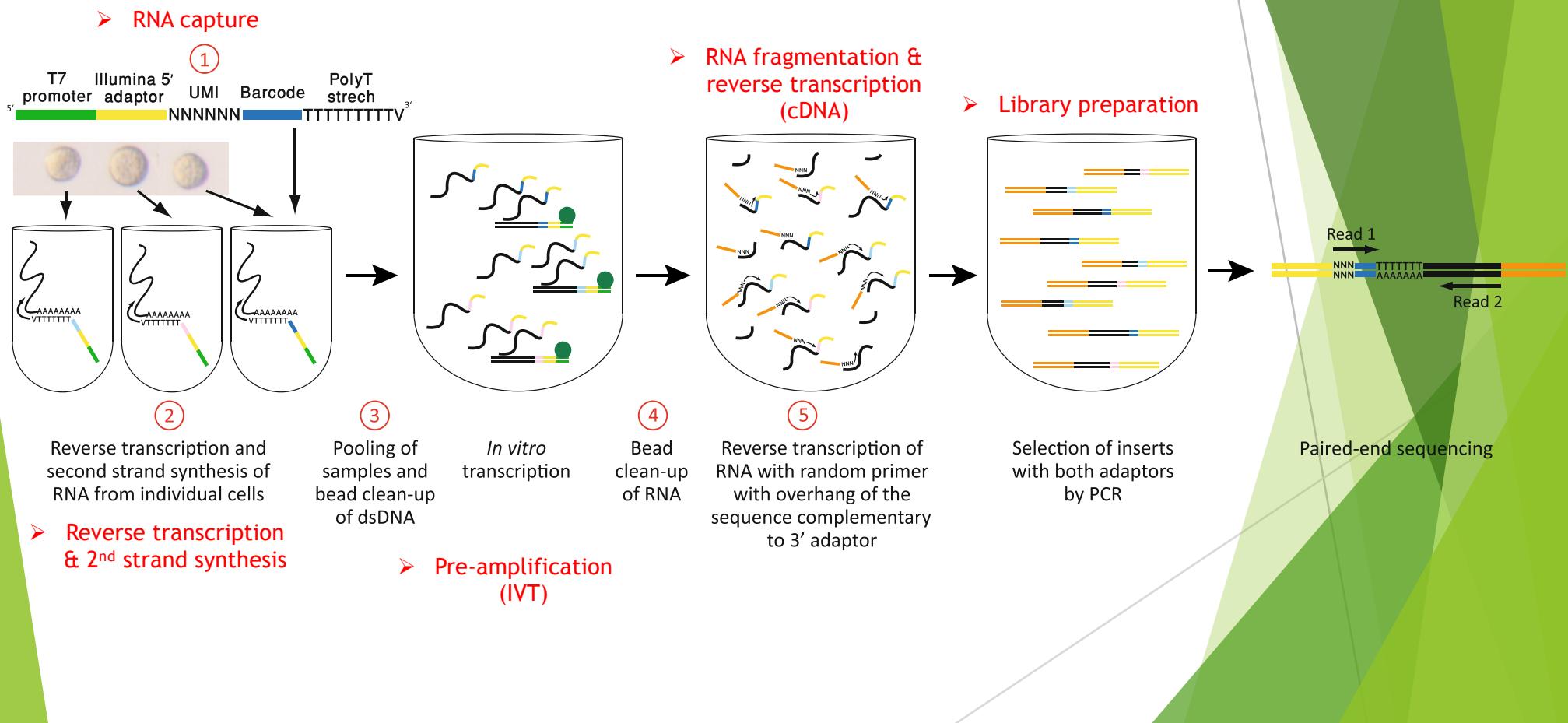
SMART-seq2

Library preparation

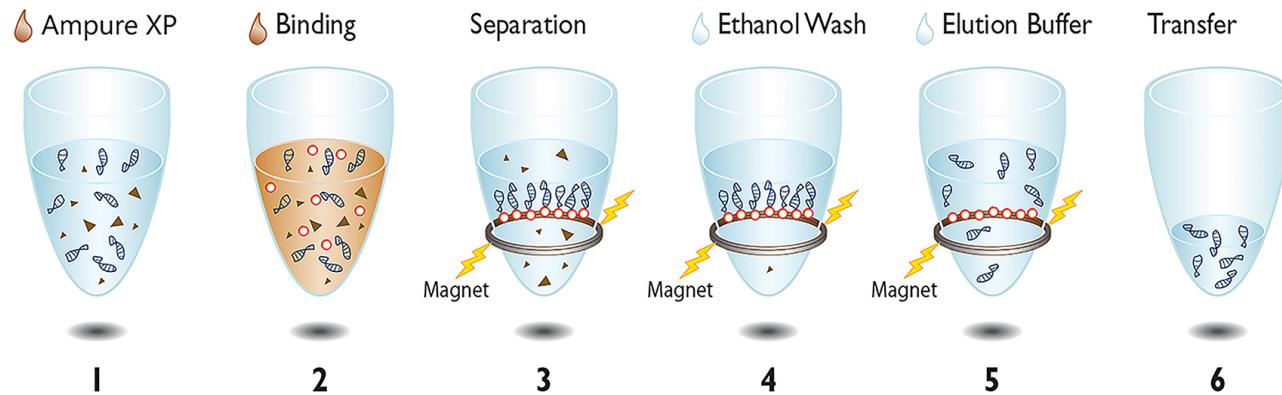


- amplification with few PCR cycles
- **tagmentation:** combining fragmentation and sequencing adapter integration
 - hyperactive derivative of the the Tn5 transposase **cuts** the cDNA and **ligates** sequencing adapters

CEL-seq2



SPRI Bead technology

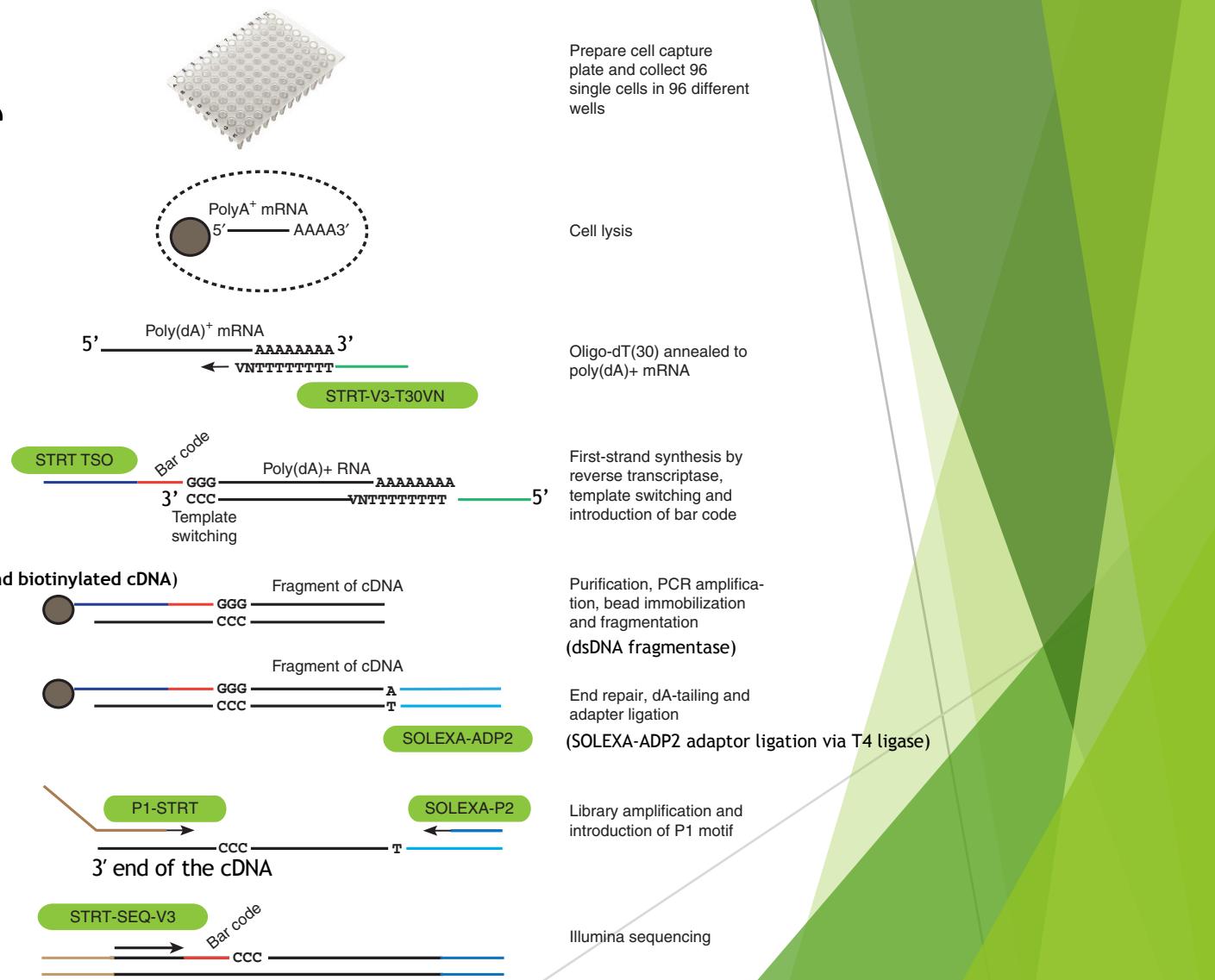


- **Solid Phase Reversible Immobilization**
- Carboxyl coated magnetic particles suspended in a solution of 10% PEG and 1.25M NaCl
- Reversibly binds DNA
 - Hawkins, et al. (1994) DNA purification and isolation using a solid-phase. Nucleic Acids Research, 22(21):4543-4544

STRT-seq: single-cell tagged reverse transcription sequencing

An upstream sequence must be introduced at the 3' end of the cDNA (5' end of the mRNA) to serve as template for the amplification using a universal primer (**biotinylated primer**)

In this method, the sequenced fragments correspond to a template-switching site located preferentially at the **5' end of mRNA**, which can be used to analyze promoter usage in single cells, to characterize **transcription start sites** and to analyze **enhancer elements**.



Comparison

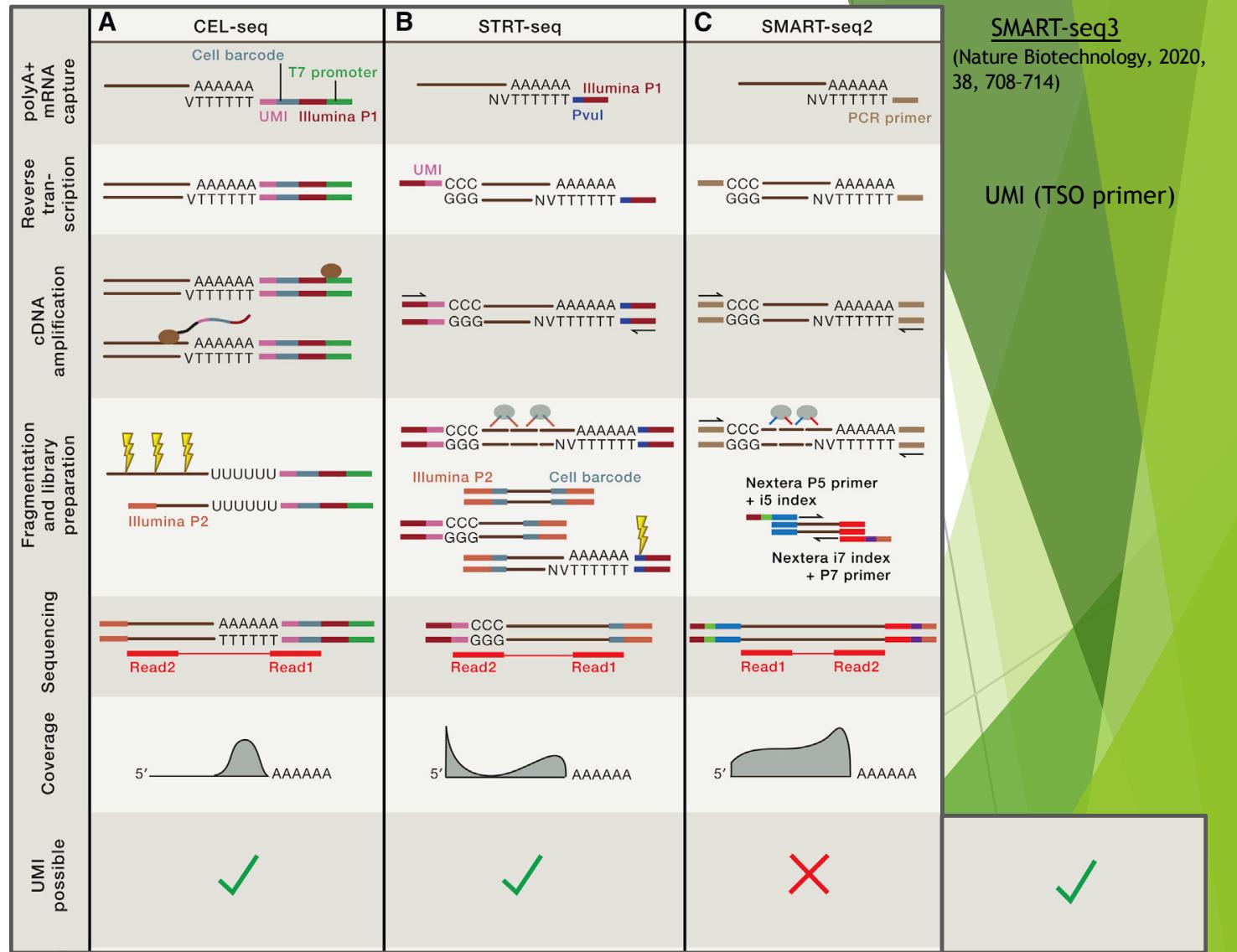
Leading Edge
Primer

Cell

Design and Analysis of Single-Cell Sequencing Experiments

Dominic Grün^{1,2,3} and Alexander van Oudenaarden^{1,2,*}

Cell, 2015, 163, 799

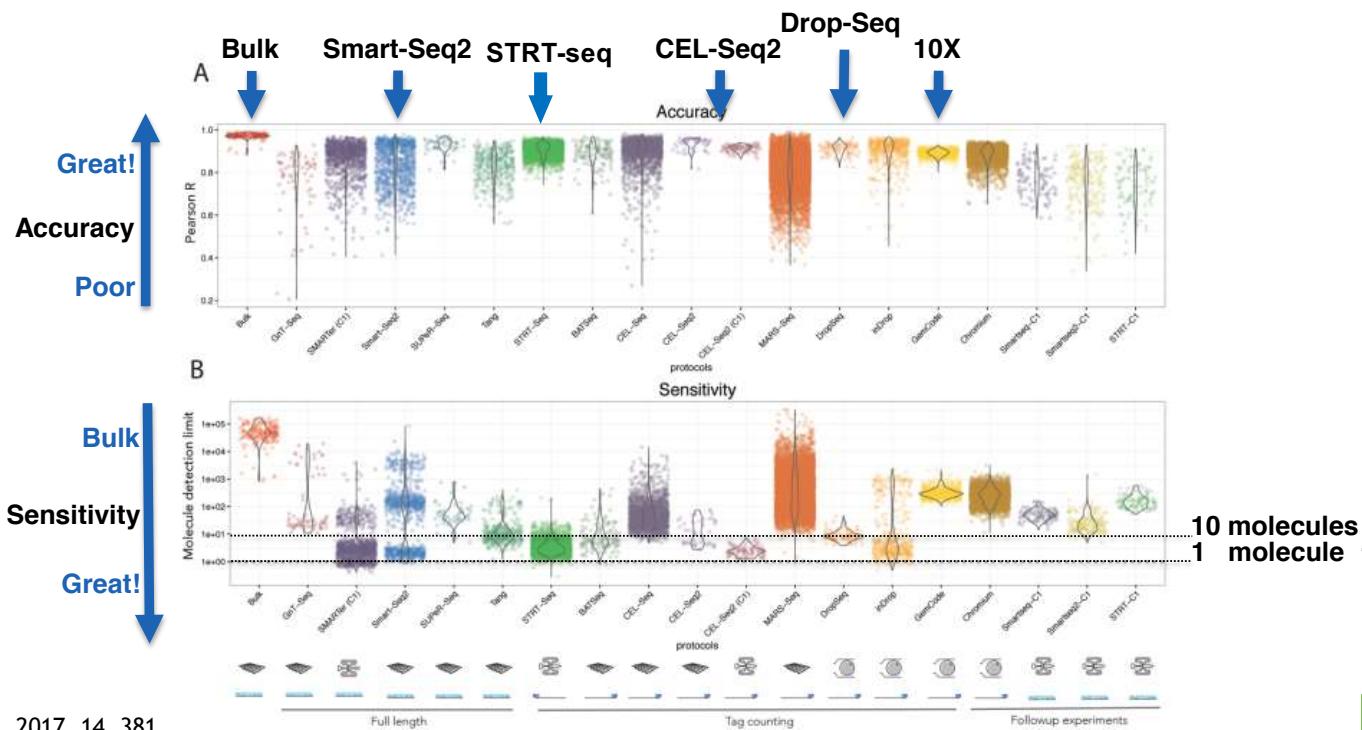


Comparison

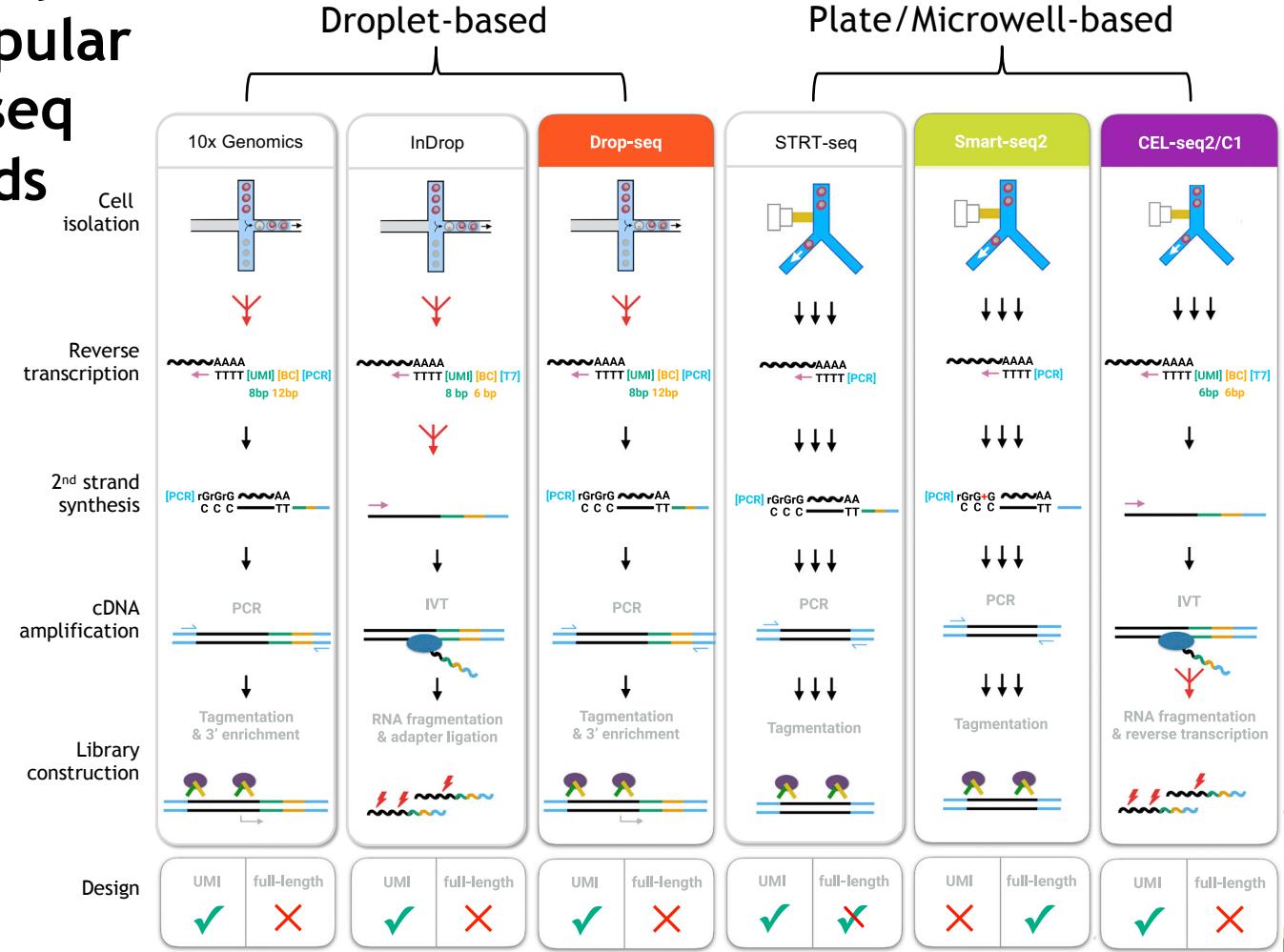
ANALYSIS

Power analysis of single-cell RNA-sequencing experiments

Valentine Svensson^{1,2,6}, Kedar Nath Natarajan^{1,2,6}, Lam-Ha Ly², Ricardo J Miragaia^{2,3}, Charlotte Labalette^{2,4,5}, Iain C Macaulay², Ana Cvejic^{2,4,5} & Sarah A Teichmann^{1,2}



Summary of the popular scRNAseq methods

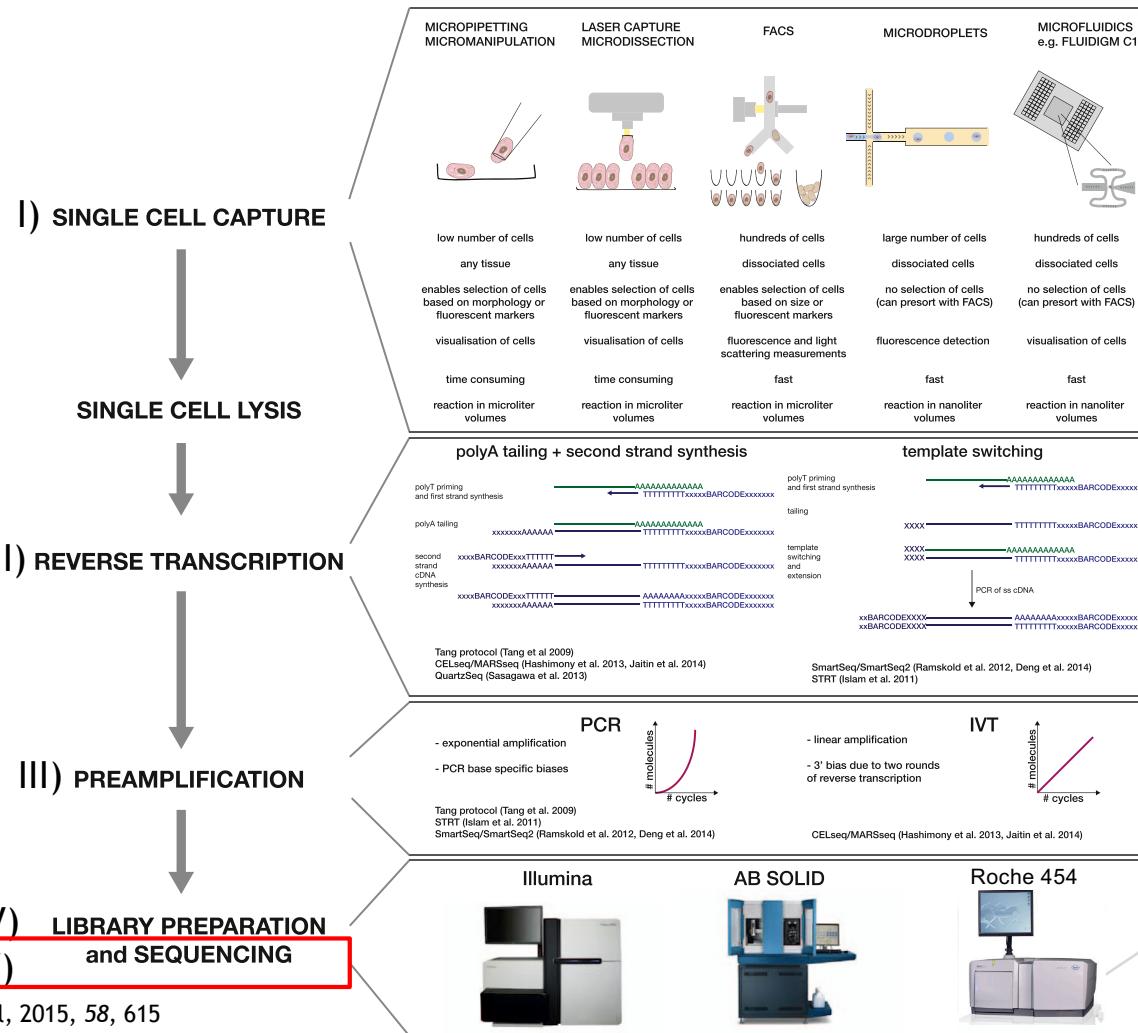


Which method should I use?

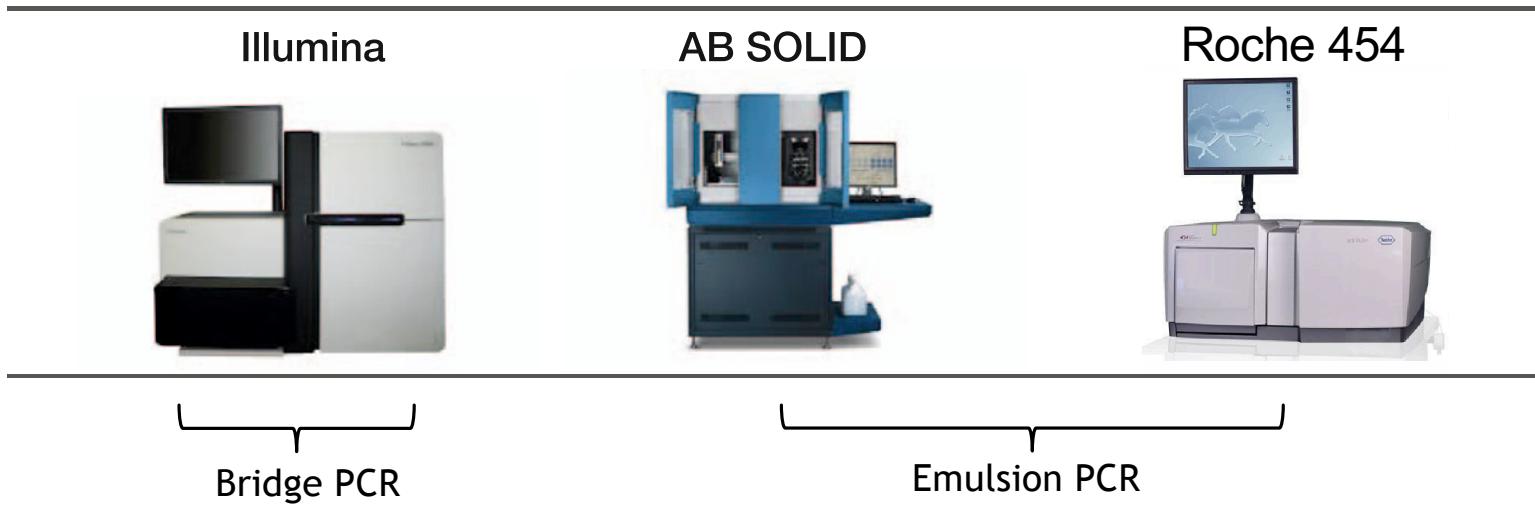


- If you want to study overall variability in transcription of cells within or across different tissues? **Many cells (hundreds to thousands); droplet-based methods**
- If you want to look at a few genes associated with a specific process, such as cell death? **Plate-based methods (deeper sequencing depth)**
- 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 (STRT-seq?)**
- To have less errors? **IVT-based amplification method (CEL-seq, InDrop)**
- To study transcription start sites? **STRT-seq, SMART-seq**

Single-cell RNA sequencing experiment workflow

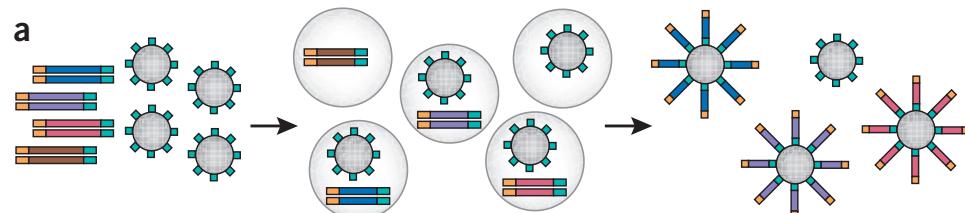
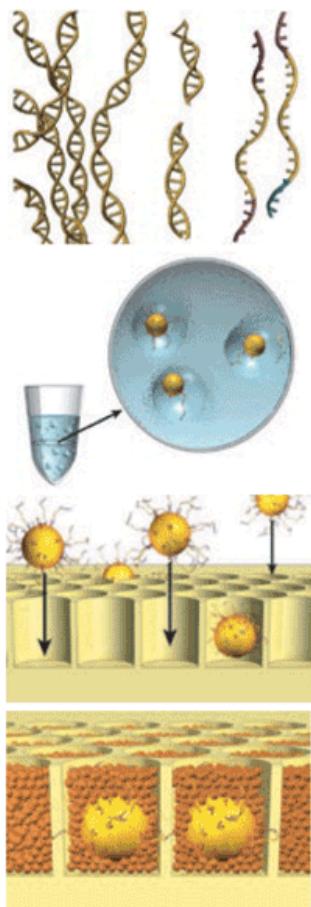


V) Sequencing



Next-generation sequencing platforms

1) Emulsion PCR



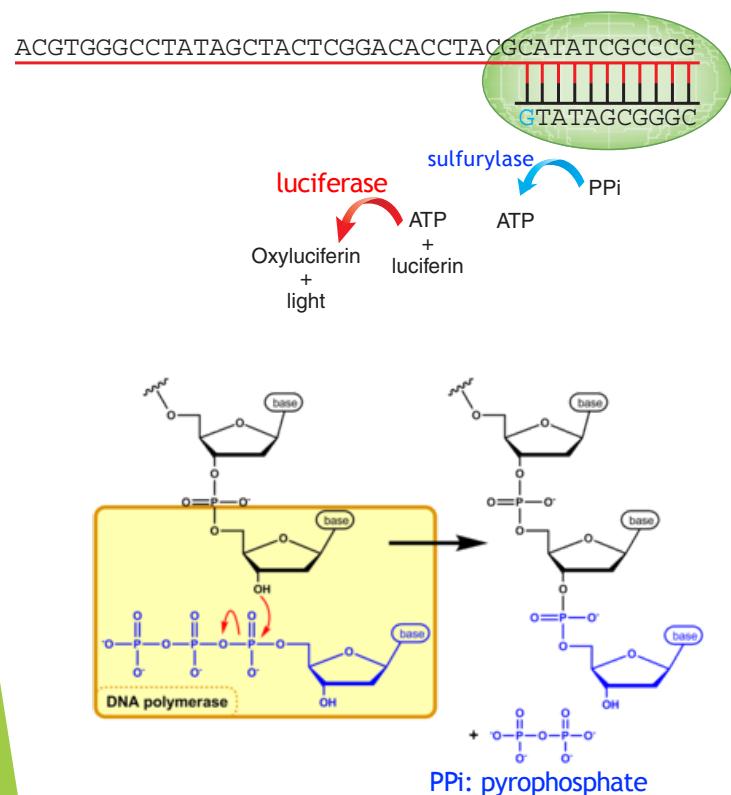
- Fragments, with adaptors, are PCR amplified within a water drop in oil
- One primer is attached to the surface of a bead
- Used by Roche 454 and AB SOLiD

Nat Biotechnol, 2008, 16, 1117

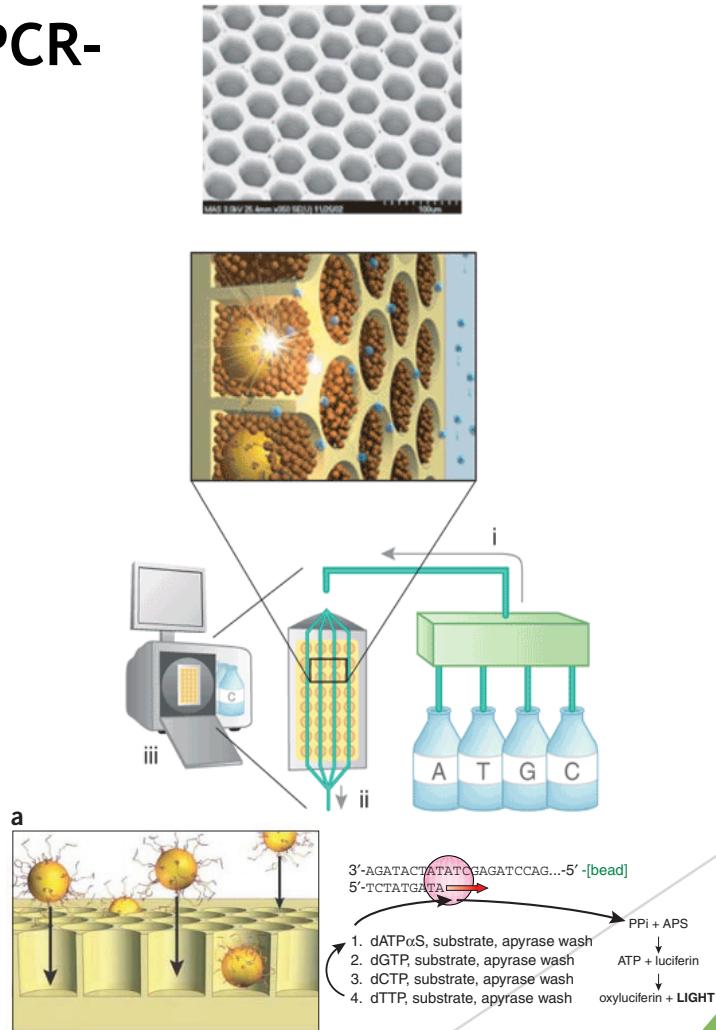
Nature Biotechnology, 2008, 26, 1135-1145

Next-generation sequencing platforms

Pyrosequencing (emulsion PCR-based seq, used by 454)

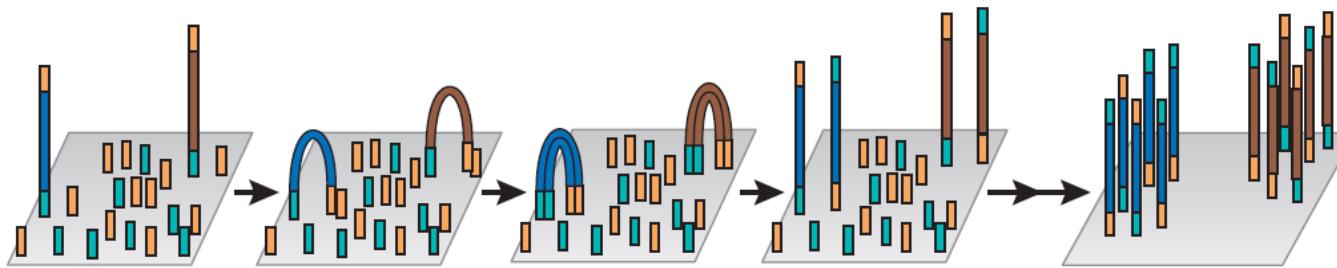


Nat Biotechnol, 2008, 16, 1117



Next-generation sequencing platforms

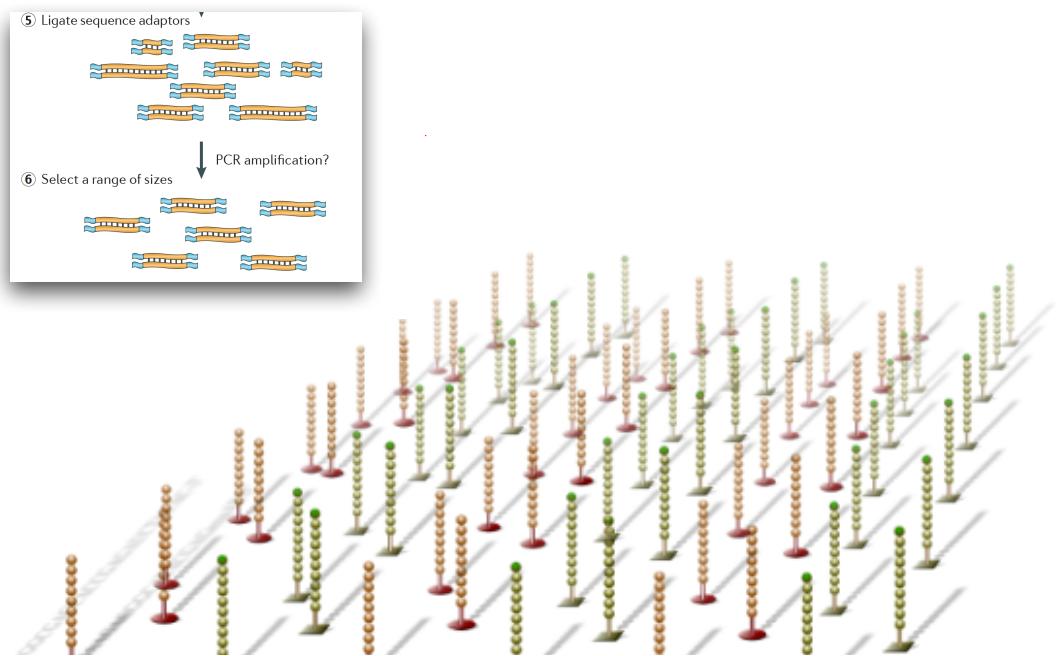
2) Bridge PCR (used by Illumina)



Illumina Sequencing

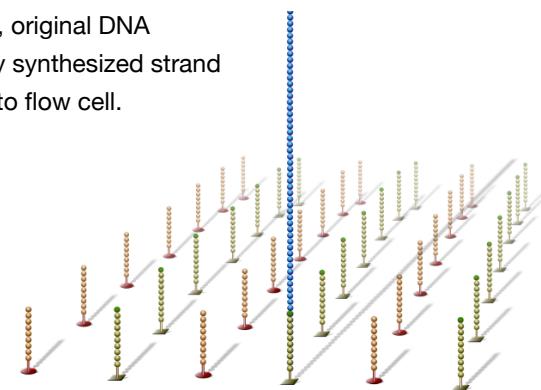
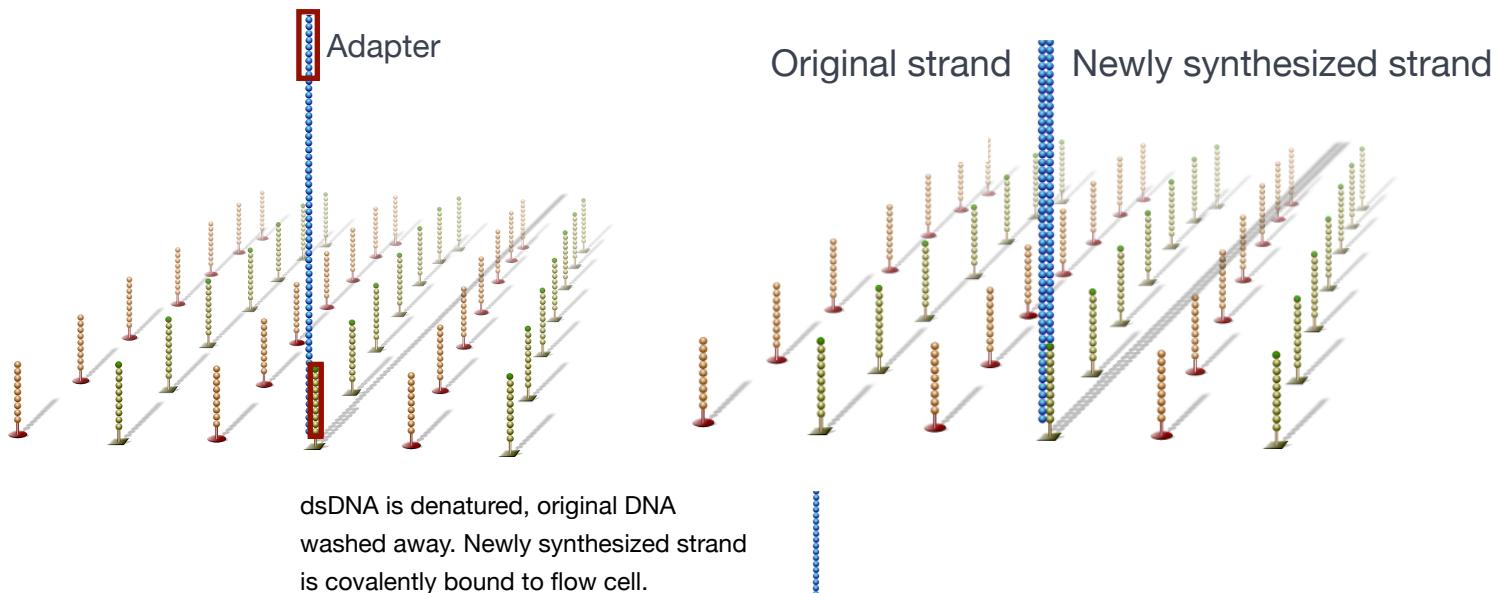
Flow cell

TTAATGATAACGGGACCAACCGAGAUCTACAC-3'
TTTCAAGCAGAAAGACGGCATACGGAGoxoAT-3'



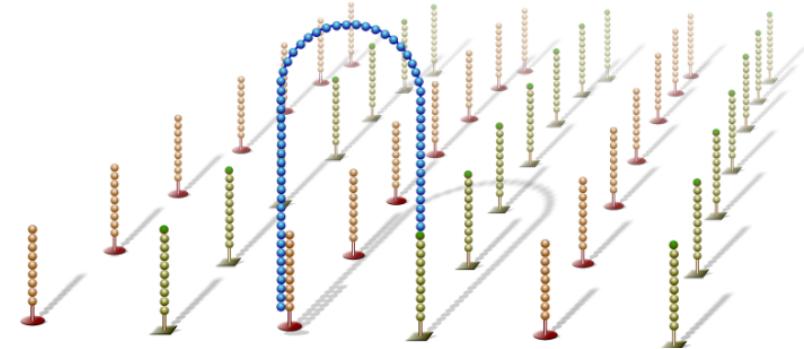
Illumina Sequencing

Cluster generation

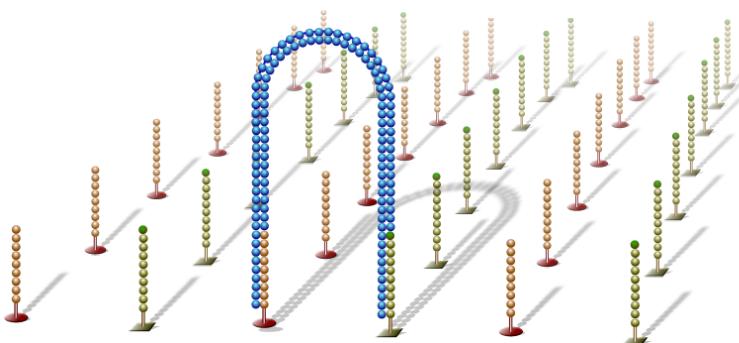


Illumina Sequencing

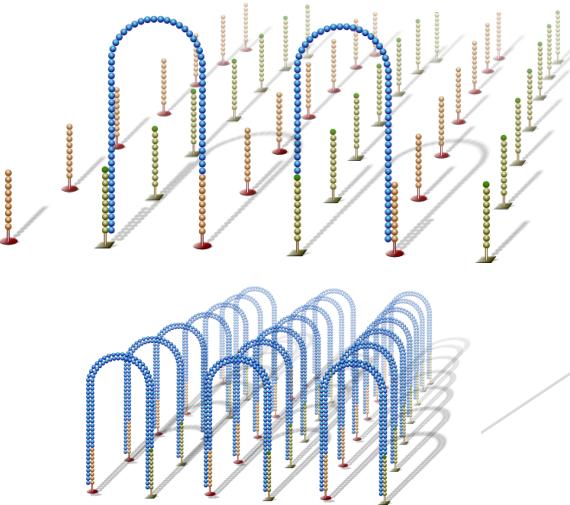
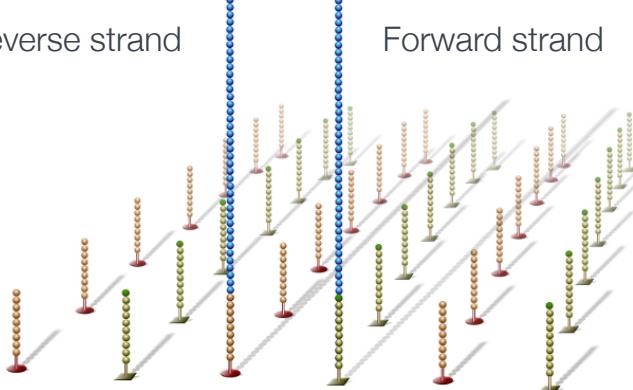
Bridge amplification



Reverse strand

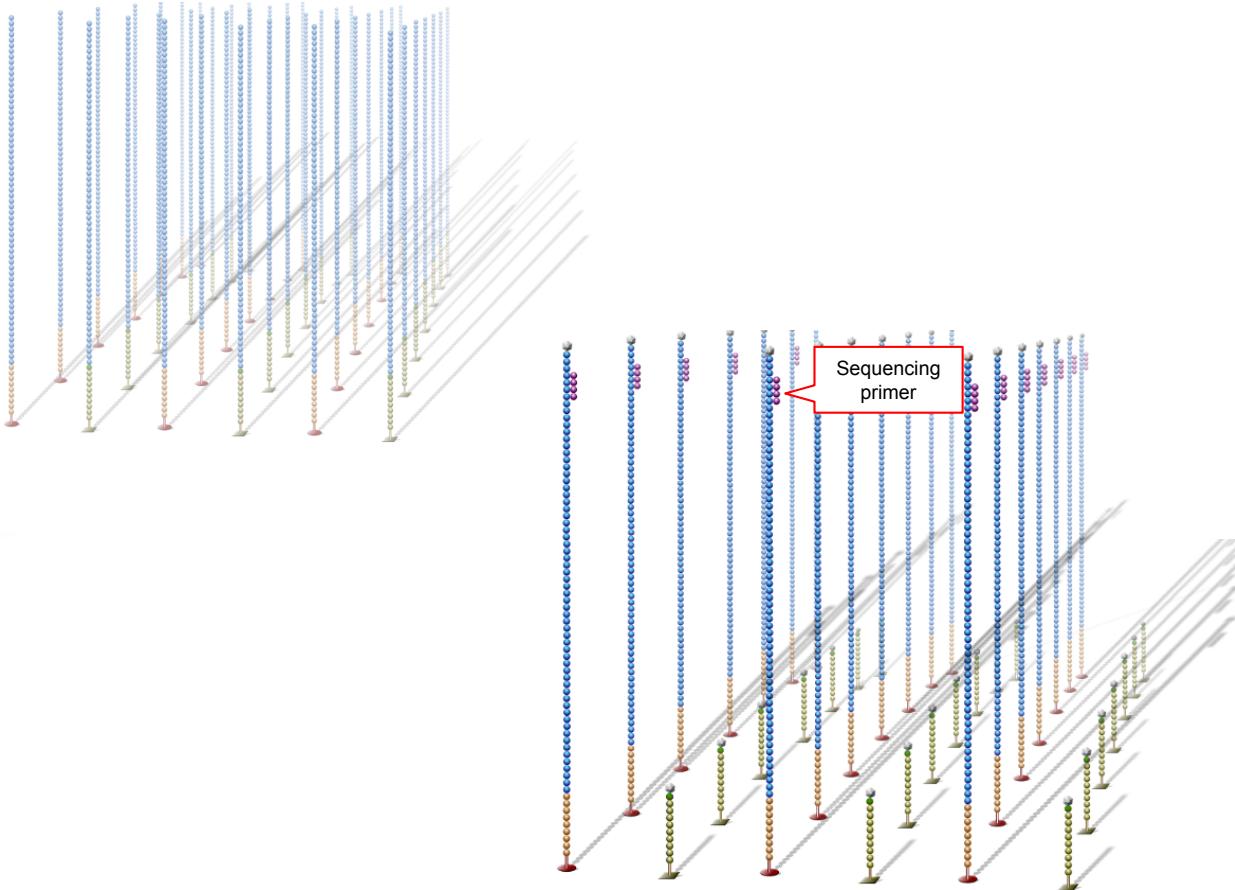
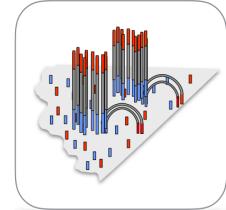


Forward strand



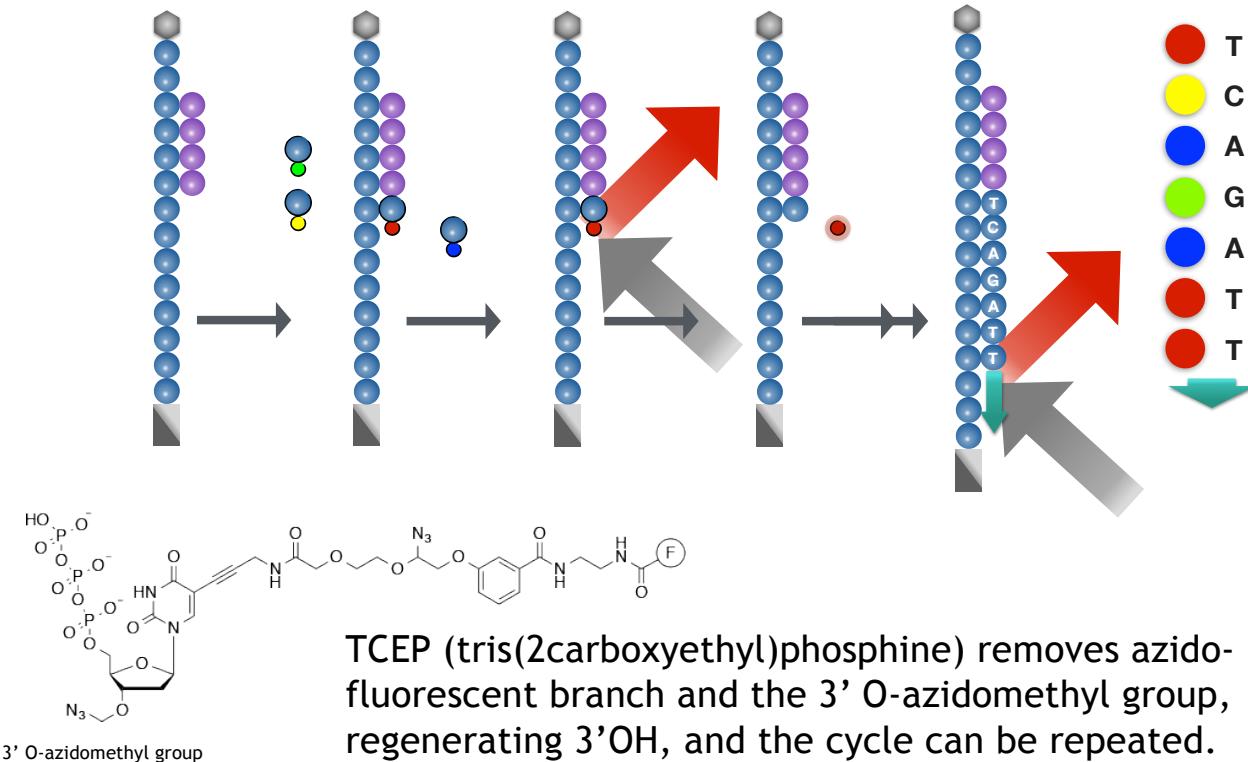
Illumina Sequencing

Preparation for sequencing



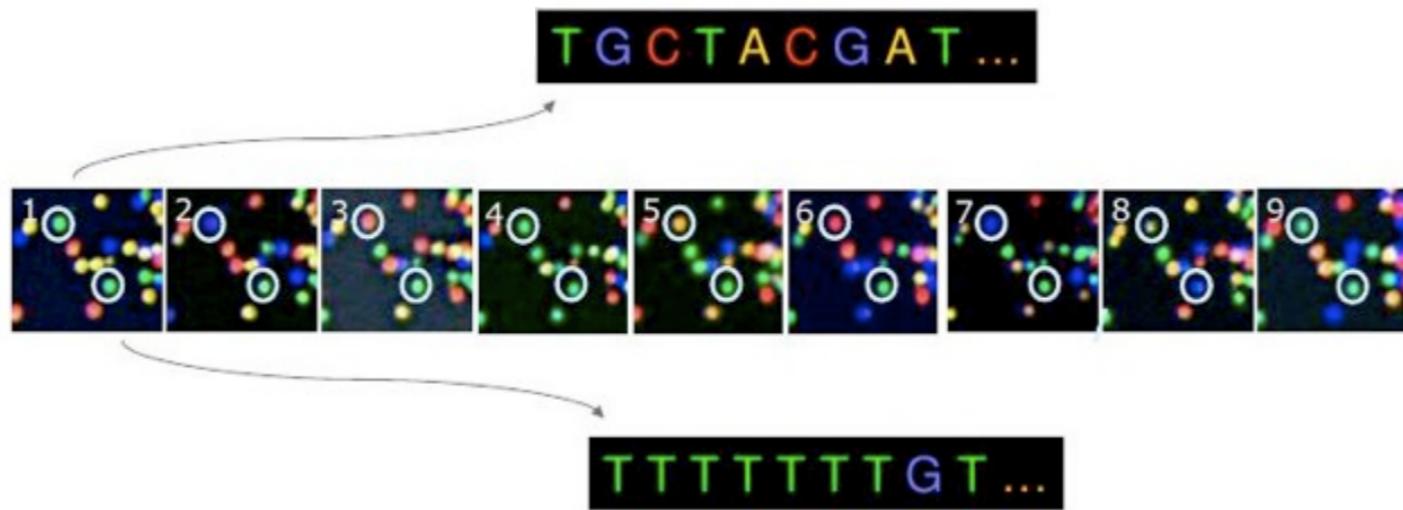
Illumina Sequencing

Sequencing by synthesis



Illumina Sequencing

Base calling



Conclusion

- **General introduction about scRNAseq**
 - scSeq vs NGS
- **Different types of plate-based scRNAseq**
 - SMART-seq2, CEL-seq2, STRT-seq
- **Workflow of different plate-based scRNAseq**
 - Single cell capture, cell lysis, reverse transcription, pre-amplification, library preparation, sequencing
- **Which method to choose?**
 - Full-length, 3'-end, 5'-end, comparing cell types btw tissues (many cells, droplet-based), deeper sequencing depth (fewer cells, plate-based)

