

# Plate-based single cell RNA sequencing (scRNAseq)

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(Single cell technology for cancer biology)

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# Outline

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

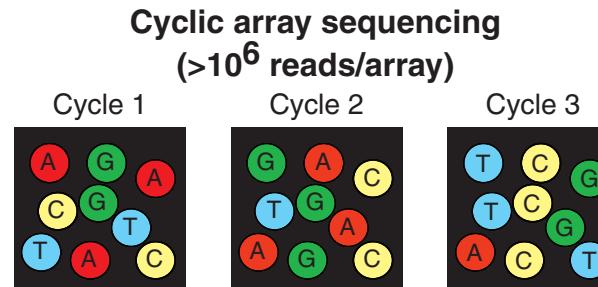
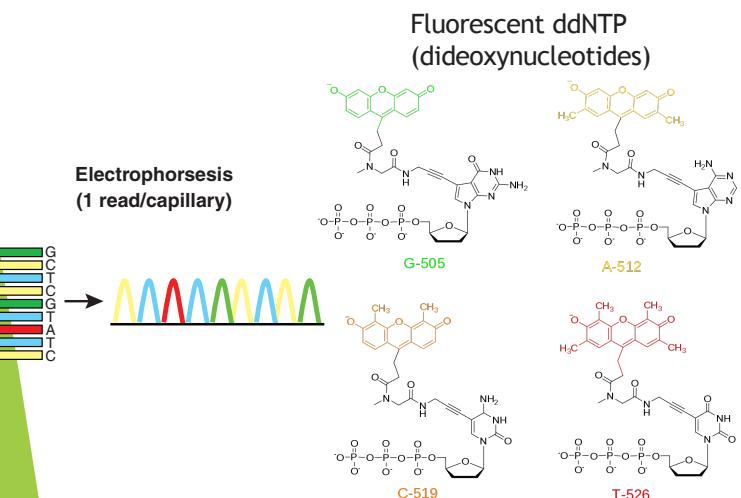
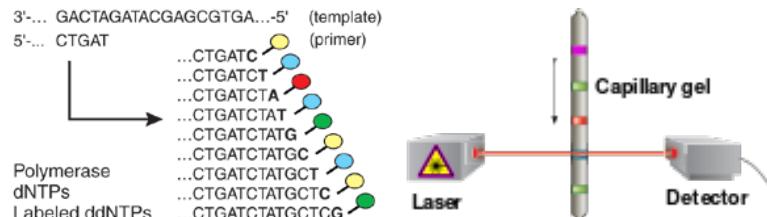


single cell sequencing  $\stackrel{?}{=}$  next-generation sequencing



# Traditional sequencing vs next-generation sequencing?

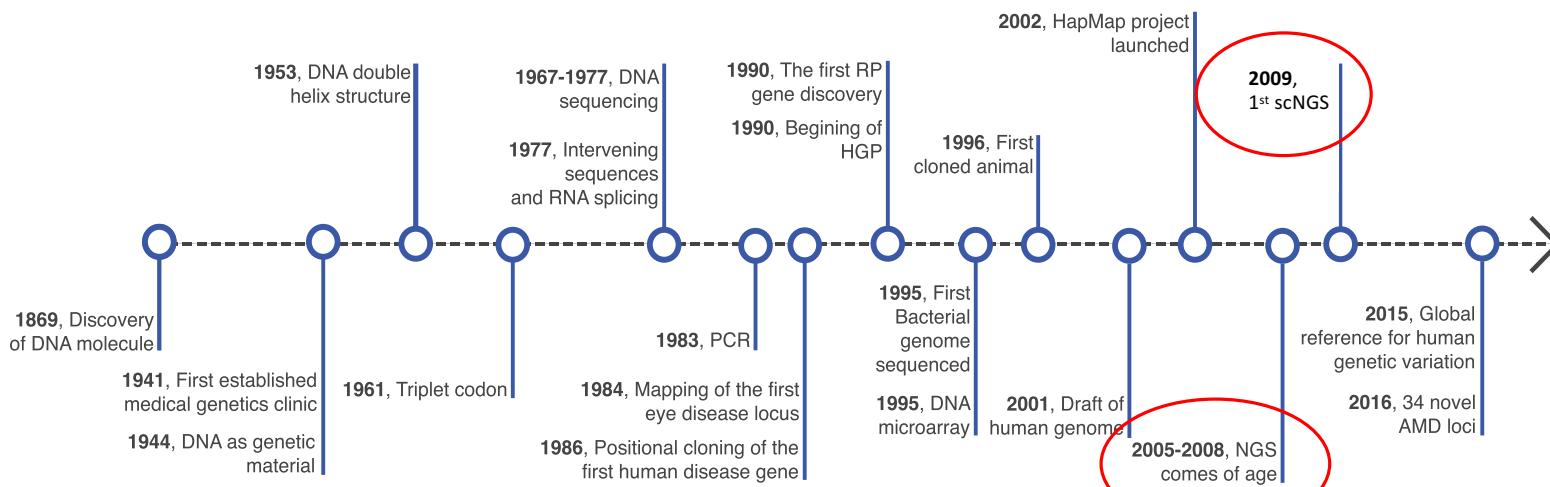
- From sanger sequencing ..... to next-generation sequencing



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

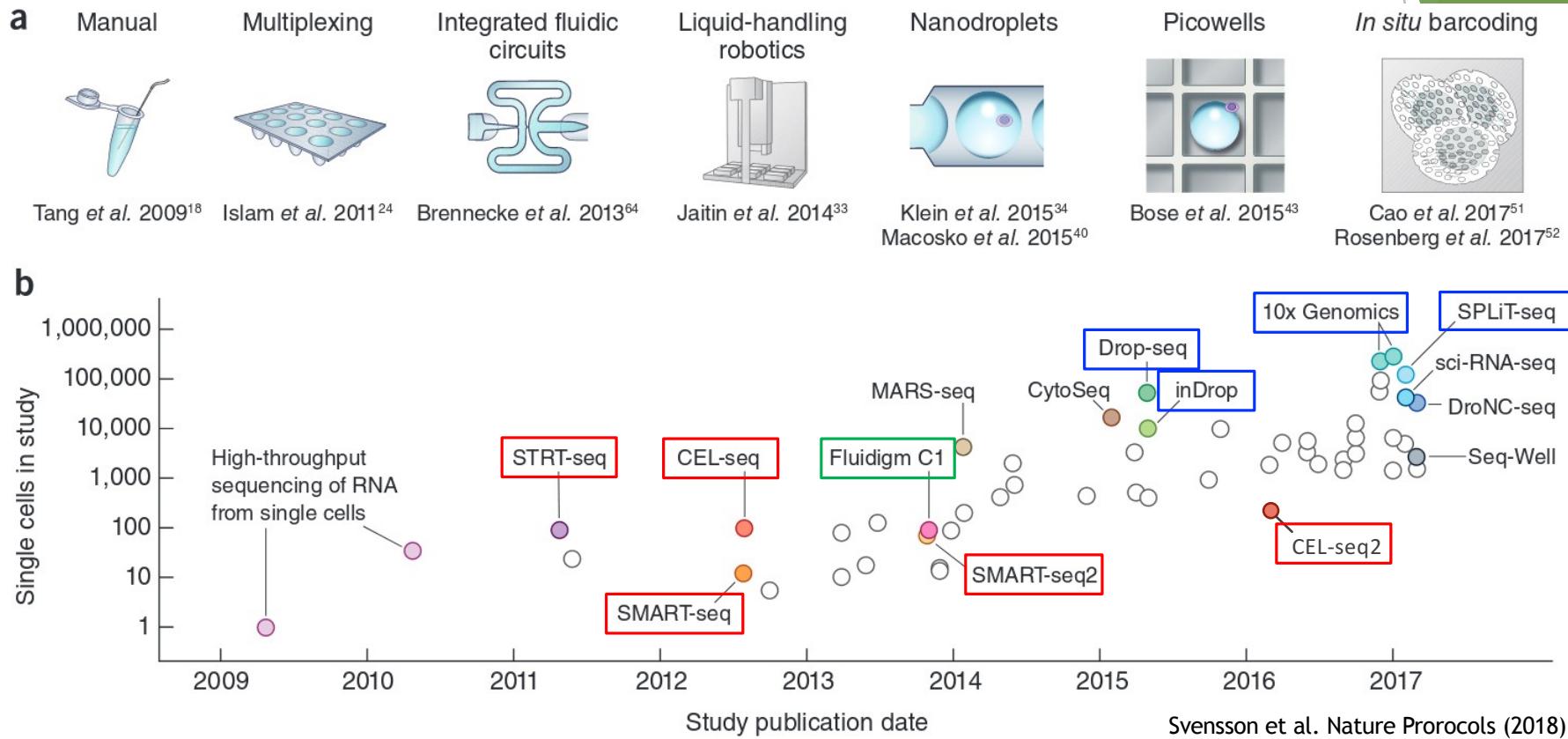
- Illumina/Solexa
- Roche 454
- ABI SOLiD
- ...

# Next-generation sequencing & single cell sequencing



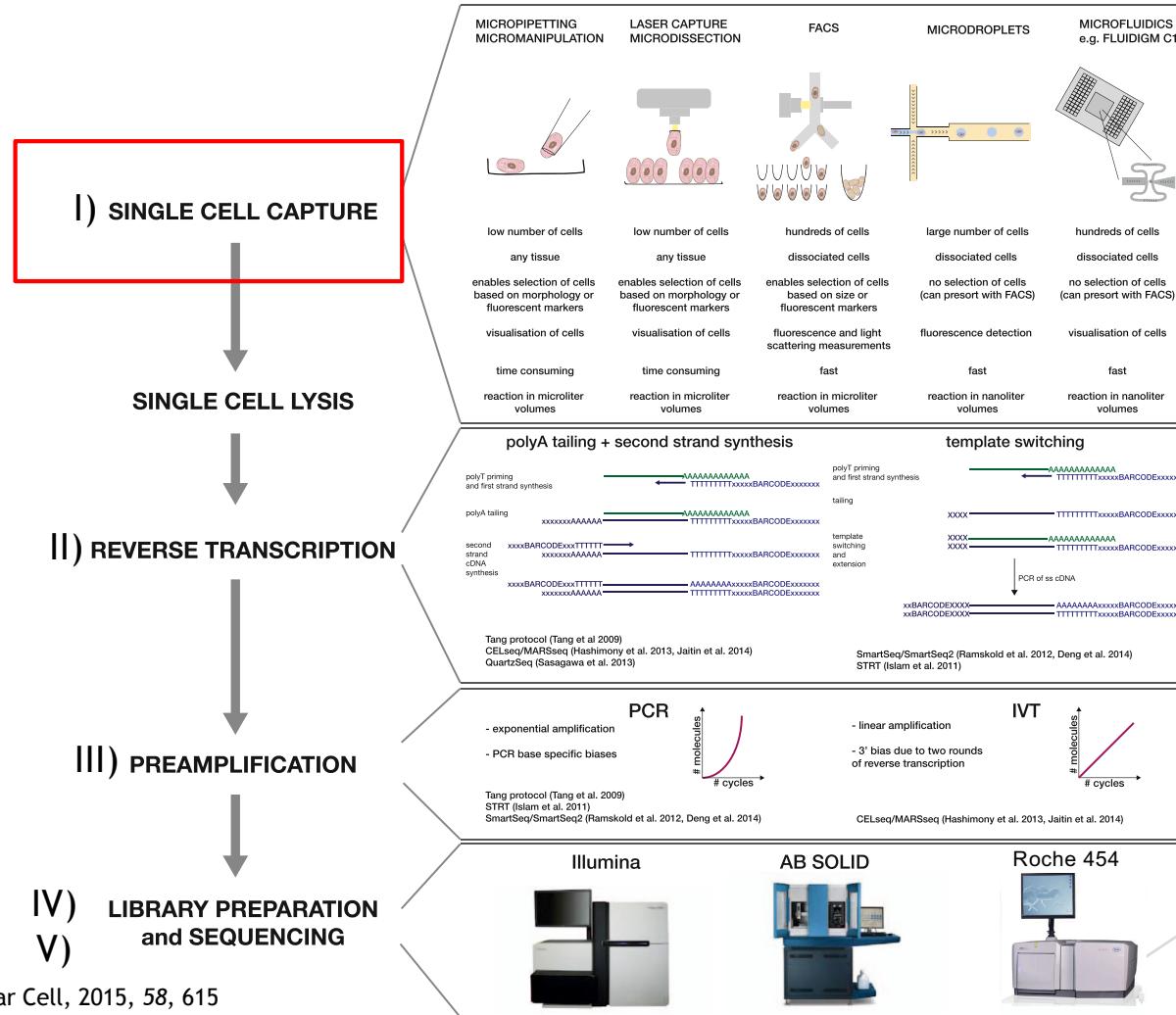
2005: 454 (Roche)  
2007: Illumina  
2008: SOLid (ABI)

# Evolution of scRNAseq techniques



- ~100s cells thanks to **multiplexing (barcode)**; ~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**

# Single-cell RNA sequencing experiment workflow



# I) Single cell capture

10X Genomics  
/Drop-seq/InDrop

Fluidigm (C1),  
Wafergen (iCell8),  
CellenOne

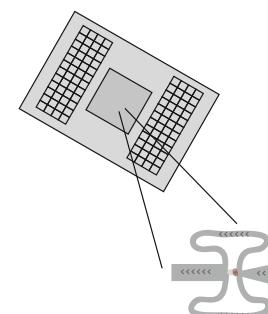
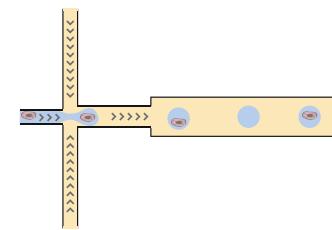
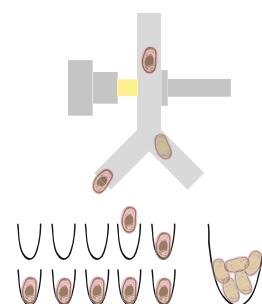
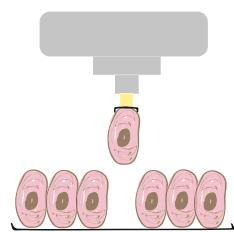
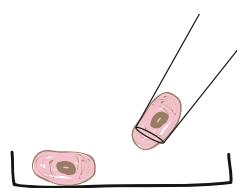
MICROPIPETTING  
MICROMANIPULATION

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

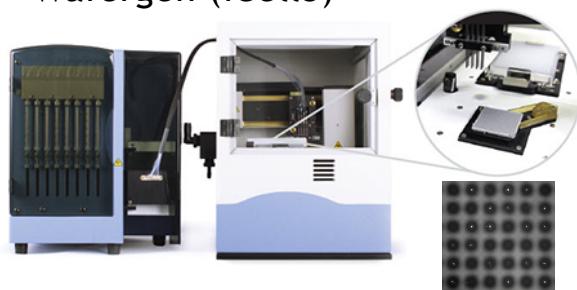
# I) Single cell capture

Fluidigm (C1):



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

Wafergen (iCell8)



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

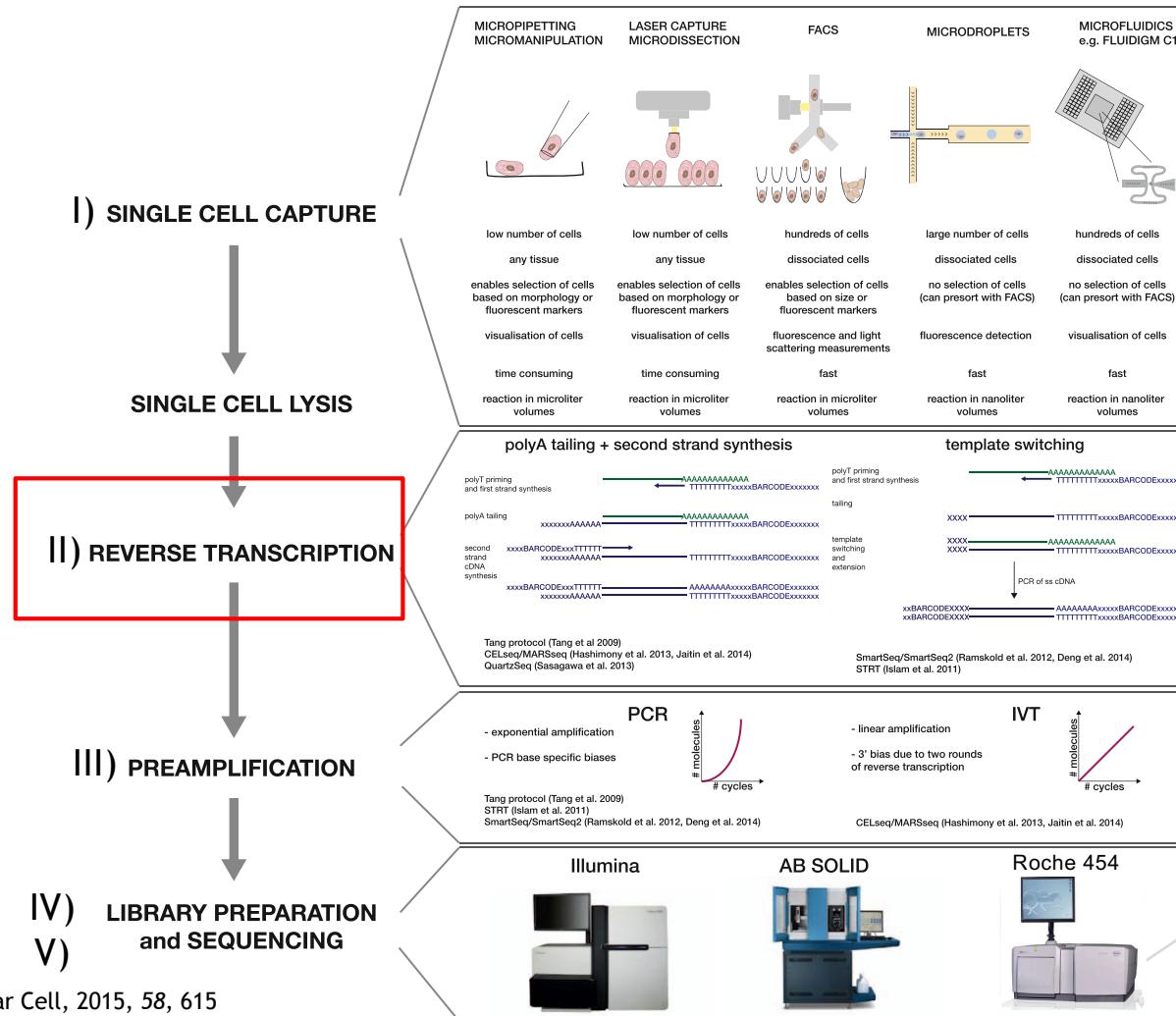


Cellenion (CellenONE)



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

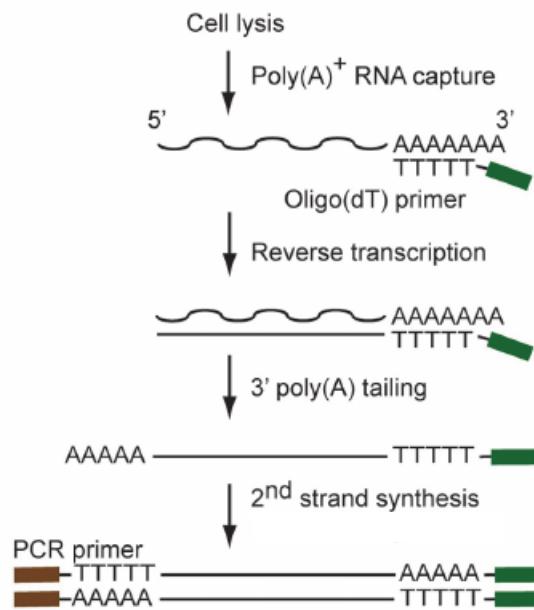
# Single-cell RNA sequencing experiment workflow



# II) Reverse transcription

Cel-Seq(2), (InDrop)

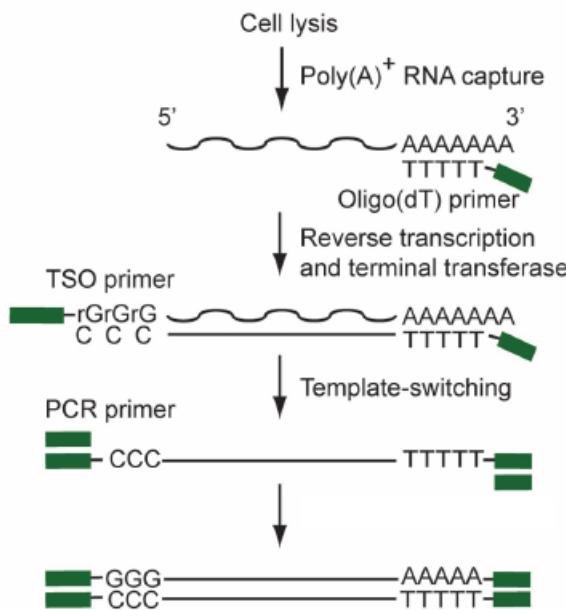
## 1) PolyA tailing + 2<sup>nd</sup> strand synthesis



PolyA tailing: added by template-free terminal transferase (in addition to dATP)

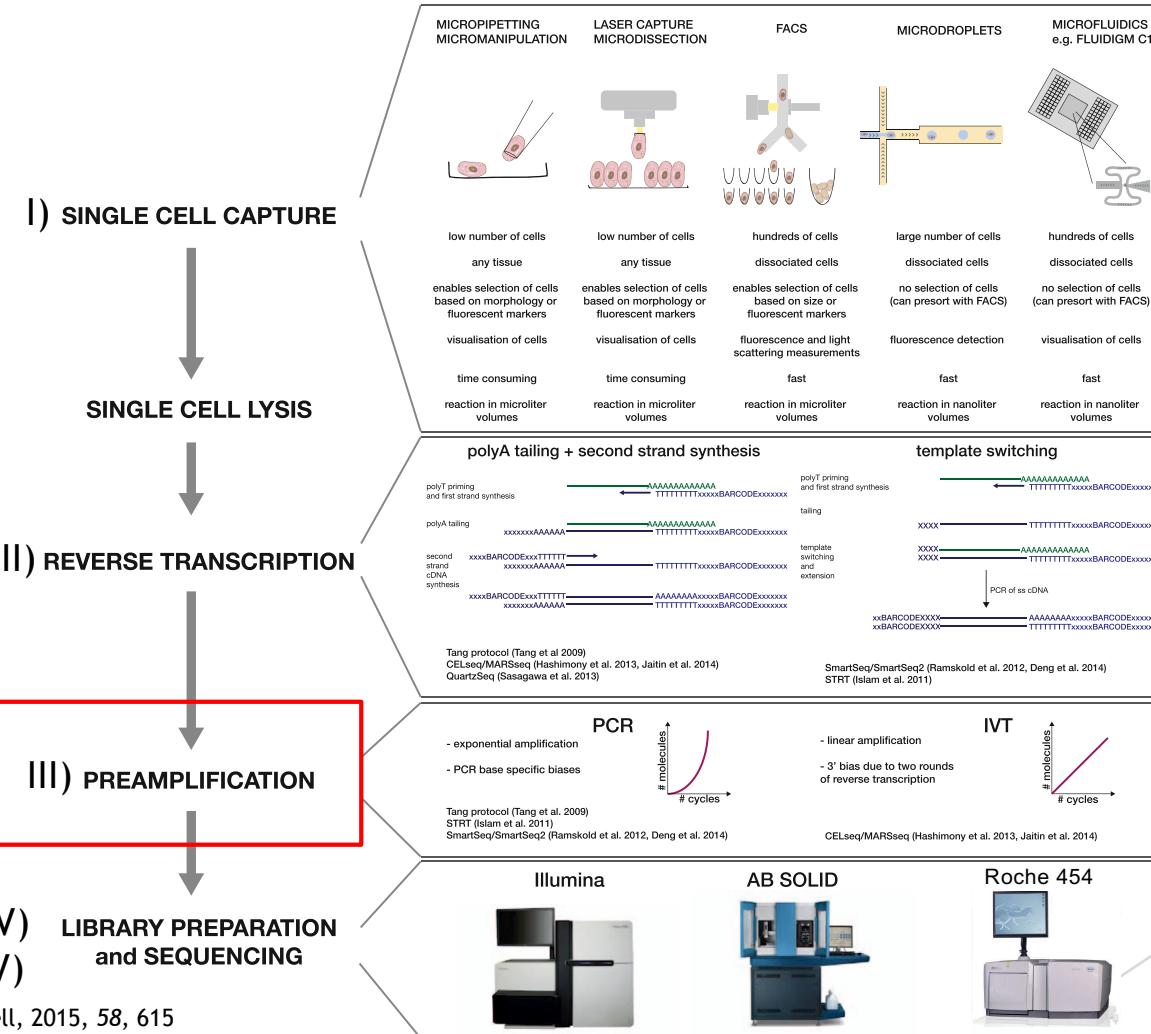
Smart-Seq(2), STRT-Seq, (Drop-seq, 10X)

## 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



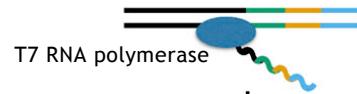
### III) Preamplification

Cel-Seq(2), (InDrop)

(In vitro transcription)

IVT

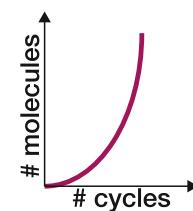
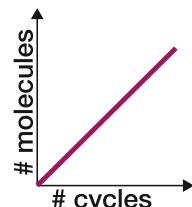
- linear amplification (slow)
- less error



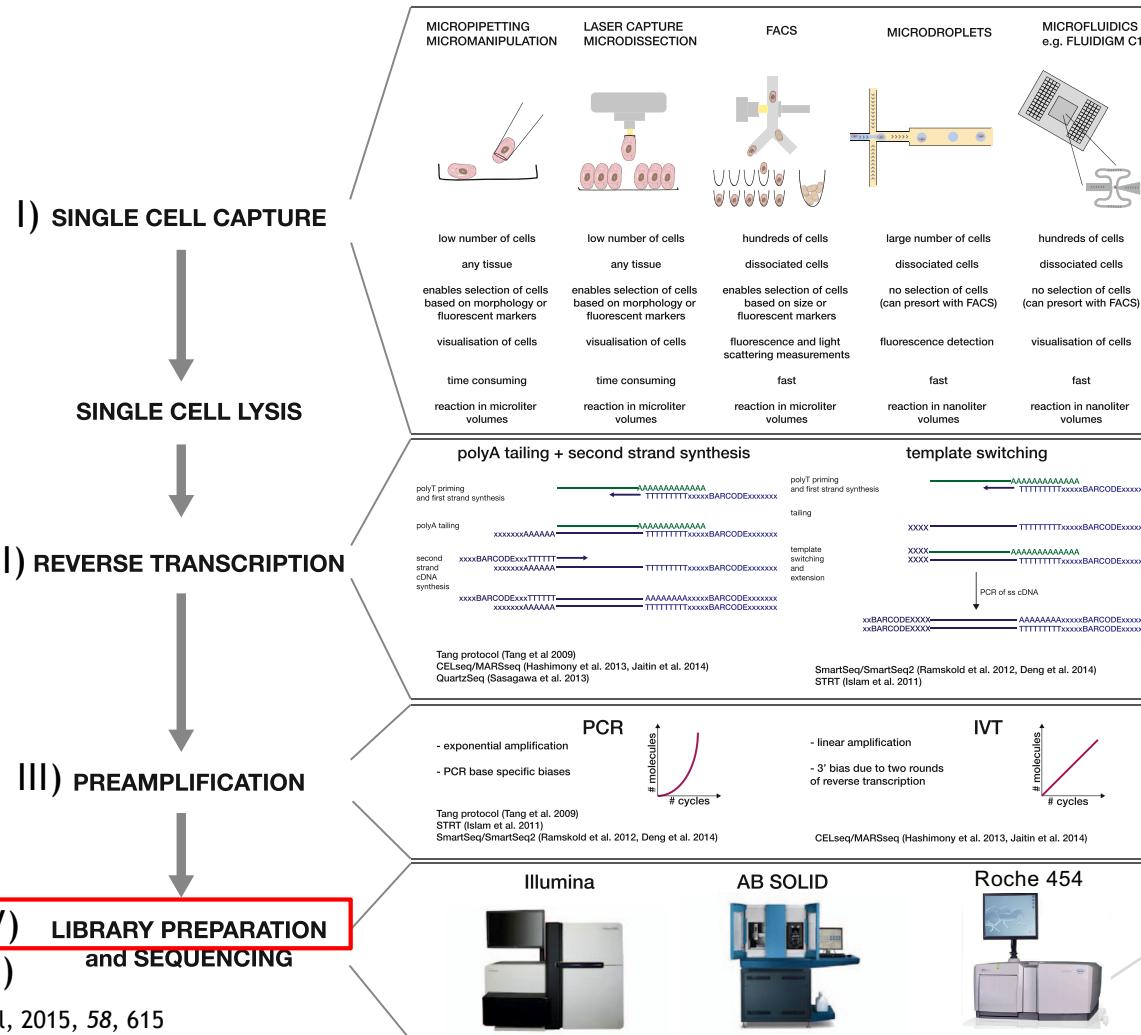
Smart-Seq(2), STRT-Seq, (Drop-seq, 10X)

PCR

- exponential amplification (fast)
- error prone



# Single-cell RNA sequencing experiment workflow



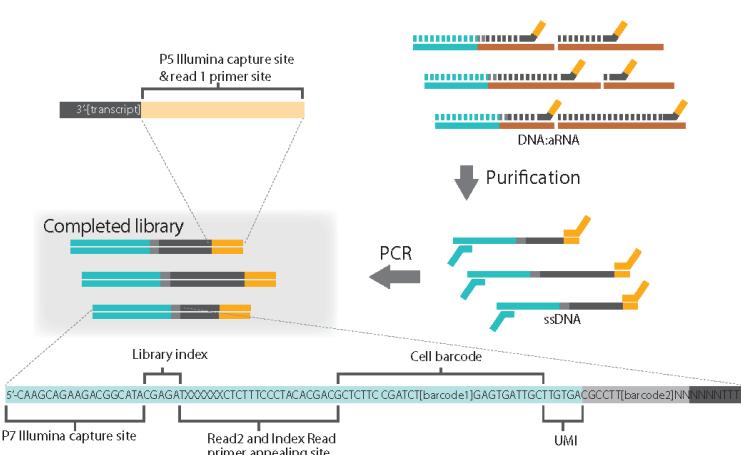
# IV) Library preparation

## Cel-Seq(2), (InDrop)

RNA fragmentation & reverse transcription (cDNA)

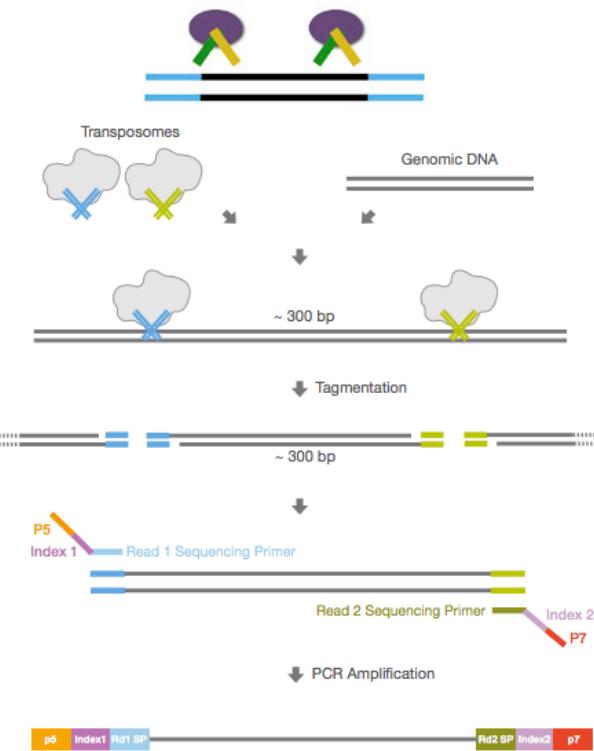


RNA Fragmentation:  
heat & mild-base



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

Tagmentation



## Three most popular plate-based scRNASeq

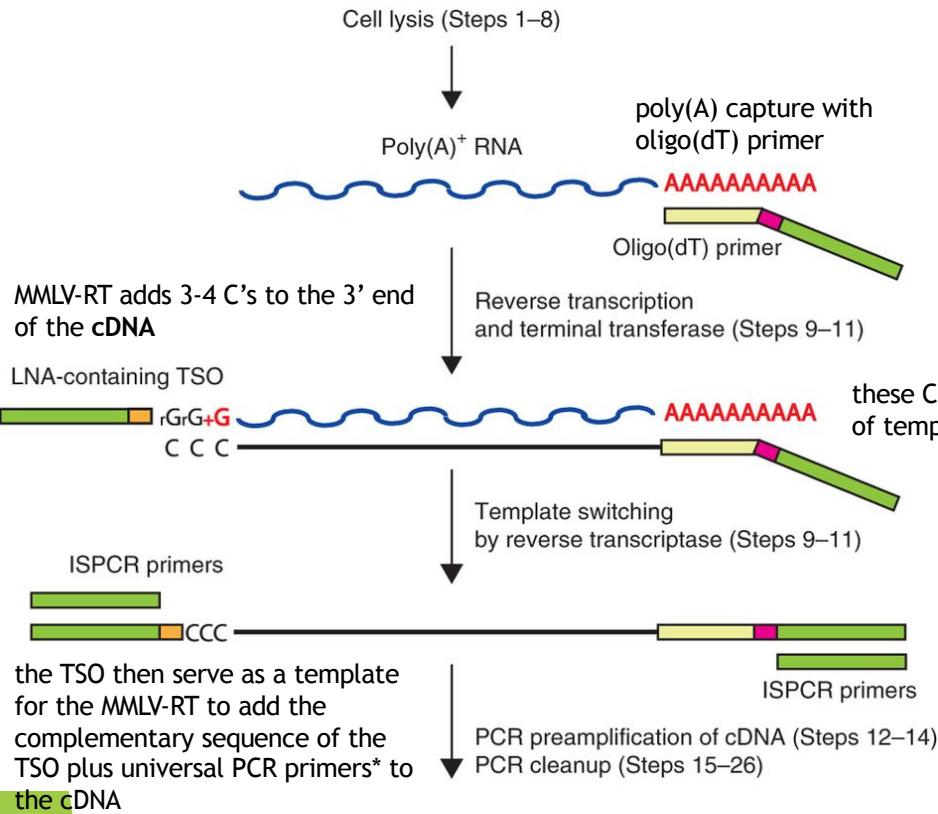
- SMART-seq2
- CEL-seq2
- STRT-seq

# SMART-seq2

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

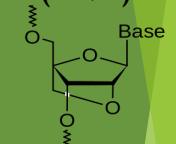


## RNA capture and cDNA synthesis



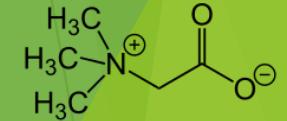
- A modified guanosine (a locked nucleic acid, LNA) is incorporated in TSO: enhanced thermal stability & anneal strongly to the untemplated 3' extension of the cDNA

locked nucleic acid (LNA)



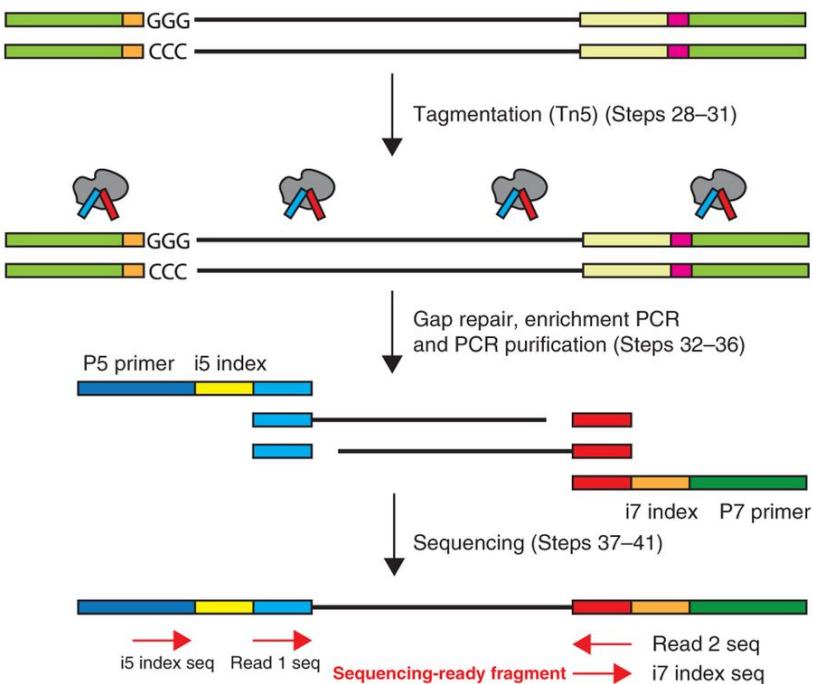
- Betaine**, a methyl group donor, can remove the steric hindrance of secondary structures of RNAs (such as hairpins or loops) so that early termination of chain elongation can be blocked.

Betaine



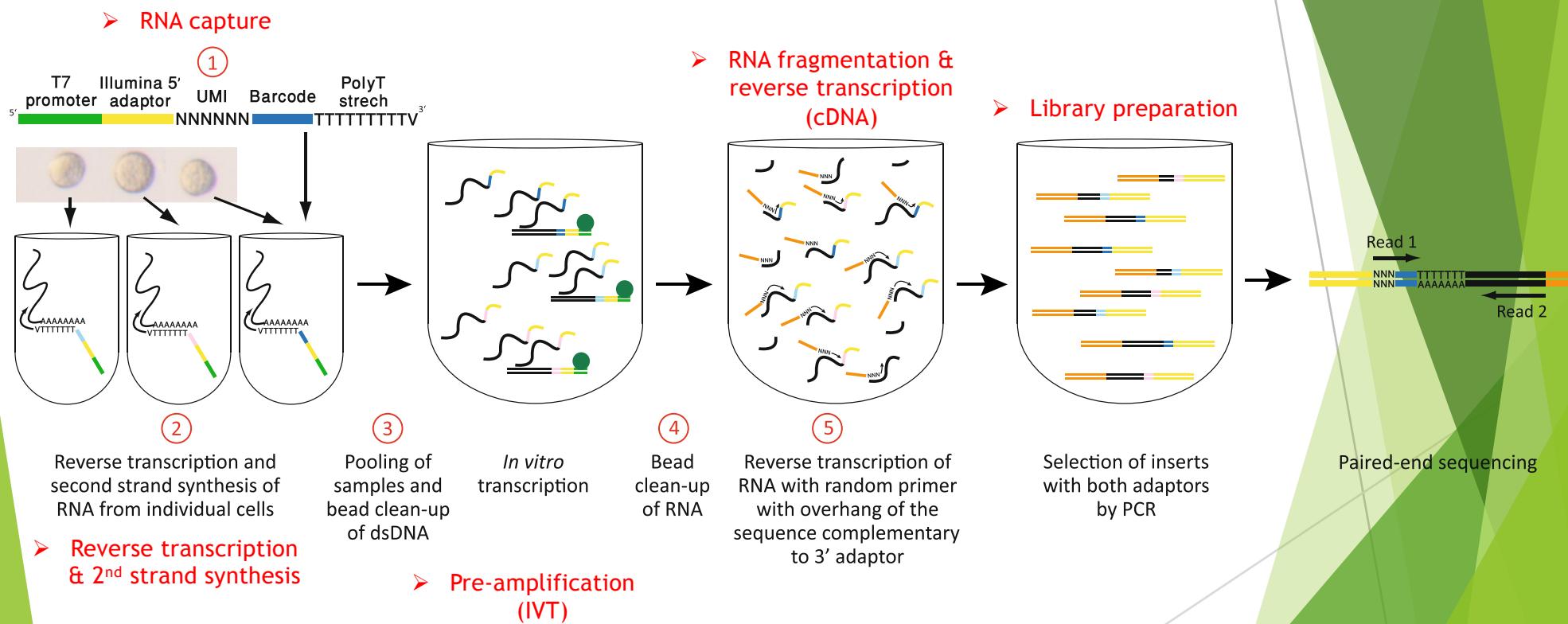
# SMART-seq2

## Library preparation

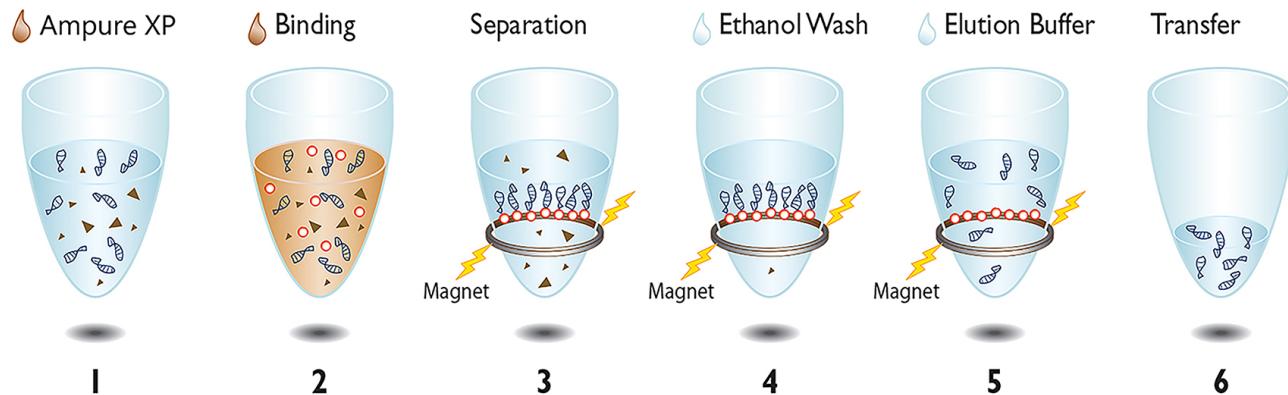


- amplification with few PCR cycles
- **tagmentation:** combining fragmentation and sequencing adapter integration
  - hyperactive derivative of the the Tn5 transposase **cuts the cDNA and ligates sequencing adapters**

# CEL-seq2



# SPRI Bead technology

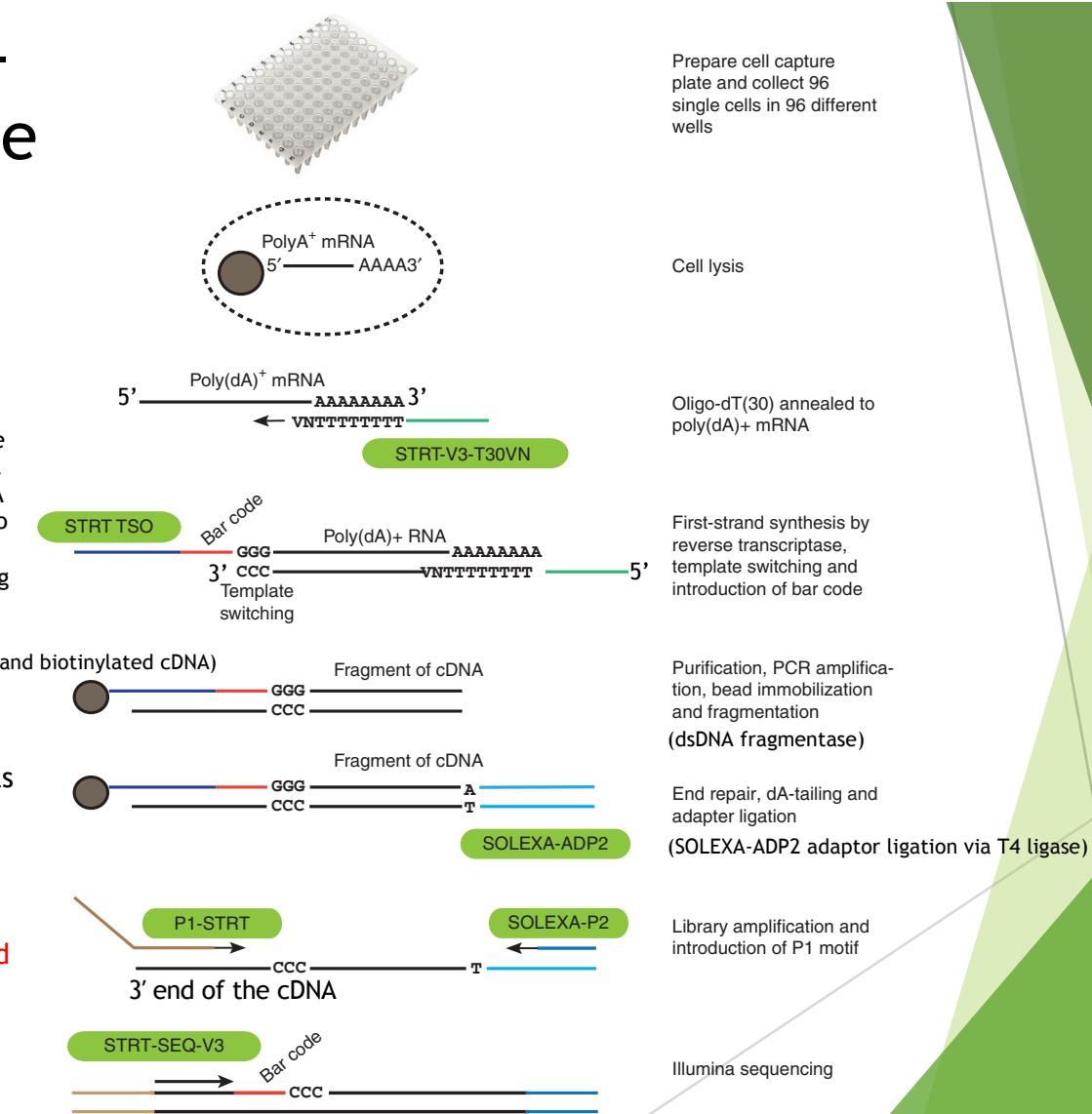


- **Solid Phase Reversible Immobilization**
- Carboxyl coated magnetic particles suspended in a solution of 10% PEG and 1.25M NaCl
- **Reversibly binds DNA**
  - Hawkins, et al. (1994) DNA purification and isolation using a solid-phase. Nucleic Acids Research, 22(21):4543-4544

# 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

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**.

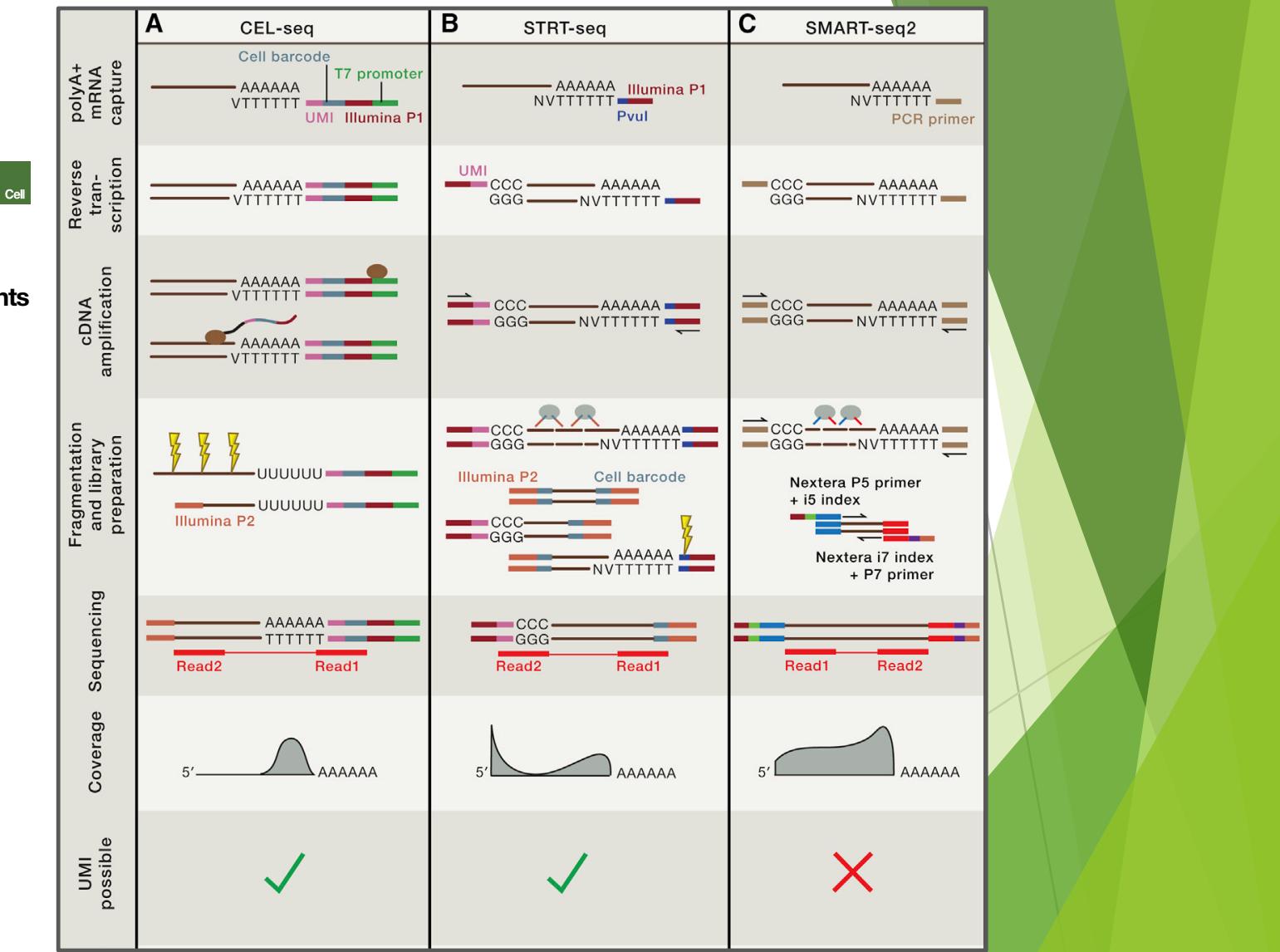


# Comparison

Leading Edge  
Primer

## Design and Analysis of Single-Cell Sequencing Experiments

Dominic Grün<sup>1,2,3</sup> and Alexander van Oudenaarden<sup>1,2,\*</sup>

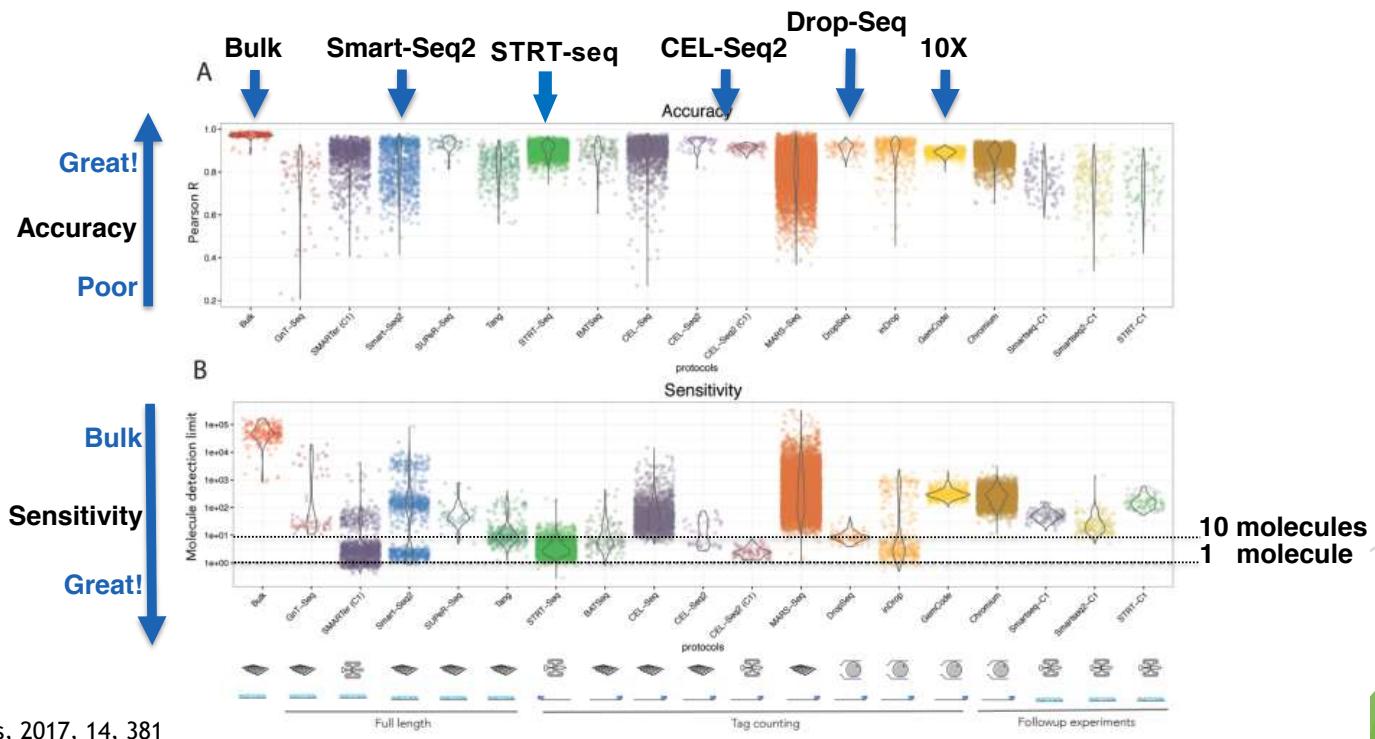


# Comparison

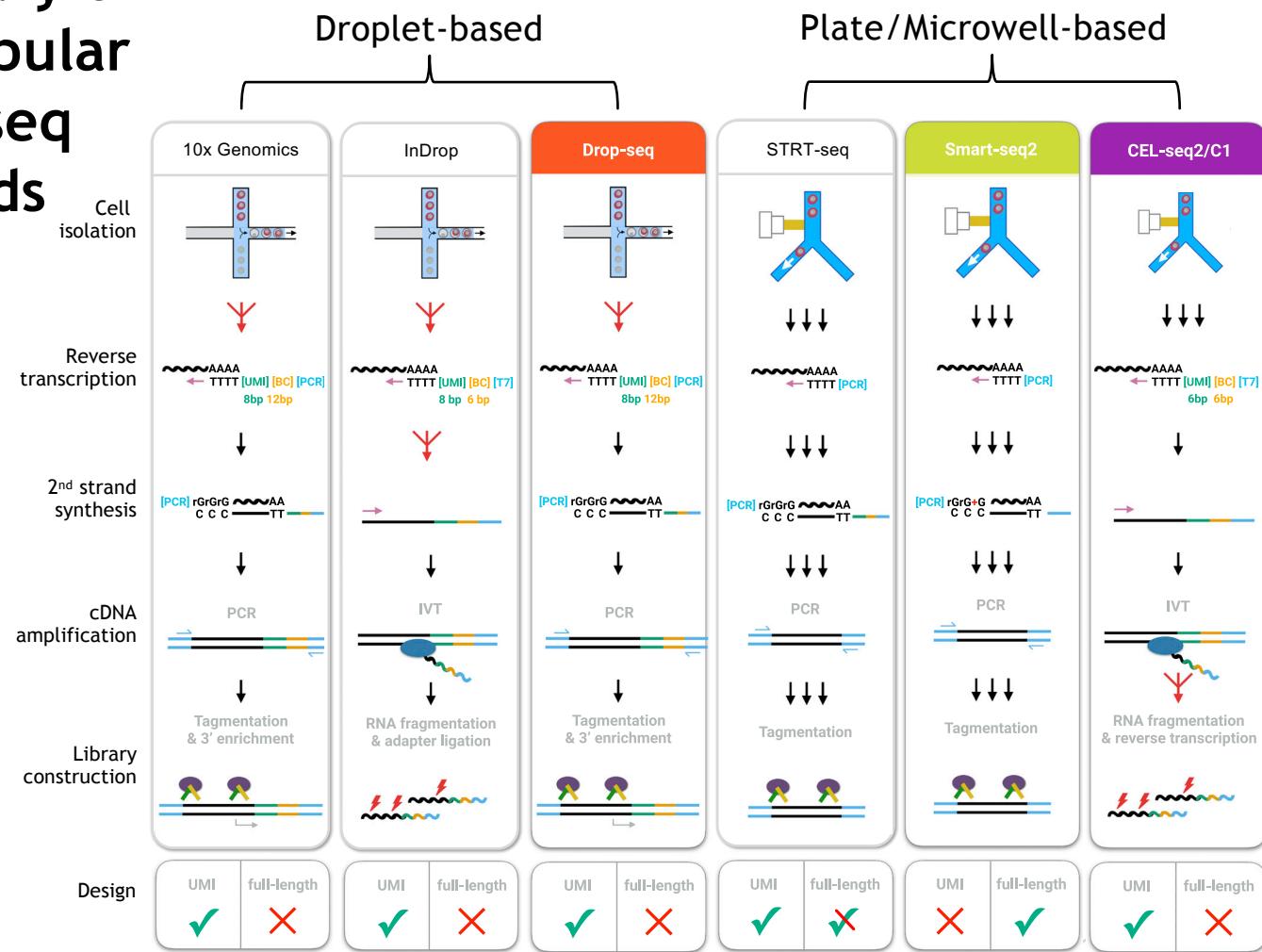
ANALYSIS

## Power analysis of single-cell RNA-sequencing experiments

Valentine Svensson<sup>1,2,6</sup>, Kedar Nath Natarajan<sup>1,2,6</sup>, Lam-Ha Ly<sup>2</sup>, Ricardo J Miragaia<sup>2,3</sup>, Charlotte Labalette<sup>2,4,5</sup>, Iain C Macaulay<sup>2</sup>, Ana Cvejic<sup>2,4,5</sup> & Sarah A Teichmann<sup>1,2</sup>



# Summary of the popular scRNAseq methods

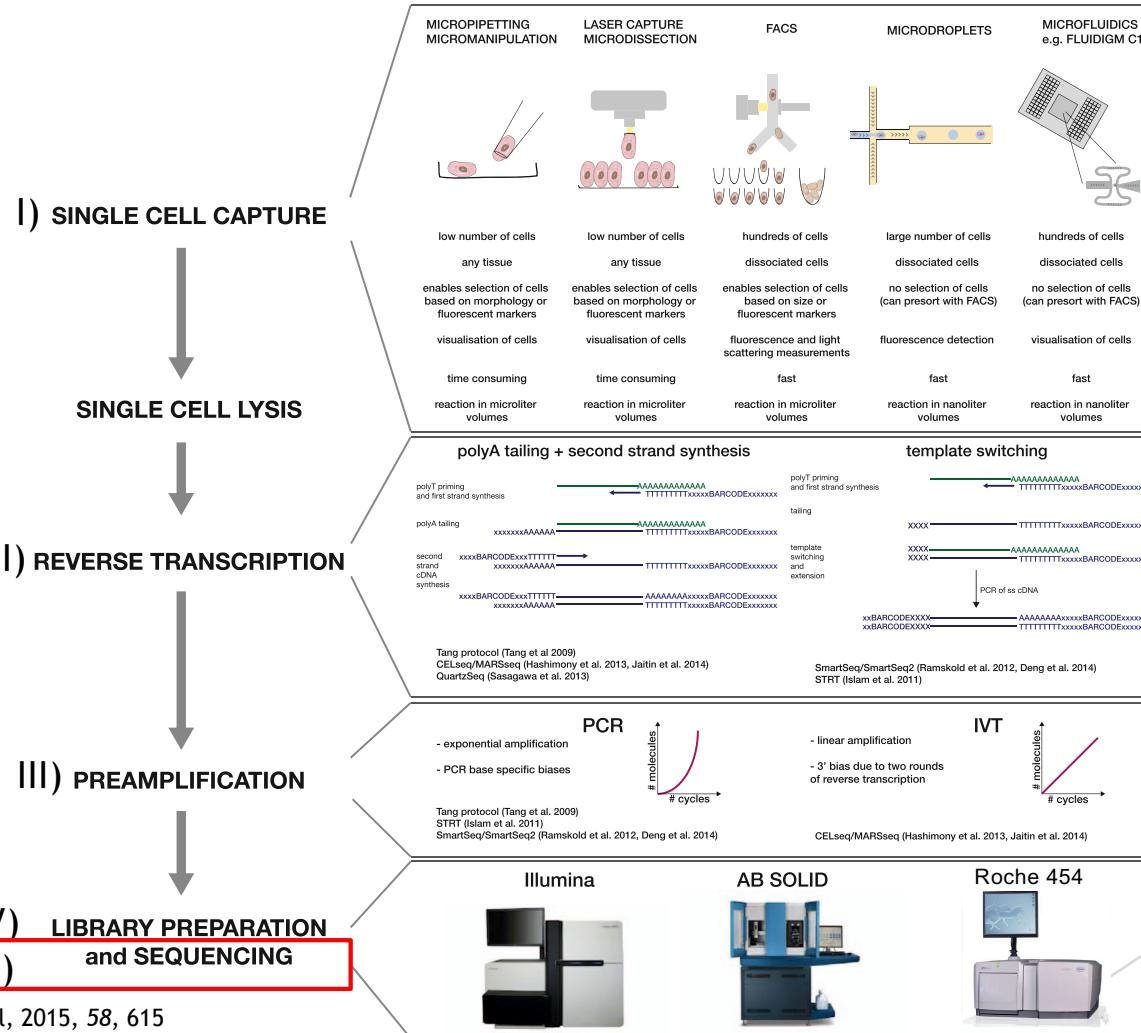


# Which method should I use?



- 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



Illumina



AB SOLID



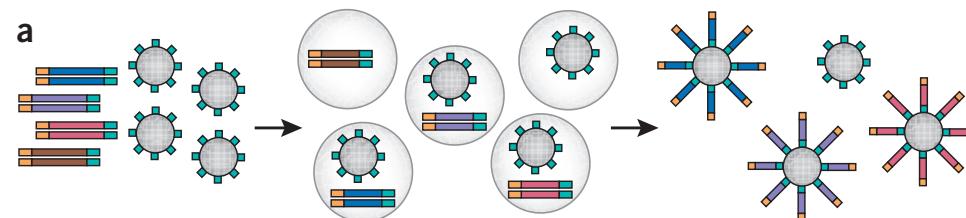
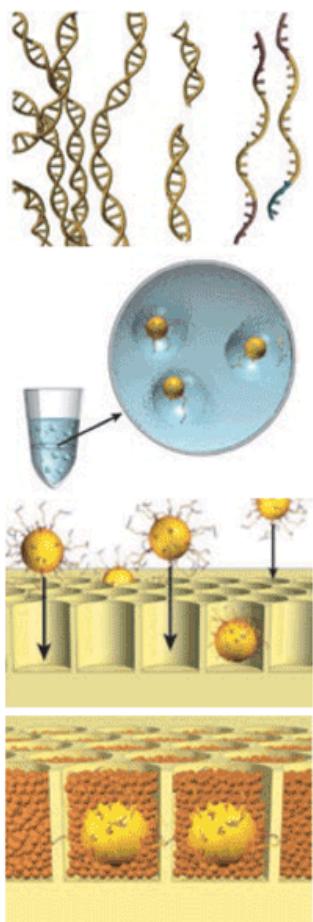
Roche 454

Bridge PCR

Emulsion PCR

# Next-generation sequencing platforms

## 1) Emulsion PCR



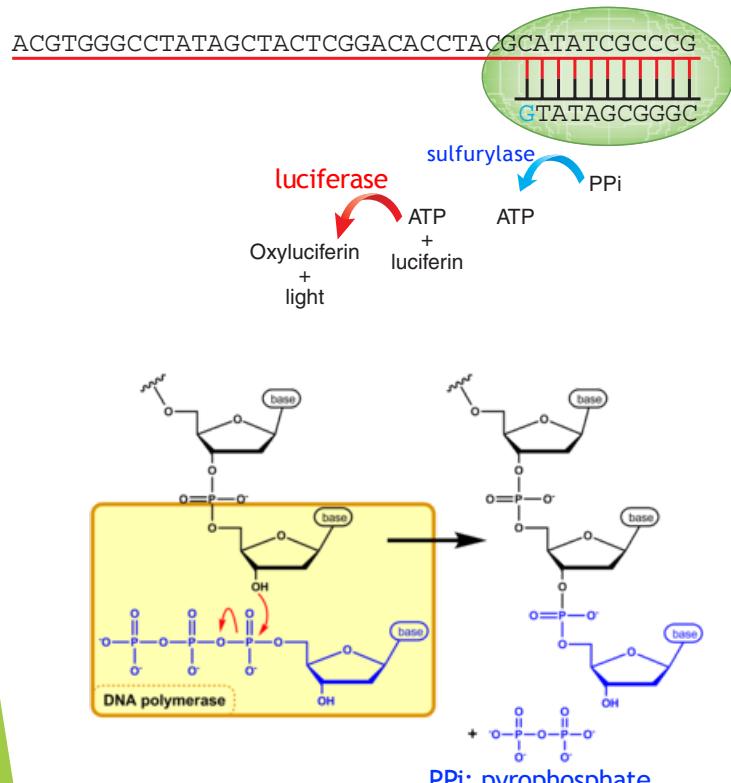
- Fragments, with adaptors, are PCR amplified within a water drop in oil
- One primer is attached to the surface of a bead
- Used by Roche 454 and AB SOLiD

Nat Biotechnol, 2008, 16, 1117

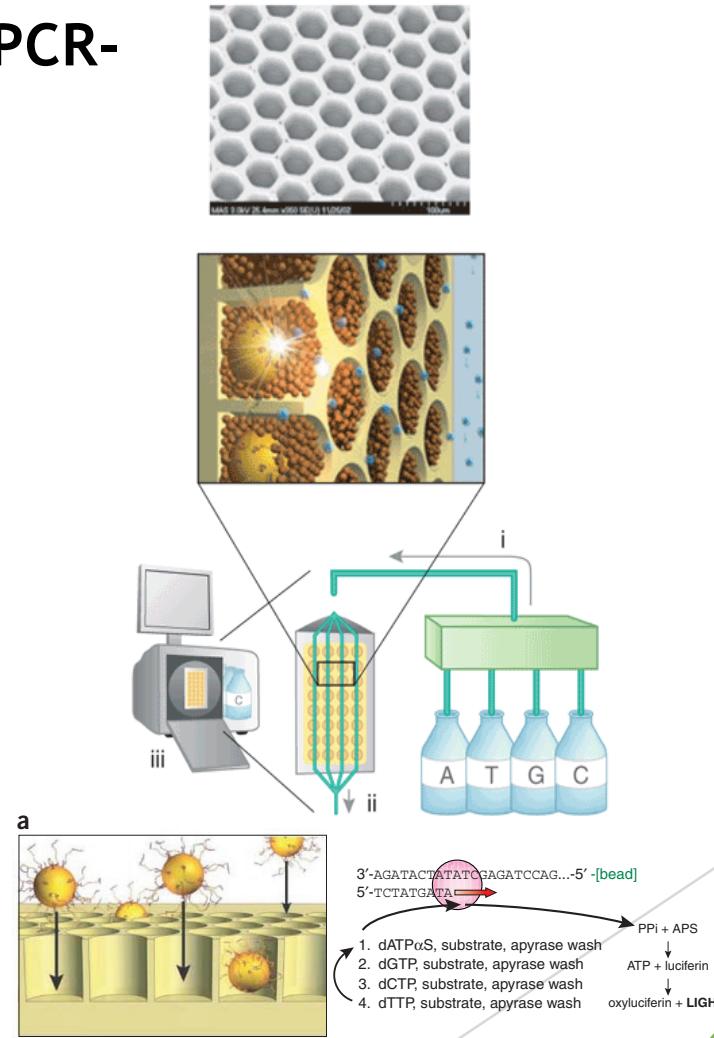
Nature Biotechnology, 2008, 26, 1135-1145

# Next-generation sequencing platforms

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

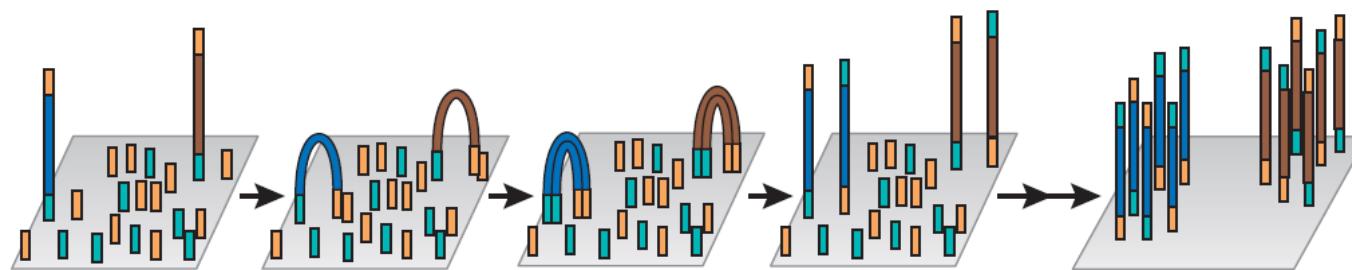


Nat Biotechnol, 2008, 16, 1117



# Next-generation sequencing platforms

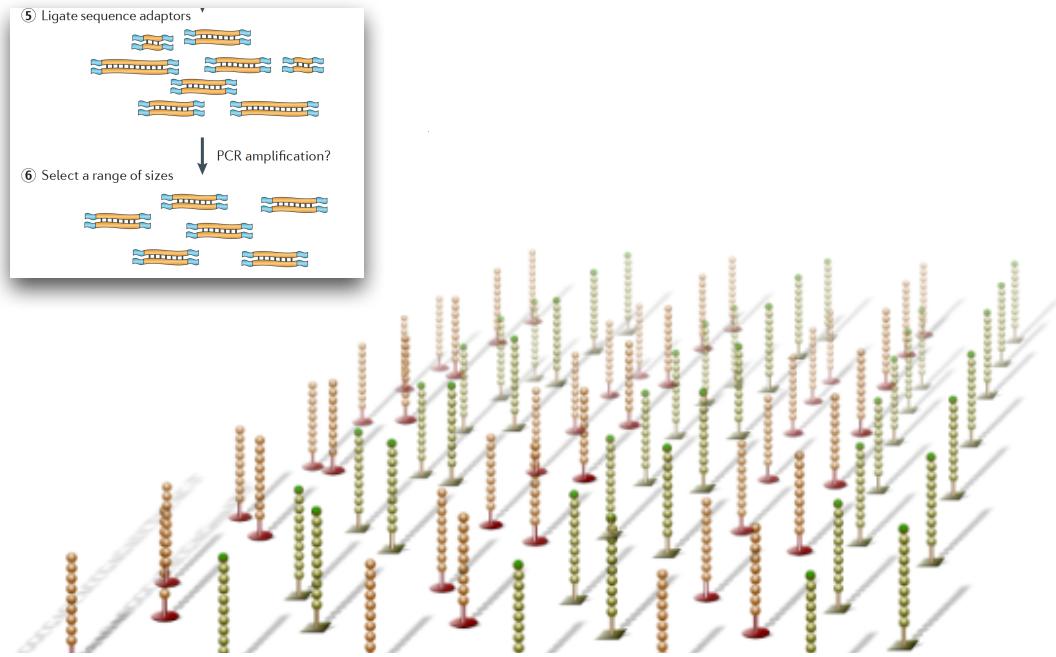
## 2) Bridge PCR (used by Illumina)



# Illumina Sequencing

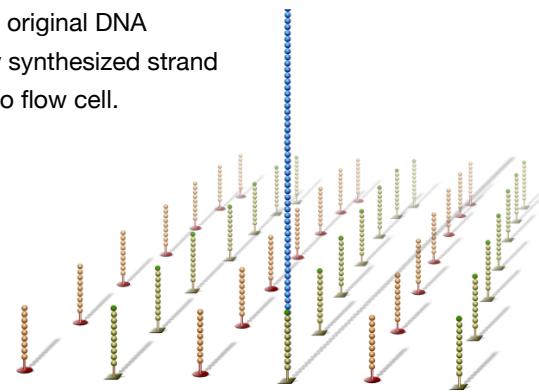
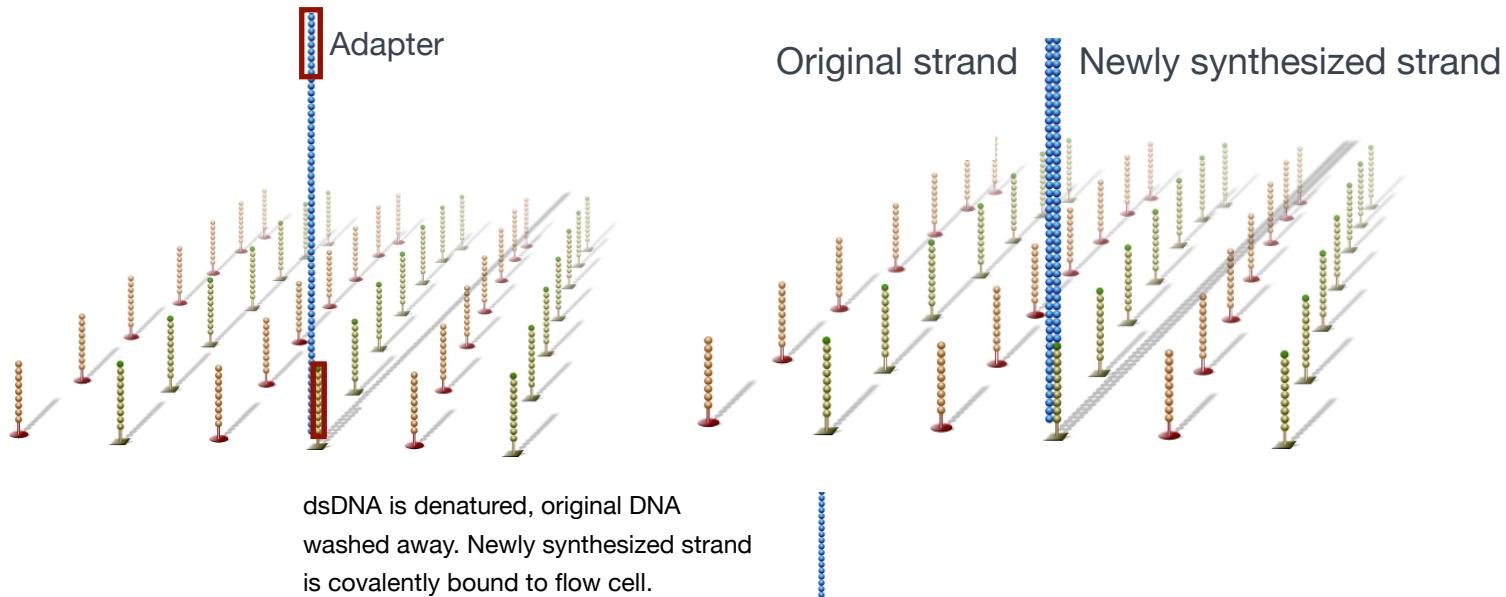
## Flow cell

TTATGATAACGGGACCCGAGAUCTACAC-3'  
TTCAAGCAGAACAGCGCATACGA~~Goxo~~AT-3'



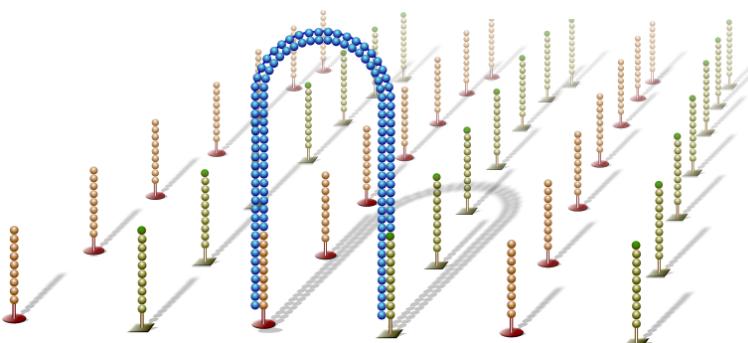
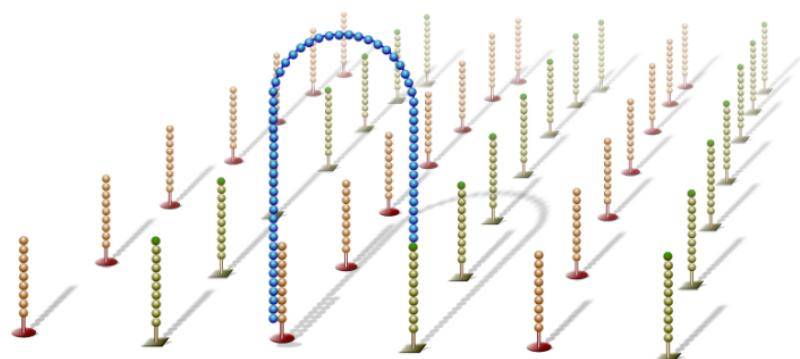
# Illumina Sequencing

## *Cluster generation*



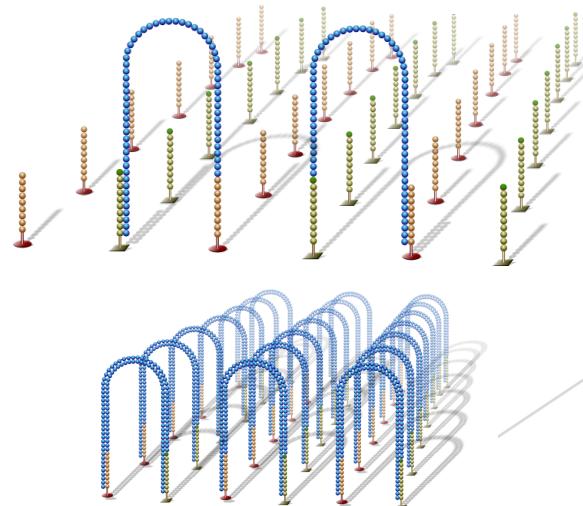
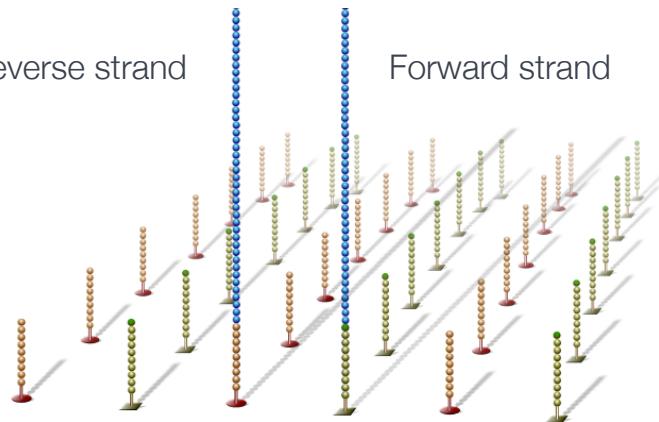
# Illumina Sequencing

## *Bridge amplification*



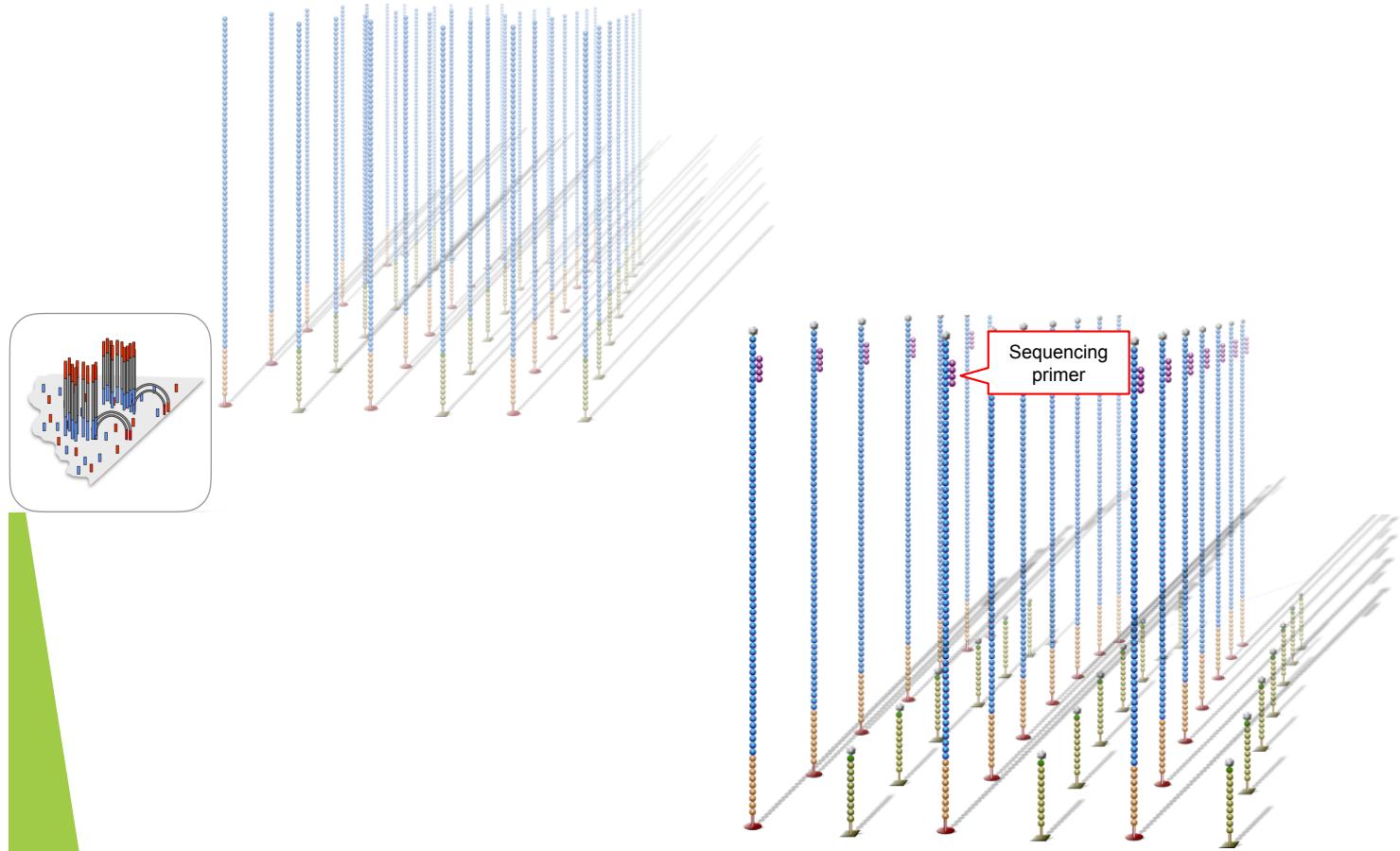
Reverse strand

Forward strand



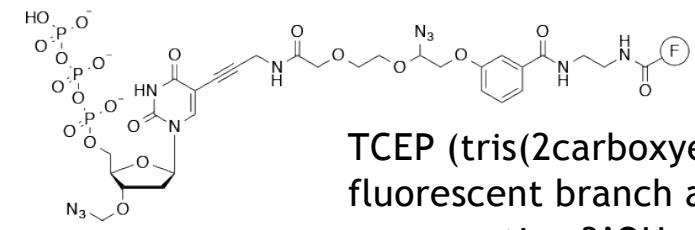
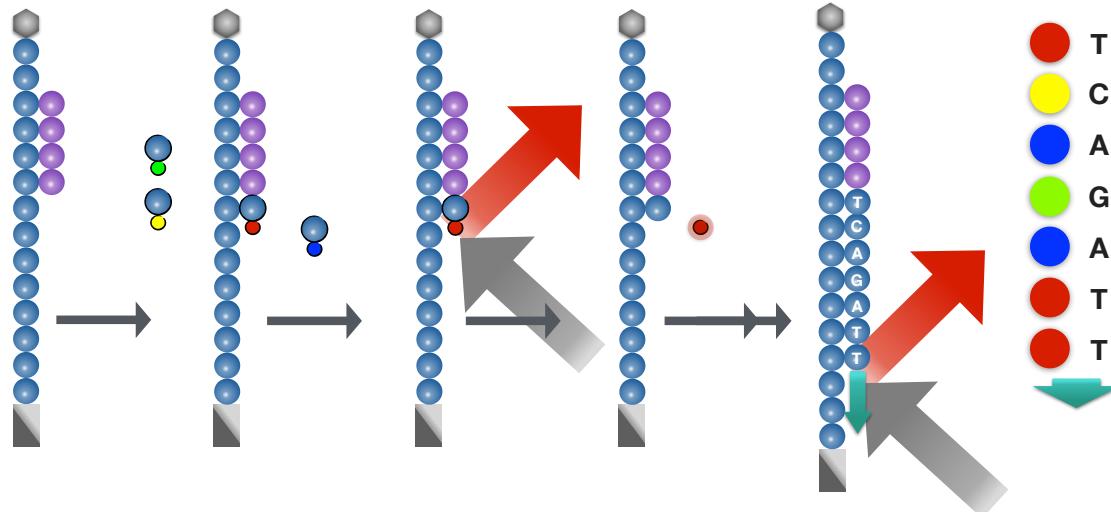
# Illumina Sequencing

## *Preparation for sequencing*



# Illumina Sequencing

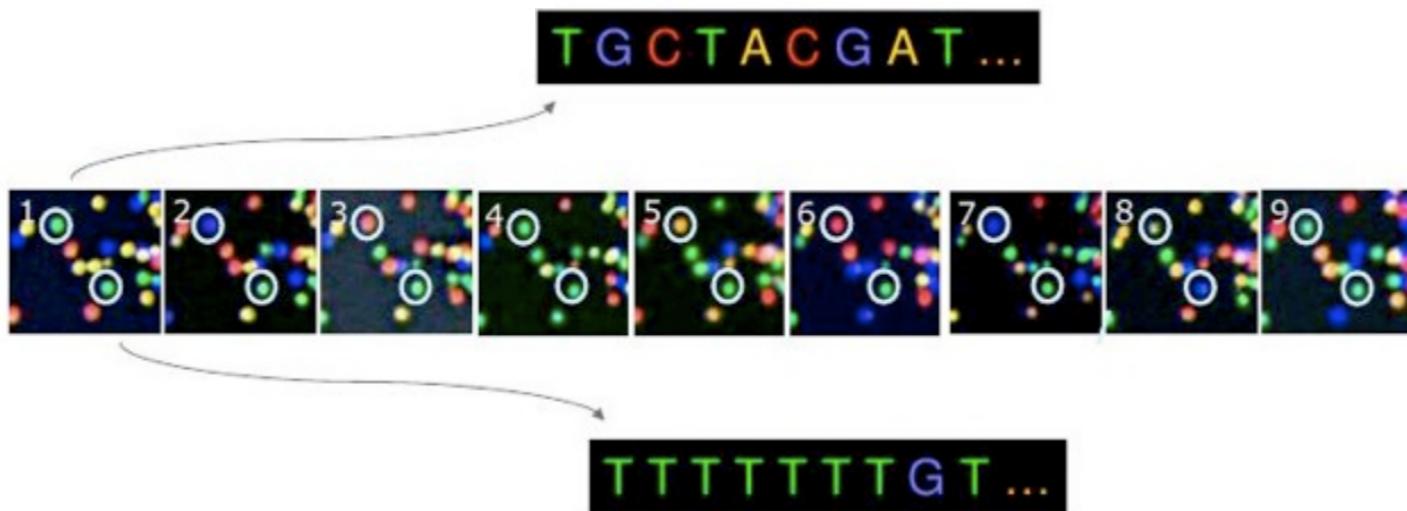
## *Sequencing by synthesis*



TCEP (tris(2carboxyethyl)phosphine) removes azido-fluorescent branch and the 3' Oazidomethyl group, regenerating 3'OH, and the cycle can be repeated.

# 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)

