

Technical overview of single-cell transcriptomics methods.

1. collecting single cells.
2. general scRNA-seq methods for plate / C1 formats: template-switch versus T7.
3. droplet-based scRNA-seq: Drop-Seq / InDrop / 10x platforms.
4. Combinatorial indexing approaches for scRNA-seq.
5. Integrated multi-omics approaches.

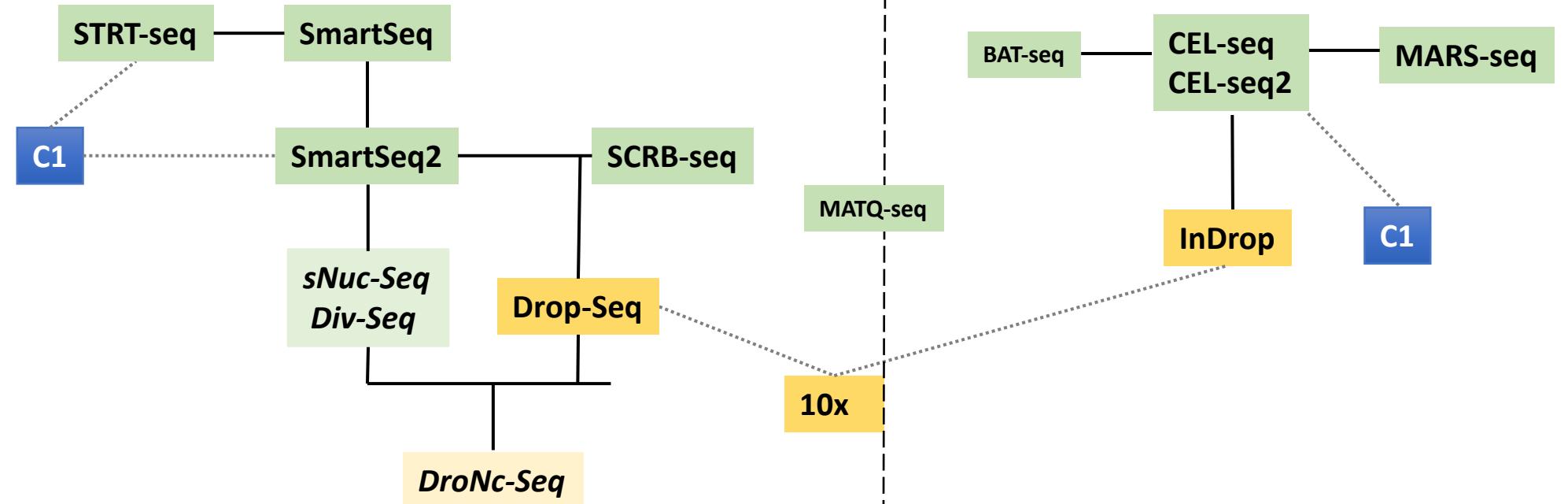
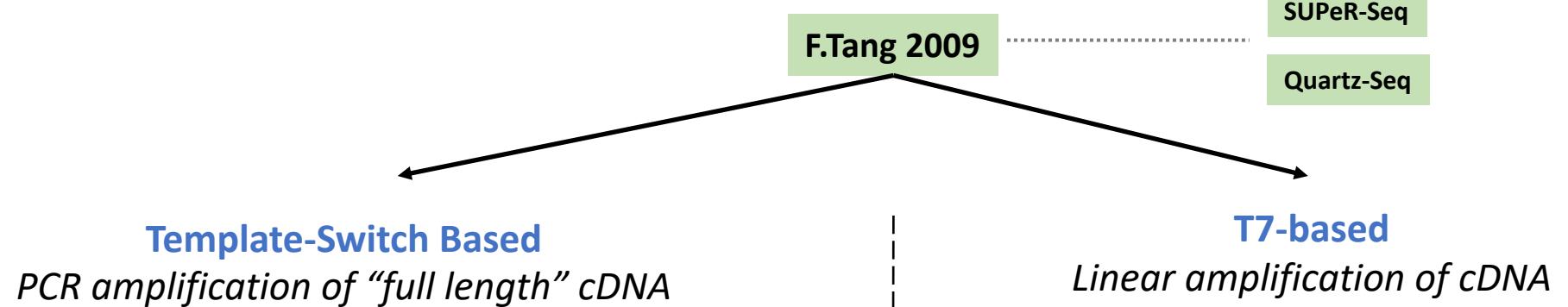
Sébastien Smallwood – FMI
sebastien.smallwood@fmi.ch

SIB - Single-cell RNA-Seq Analysis (Bern 2018)

How to select the right protocol for a given biological question?

The image shows the cover of a scientific article. At the top left is the 'nature protocols' logo. To the right of the logo, the text 'REVIEW ARTICLE' is displayed in white capital letters. Below that is a URL: <https://doi.org/10.1038/s41596-018-0073-y>. The main title of the article is 'Tutorial: guidelines for the experimental design of single-cell RNA sequencing studies'. Below the title, the authors are listed: Atefeh Lafzi^{1,5}, Catia Moutinho^{1,5}, Simone Picelli^{2,4}, and Holger Heyn^{1,3*}.

- How many cells do I need to sequence?
- Are my cells precious or can they be obtained in large numbers and collection repeated easily?
- How can I collect cells (viability +++)? How many cells can I collect at a given time?
- Available equipment / Cost considerations...



Combinatorial indexing

sci-RNA-seq

SPLiT-seq

Nanowells

STRT-seq-2i

Seq-Well

Microwell-seq

F.Tang 2009

SUPeR-Seq

Quartz-Seq

Template-Switch Based
PCR amplification of “full length” cDNA

T7-based
Linear amplification of cDNA

STRT-seq

MARS-seq

C1

C1

⇒ **Many methods, many “copies”**
⇒ **common / specific issues...**
little extensive comparisons...

DroNc-Seq

Combinatorial indexing

sci-RNA-seq

SPLiT-seq

Nanowells

Seq-Well

STRT-seq-2i

Collecting single cells

Plate format

- **FACS (cells + nuclei)** directly in lysis buffer.
 - Accurate (96+384 plates), if good prep low doublets assumed.
 - Enrich for specific populations with known markers.
- **Mouth pipetting under microscope** for very precious samples (e.g. early embryos).
 - Accurate, even if doublets can be hard to discard.
 - Low throughput + time consuming + skills.
- Combination with **Patch-clamp** for neurons.
- **Laser Capture Microdissection**.
- Plates / strips with isolated single cells can be stored at -80C in lysis buffer.

C1

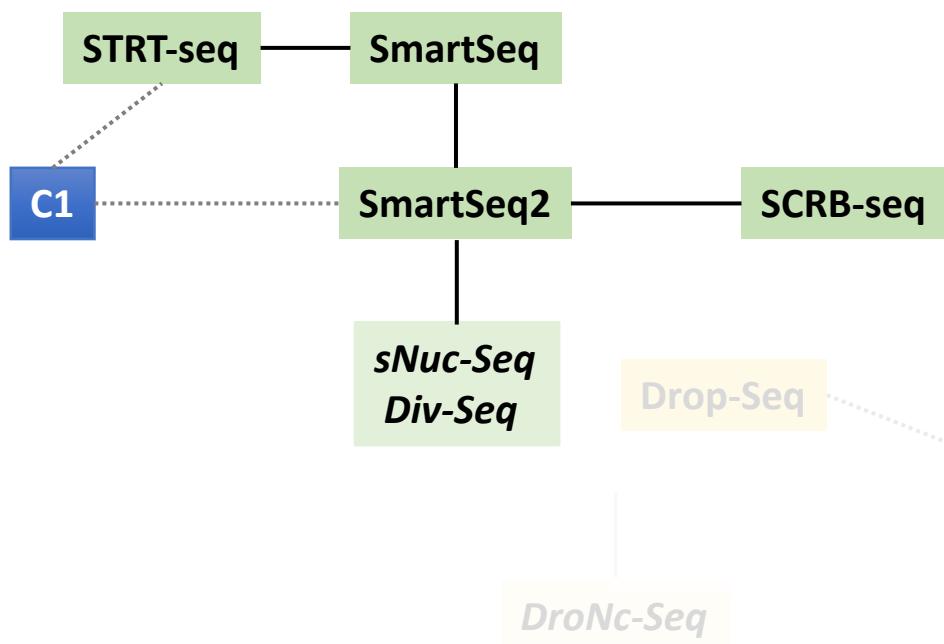
- **Cells captured directly** on the microfluidic chip.
- Possibility to visualise the cells in “high” definition on the chip (fluorescent marker?)
- Cells have to be “**healthy**” + size restrictions.
- **Capture rate low** -> not so compatible with rare cells.
- Past (?) issues with doublets.....

Droplet-based

- Cells are encapsulated **in oil droplets** using microfluidics devices.
- **Capture rate:** Drop-seq: low | InDrop: very high with optimisations | 10x ~40-50%
- **Doublets** rate can be relatively high: 1-5%.
- **can be used with fixed cells**
- **compatible with nuclei from fresh / frozen tissues: neuro+++ (DropNc-Seq / 10x)**

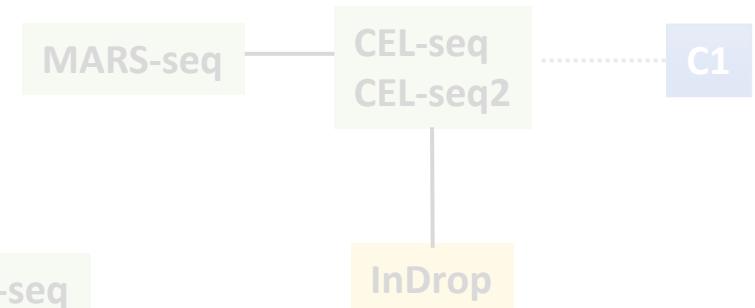
Template-Switch Based

PCR amplification of “full length” cDNA



T7-based

Linear amplification of cDNA



Combinatorial indexing

sci-RNA-seq

SPLiT-seq

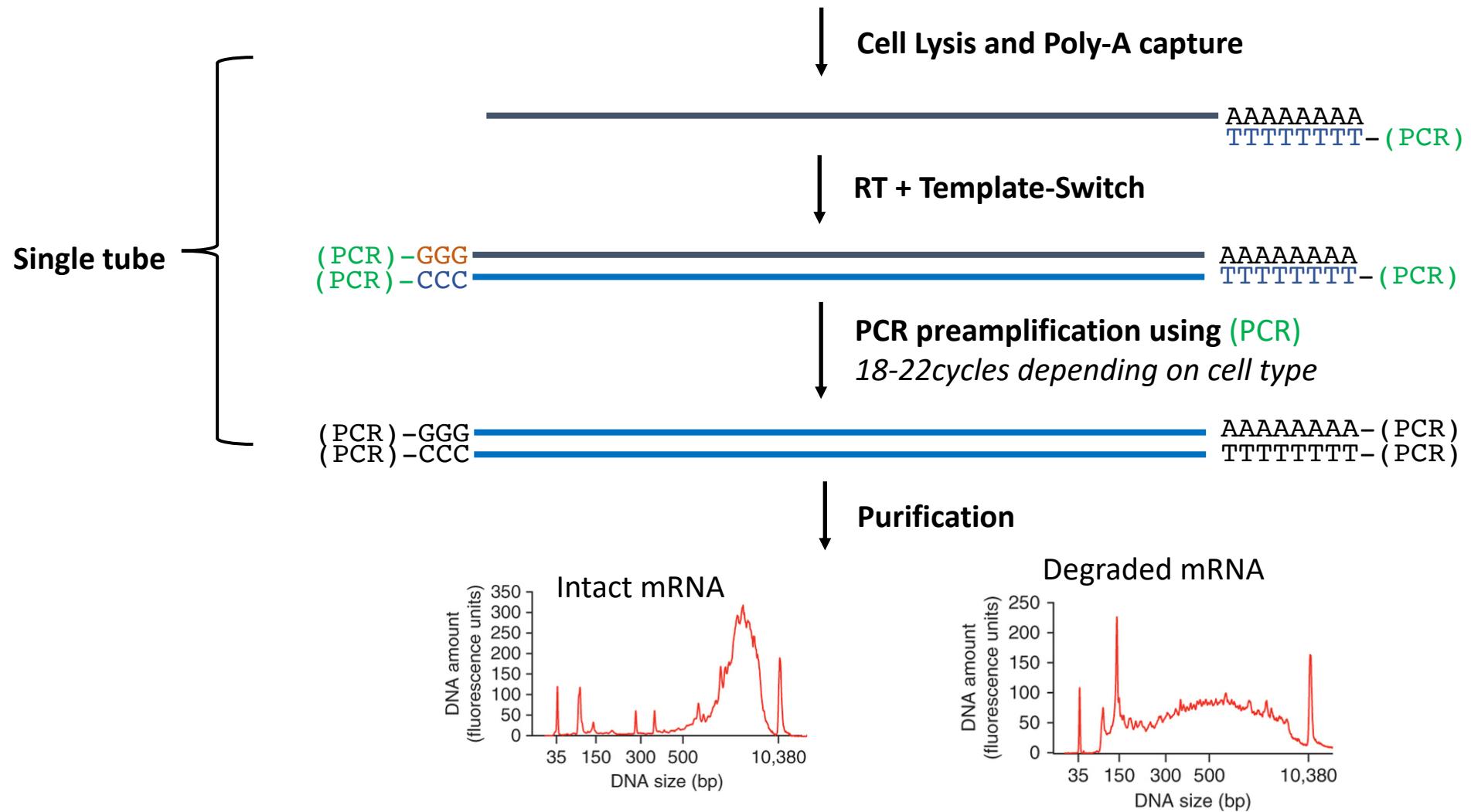
Nanowells

Seq-Well

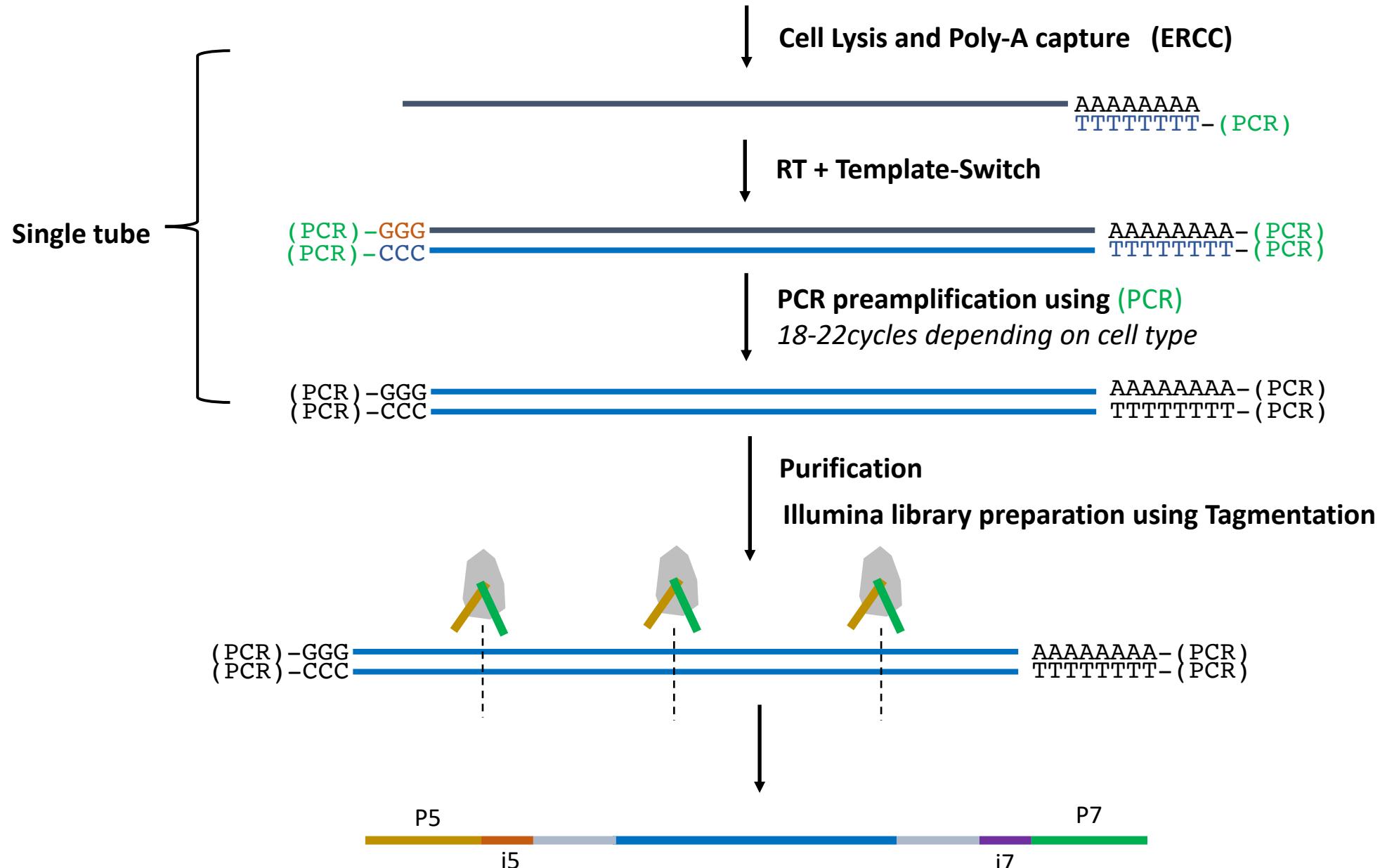
STRT-seq-2i

10x

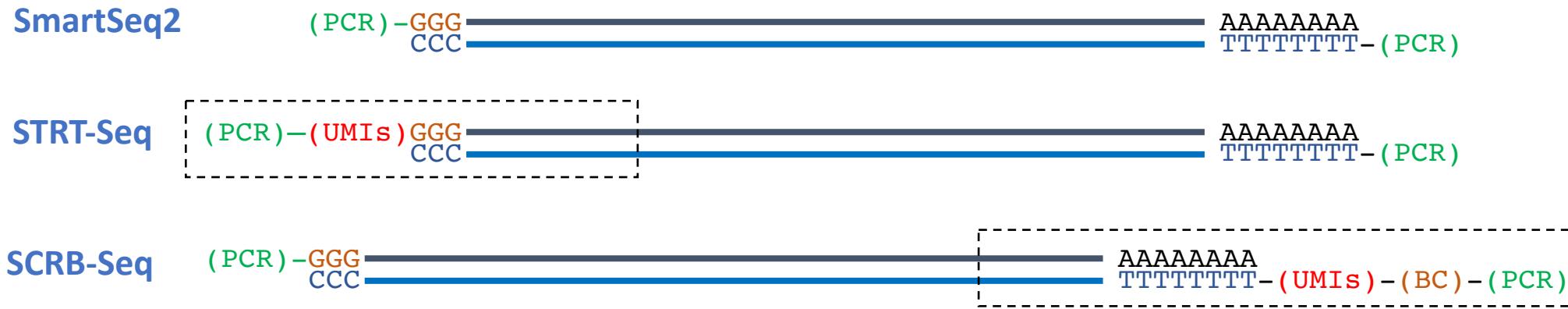
Template-Switch Based approaches with plate format: SmartSeq2



Template-Switch Based approaches with plate format: SmartSeq2



Template-Switch Based approaches: SmartSeq2 / STRT-Seq / SCRB-Seq



SmartSeq2: full length / no UMIs / ~300-500 cells per week

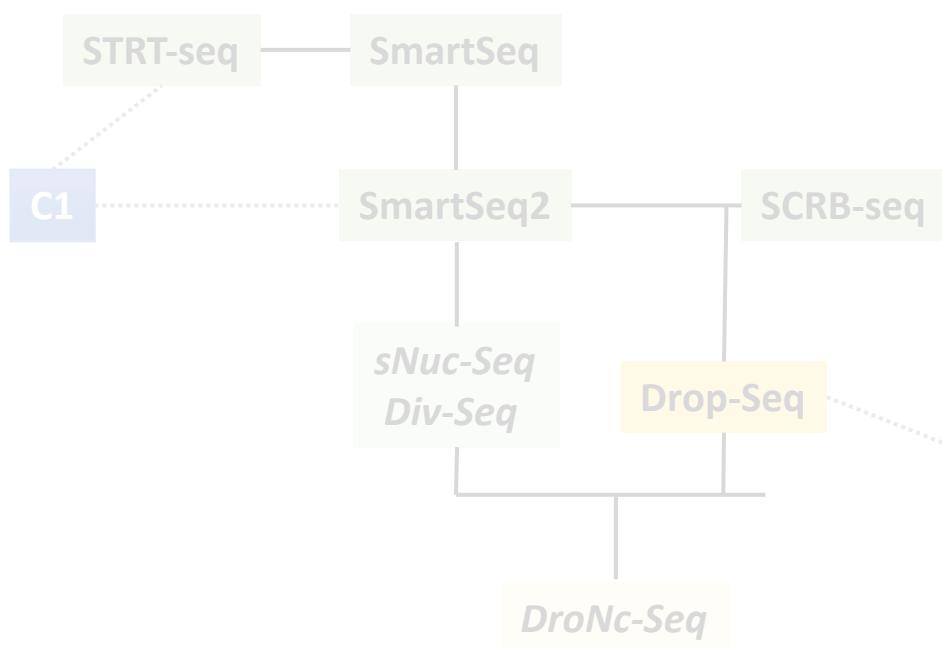
STRT-Seq: 5' sequencing / UMIs / ~300-500 cells per week

SCRB-Seq: 3' sequencing / UMIs / ~2,500-3,000 cells per week

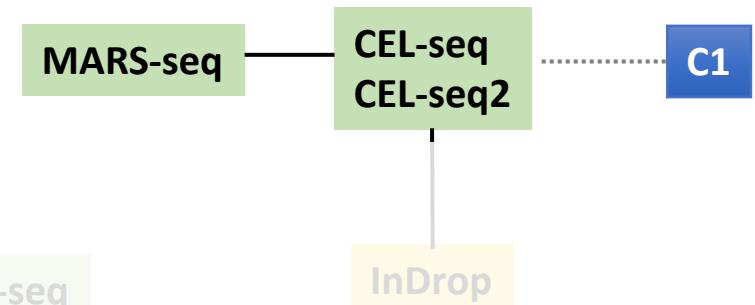
sNuc-Seq / Div-Seq -> SmartSeq2 on nuclei...

Act-seq -> SmartSeq2 on neurons with Actinomycin D for dissociation

Template-Switch Based
PCR amplification of “full length” cDNA



T7-based
Linear amplification of cDNA



Combinatorial indexing

sci-RNA-seq

SPLiT-seq

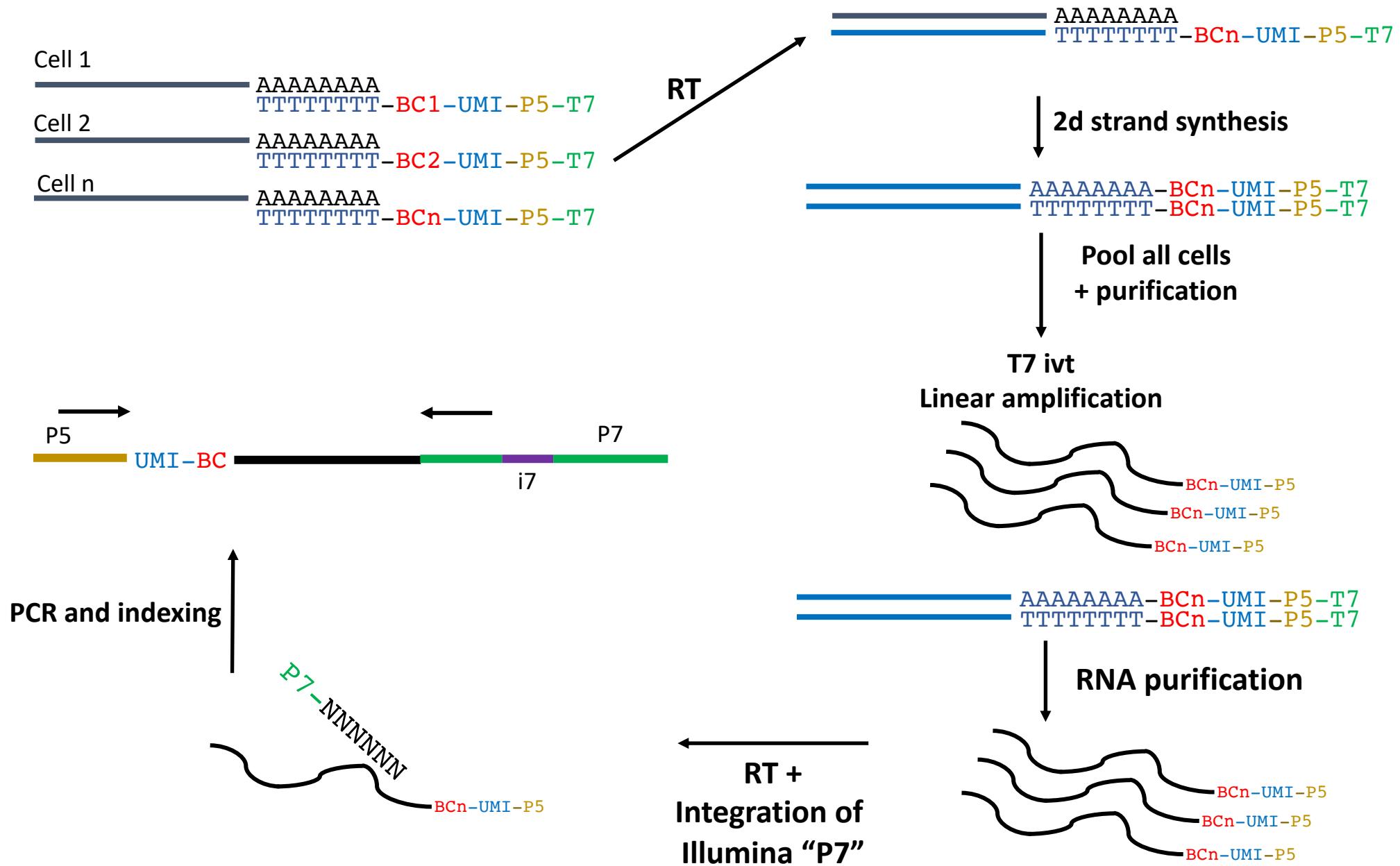
Nanowells

Seq-Well

STRT-seq-2i

T7 Based approaches – Plate format CEL-Seq2

Hashimshony et al.
Genome Biology 2016



Pros & Cons of Template-Switch versus T7.

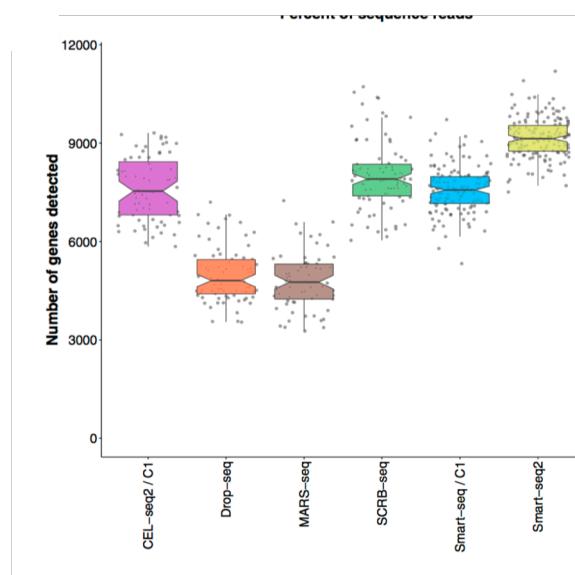
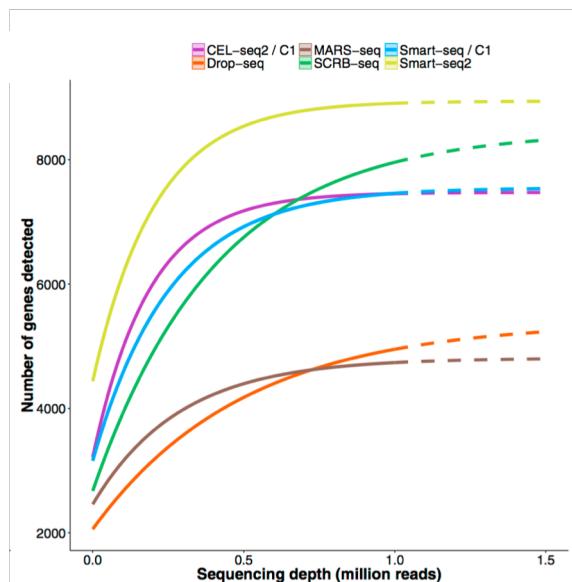
Template-switch based (e.g. SMART-Seq2):

Pros: Fast, easy and reliable. Single tube until preamp: safe for precious samples.
Cons: mRNA capture depends on poly-A capture and Template-switch efficiency.
PCR amplification -> bias (+ no UMIs for SmartSeq2)

T7 based (CEL-Seq2):

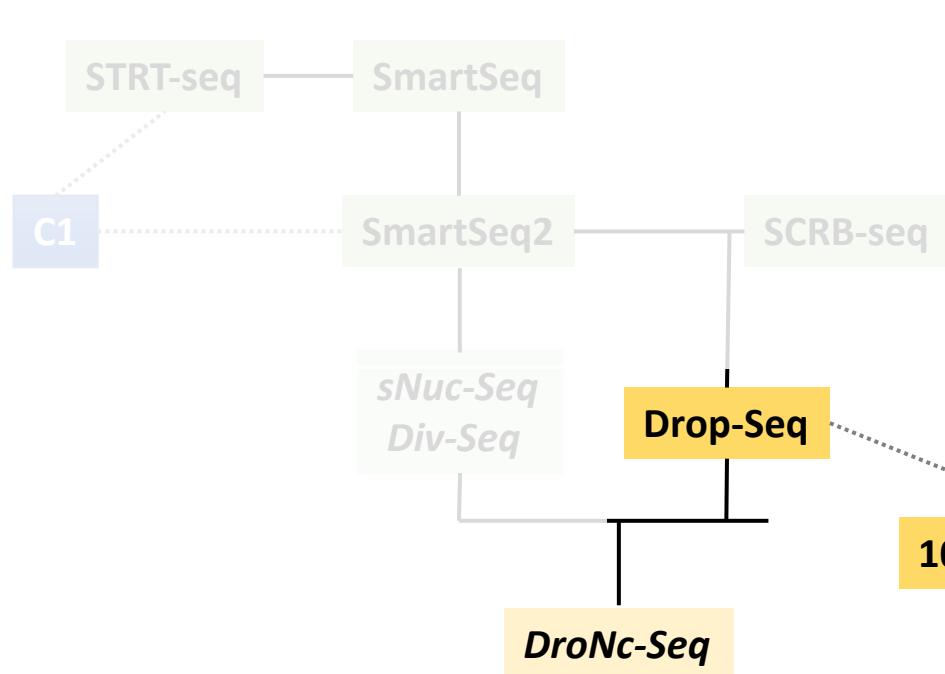
Pros: Linear Amplification. mRNA capture depends only on poly-A.
Cons: Longer and more complex protocols.
Pooling and purification of samples before amplification -> loss of material

-> Both approaches are valid. Key is mRNA capture and RT efficiency. Rest is “less” crucial



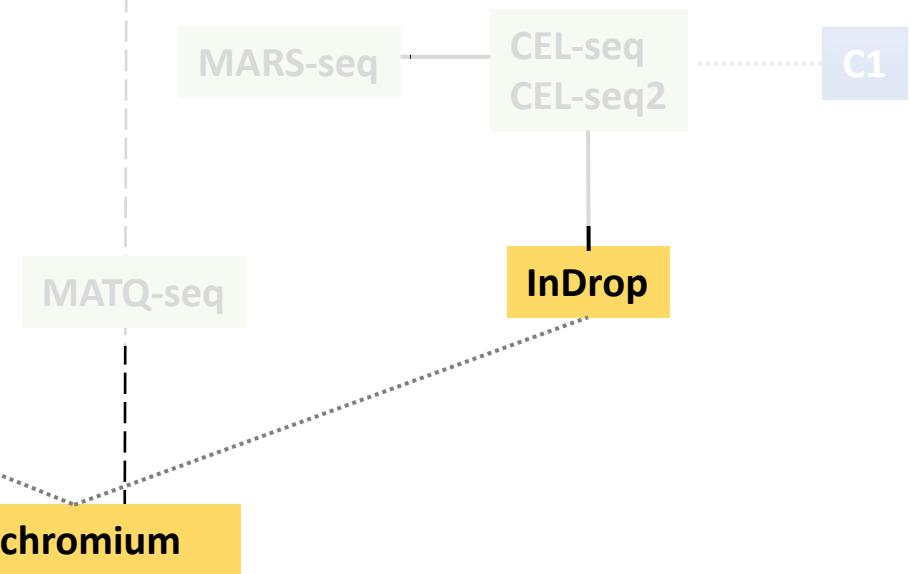
Template-Switch Based

PCR amplification of “full length” cDNA



T7-based

Linear amplification of cDNA



Combinatorial indexing

sci-RNA-seq

SPLiT-seq

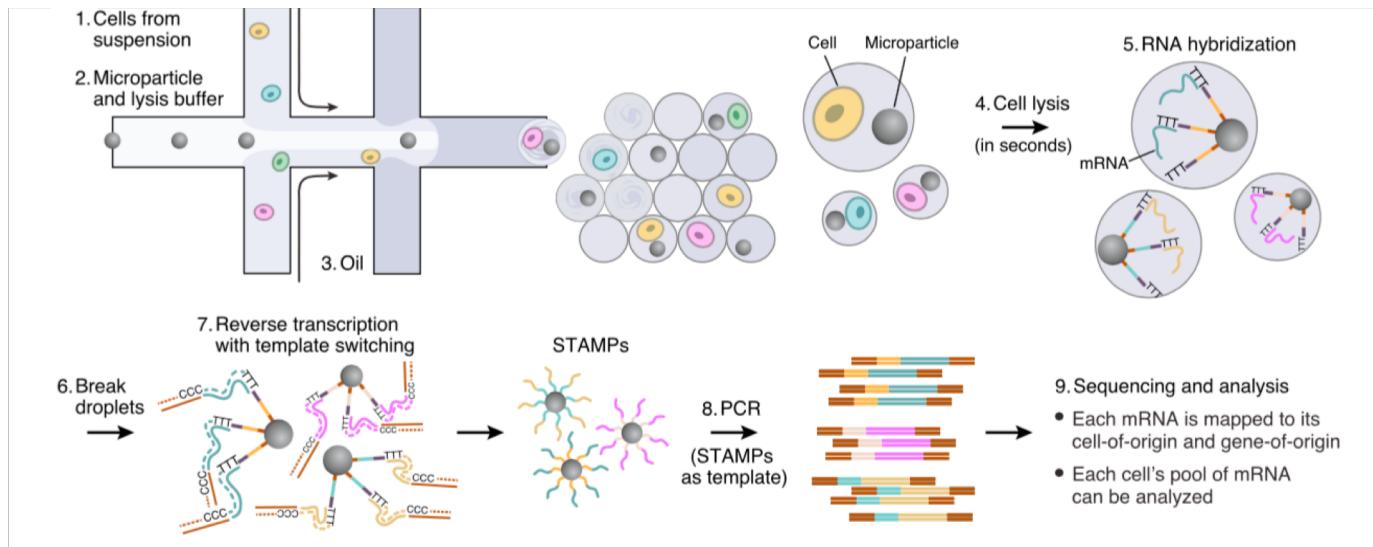
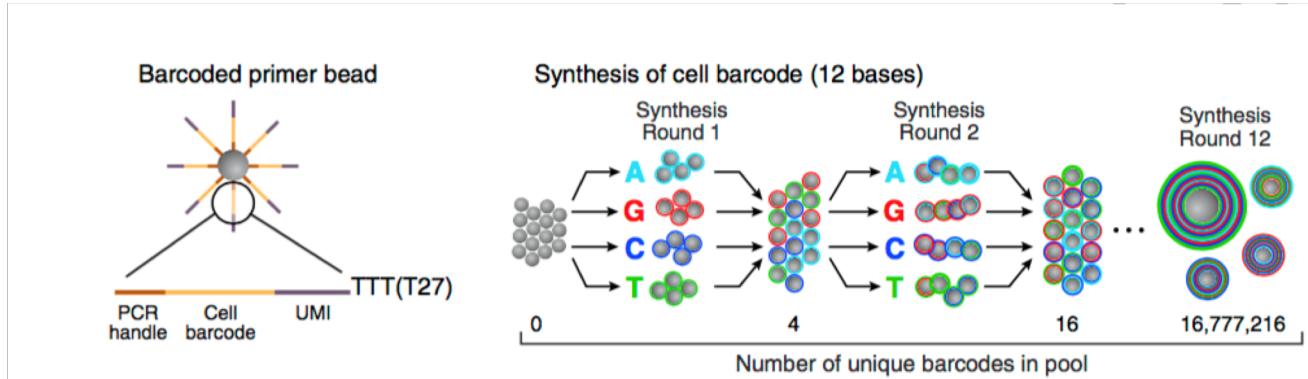
Nanowells

Seq-Well

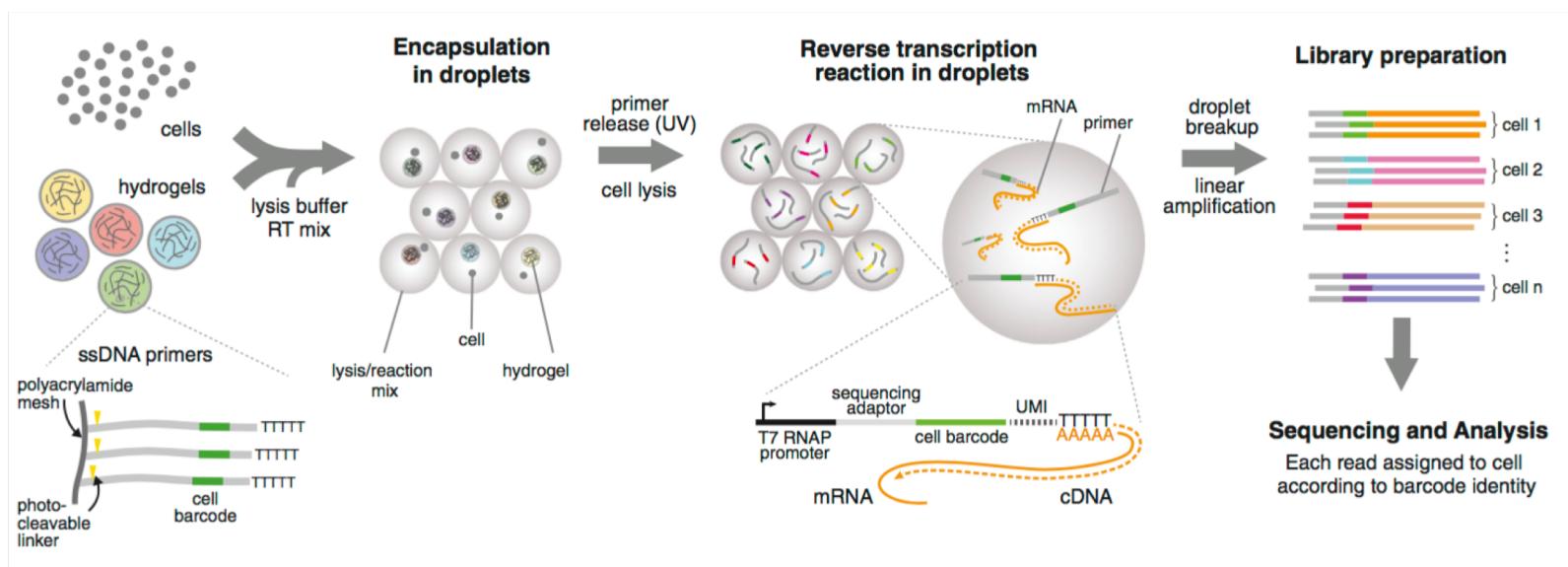
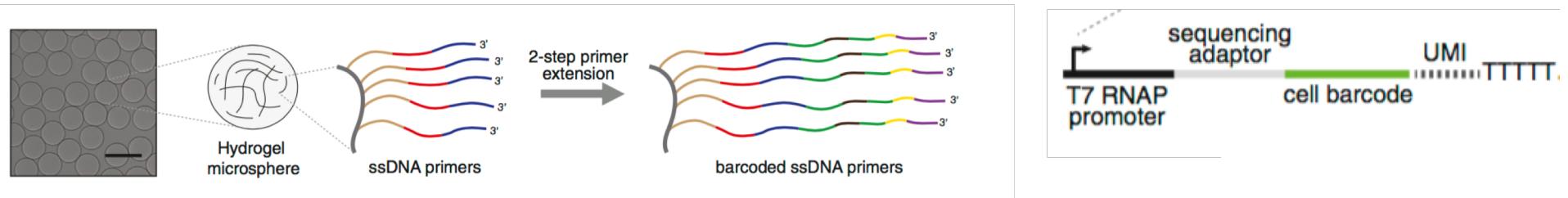
STRT-seq-2i

Drop-seq: Template-switch Based

*Drop-seq
barcodes*



InDrop: T7-based



Drop-seq versus InDrop

Drop-seq:

Pros: Widely adopted as beads barcodes / chips commercially available.

Cons: RT outside droplets - issue with "ambient" RNA (cells BC with low UMIs)
High variability between set-ups...good and bad datasets out-there...
Capture rate low.

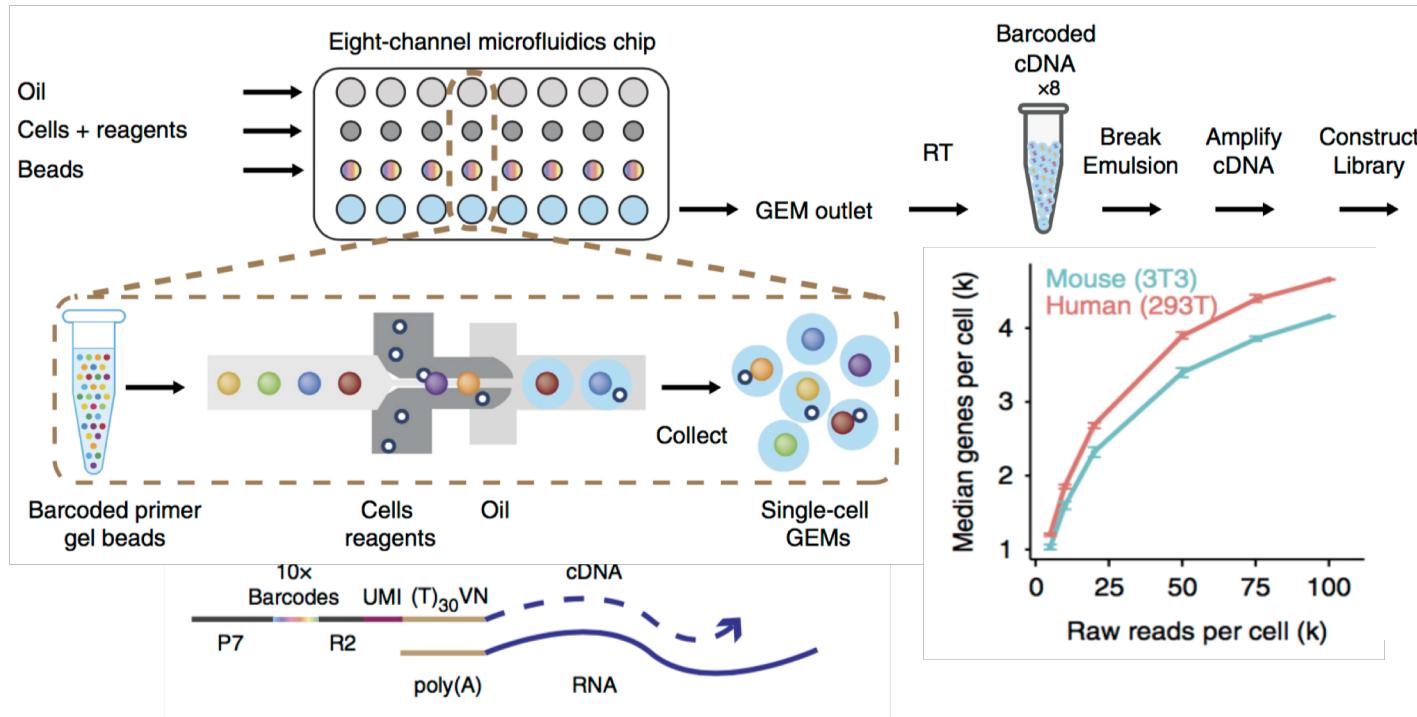
InDrop:

Pros: Quality is better than Drop-seq
Gel beads + RT within droplets (higher sensitivity).
High capture rate.

Cons: Challenging to make gel beads and barcodes (robotics helpful).
Microfluidics chips to be "made" (2 types of chips)
Now InDrop commercial solutions available

10x Chromium: a combination of Drop-seq and InDrop.

Gel beads + RT in droplets + template-switch approach

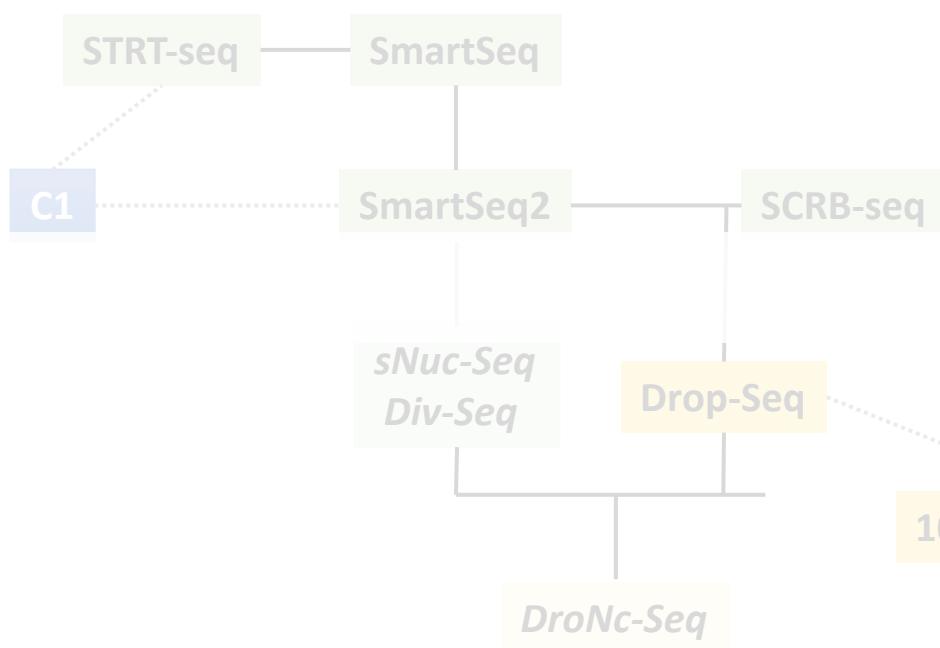


Pros: Ready box + kits: easy to use. Instrument “cheap”. High quality data

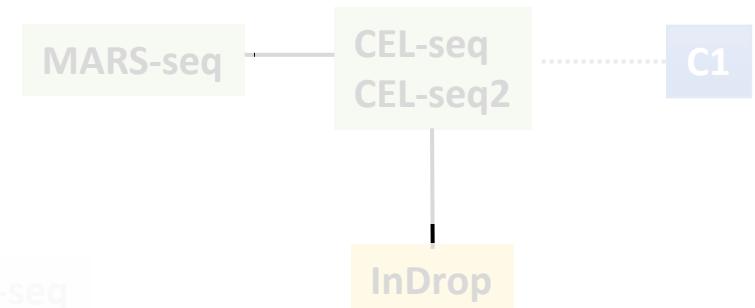
Cons: Not so convenient for low cells numbers (<10K).

Cells have to be used fresh (although cell fixation is possible)

Template-Switch Based *PCR amplification of “full length” cDNA*



T7-based *Linear amplification of cDNA*



Combinatorial indexing

sci-RNA-seq

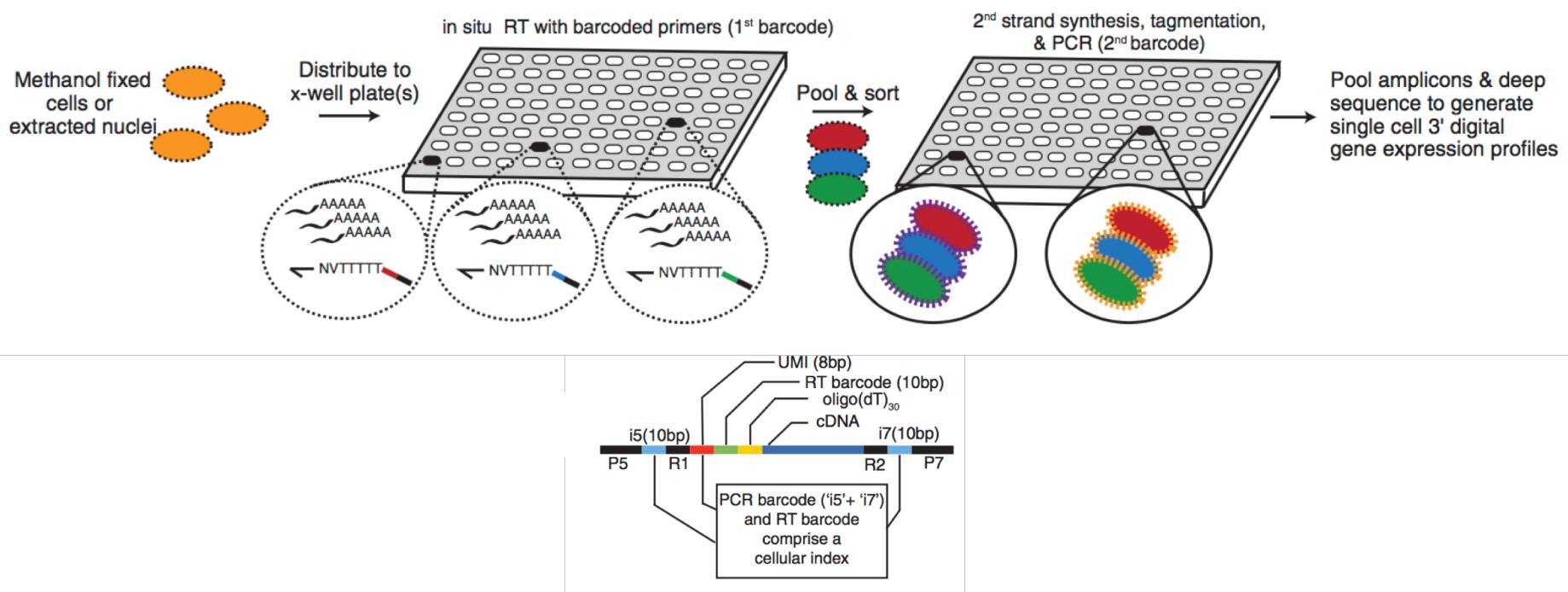
SPLiT-seq

Nanowells

Seq-Well

STRT-seq-2i

scRNA-seq using combinatorial indexing approaches: sci-RNA-seq



~4500 genes per mouse cell (3T3) - similar to 10x

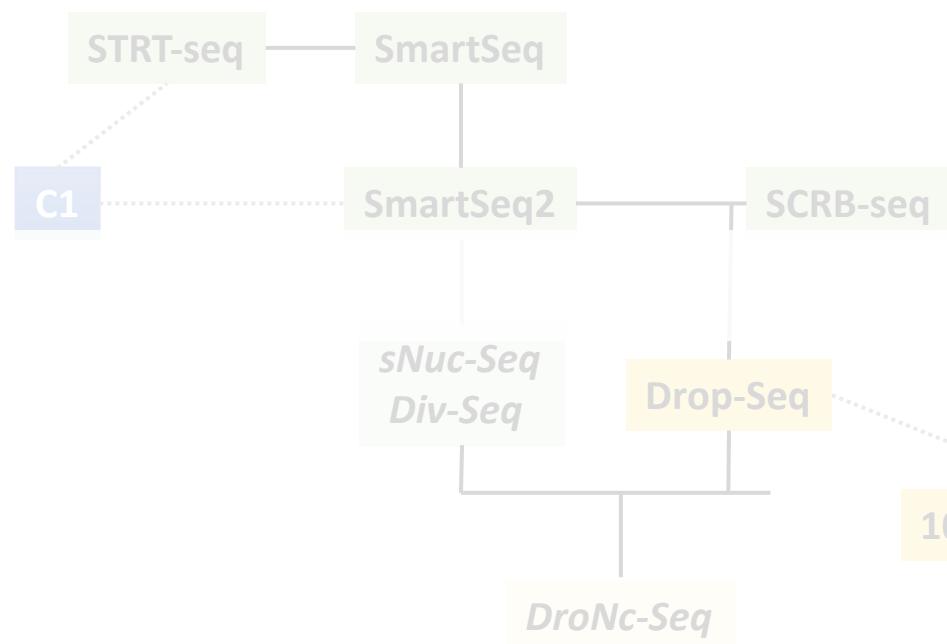
Can process 1,000s of cells per experiment (potential for 10,000s-100,000s)

Pros: do not require expensive / complicated microfluidics instruments.
compatible with cells and nuclei. Need fixation so storage +++

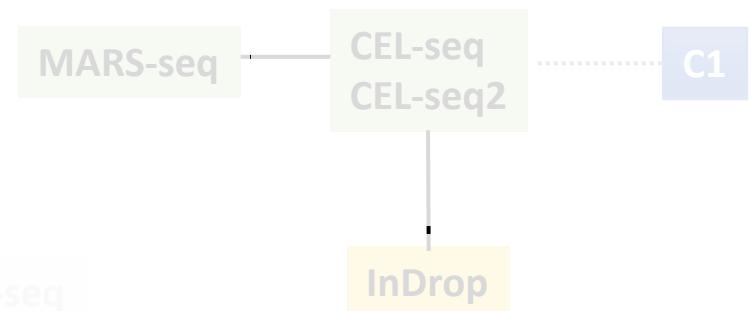
Cons: A lot of cells are required (a lot of cell loss during the multiple steps)

Cao *et al.* Science 2017

Template-Switch Based
PCR amplification of “full length” cDNA



T7-based
Linear amplification of cDNA



Combinatorial indexing

sci-RNA-seq

SPLiT-seq

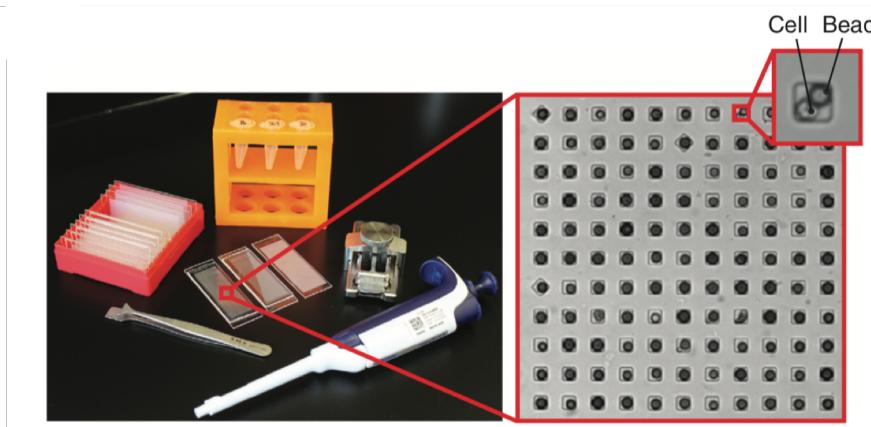
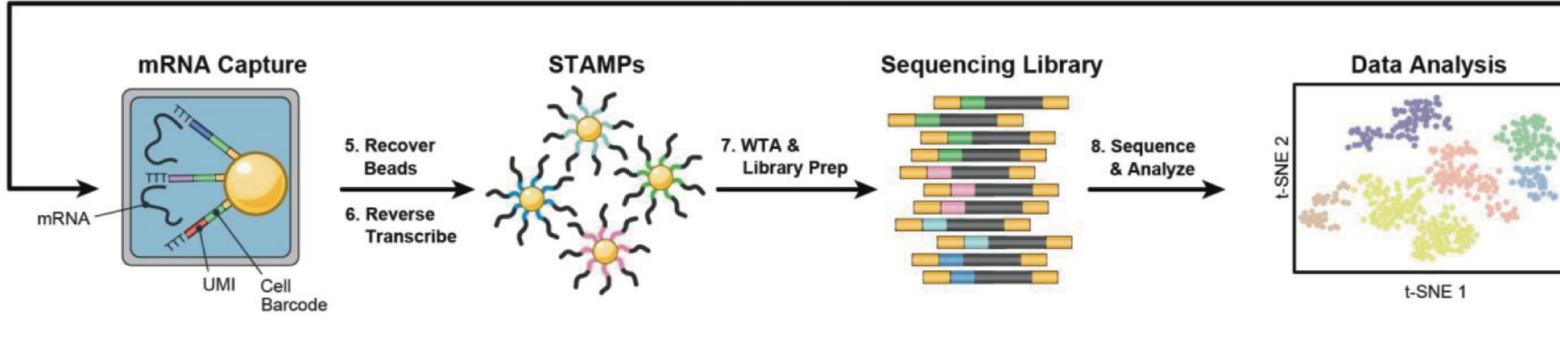
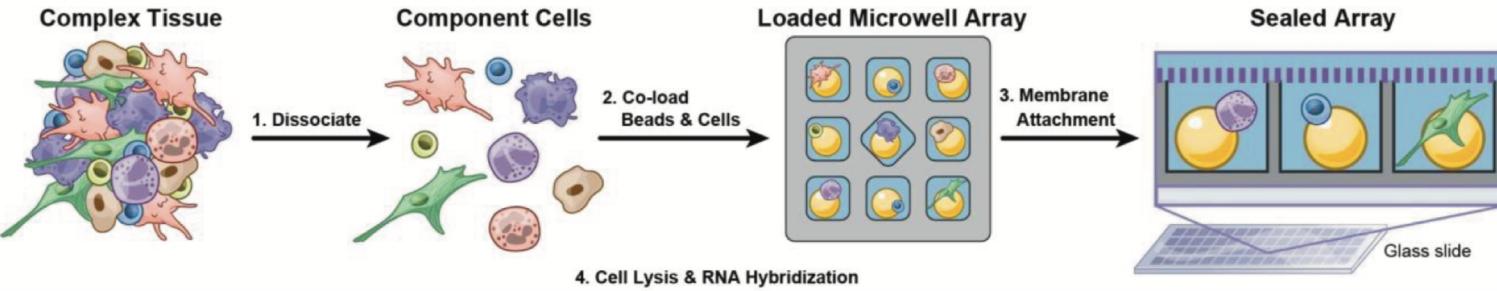
Nanowell

STRT-seq-2i

Seq-Well

Microwell-seq

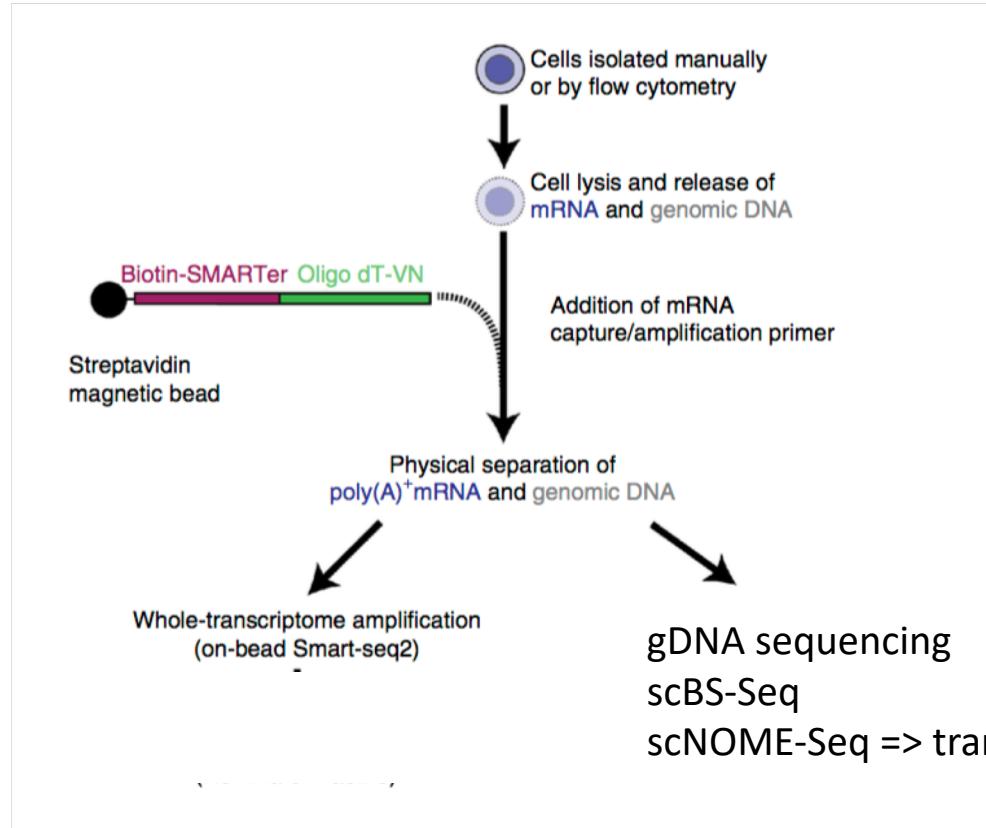
Seq-well



INTEGRATED MULTI-OMICS:

transcriptome + another layer of information from the same cell

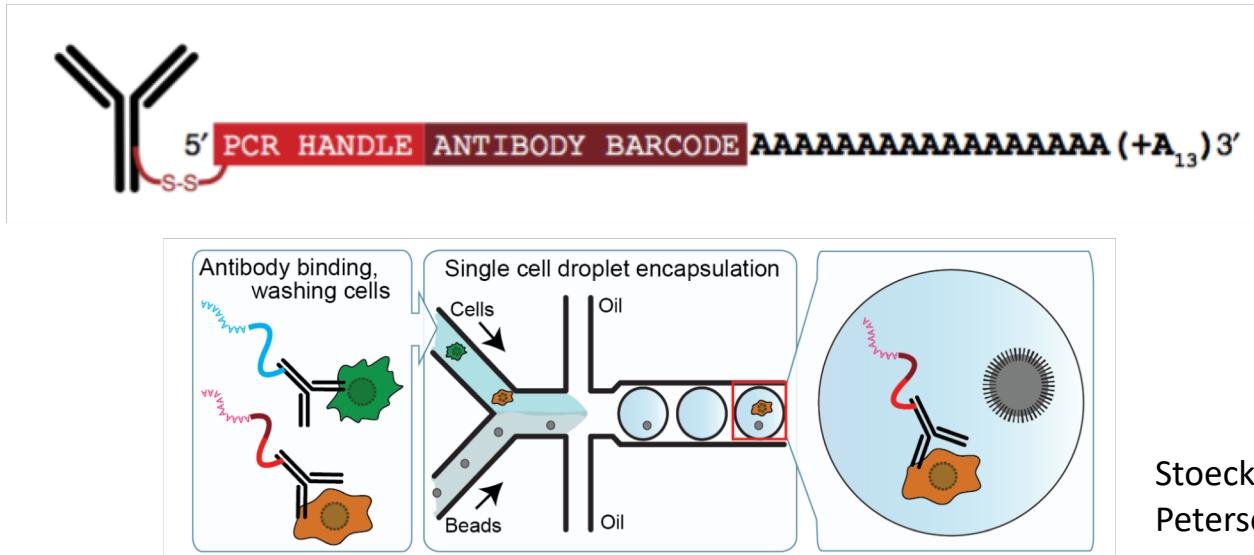
scM&T-seq: Transcriptome and DNA methylome



Smallwood *et al.* Nat. Methods 2014
Macaulay *et al.* Nat. Methods 2015
Angermueller *et al.* Nat. Methods 2016
Clark *et al.* Nat. Communications 2018

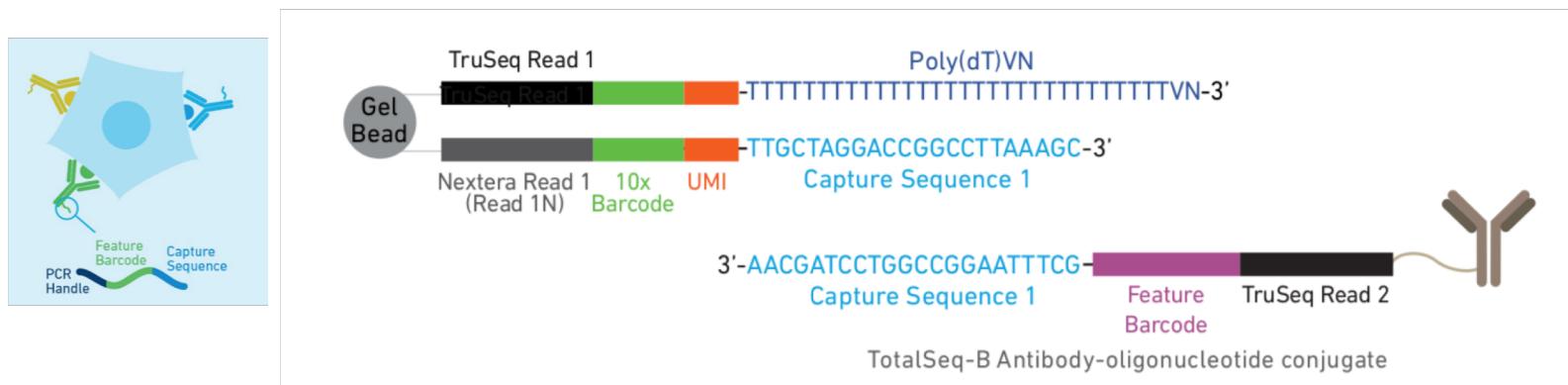
Pros: Quality is high (5000-6000 genes + 25% of the DNA methylome)
Precious samples (early embryos -> DNAm and lineage determination).
Cons: Low-Throughput + Expensive

CITE-seq / REAP-seq: cell surface markers + transcriptome



Stoeckius *et al.* Nat. Methods 2017
Peterson *et al.* Nat Biotechnology 2017

10X version (feature barcoding)



sci-CAR: Chromatin Accessibility + RNA

