

Single cell multi-omics

Miao-Ping Chien

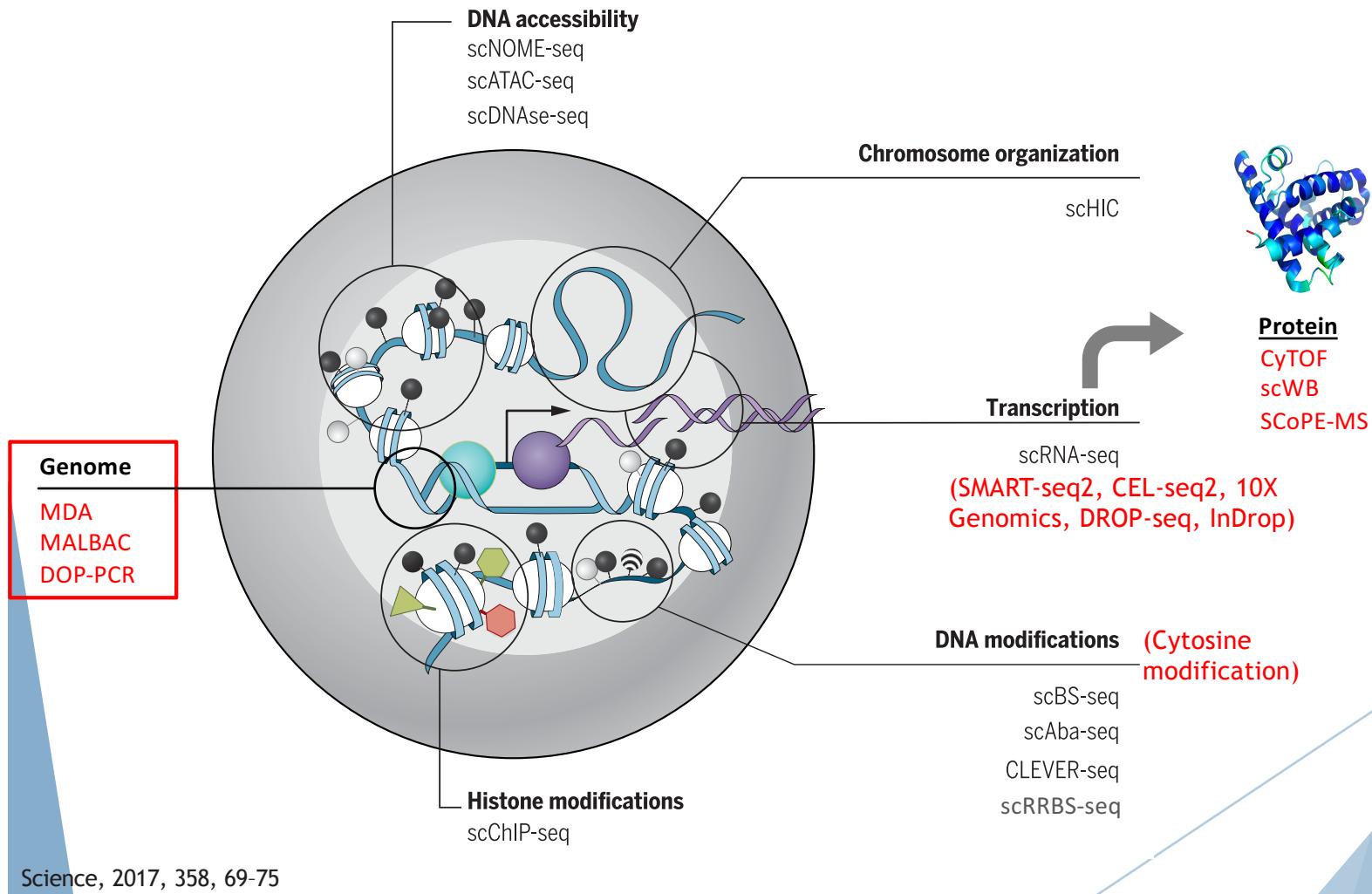
Erasmus MC, Group leader

2019 Single Cell Analysis Workshop, 2019/10/17

Outline

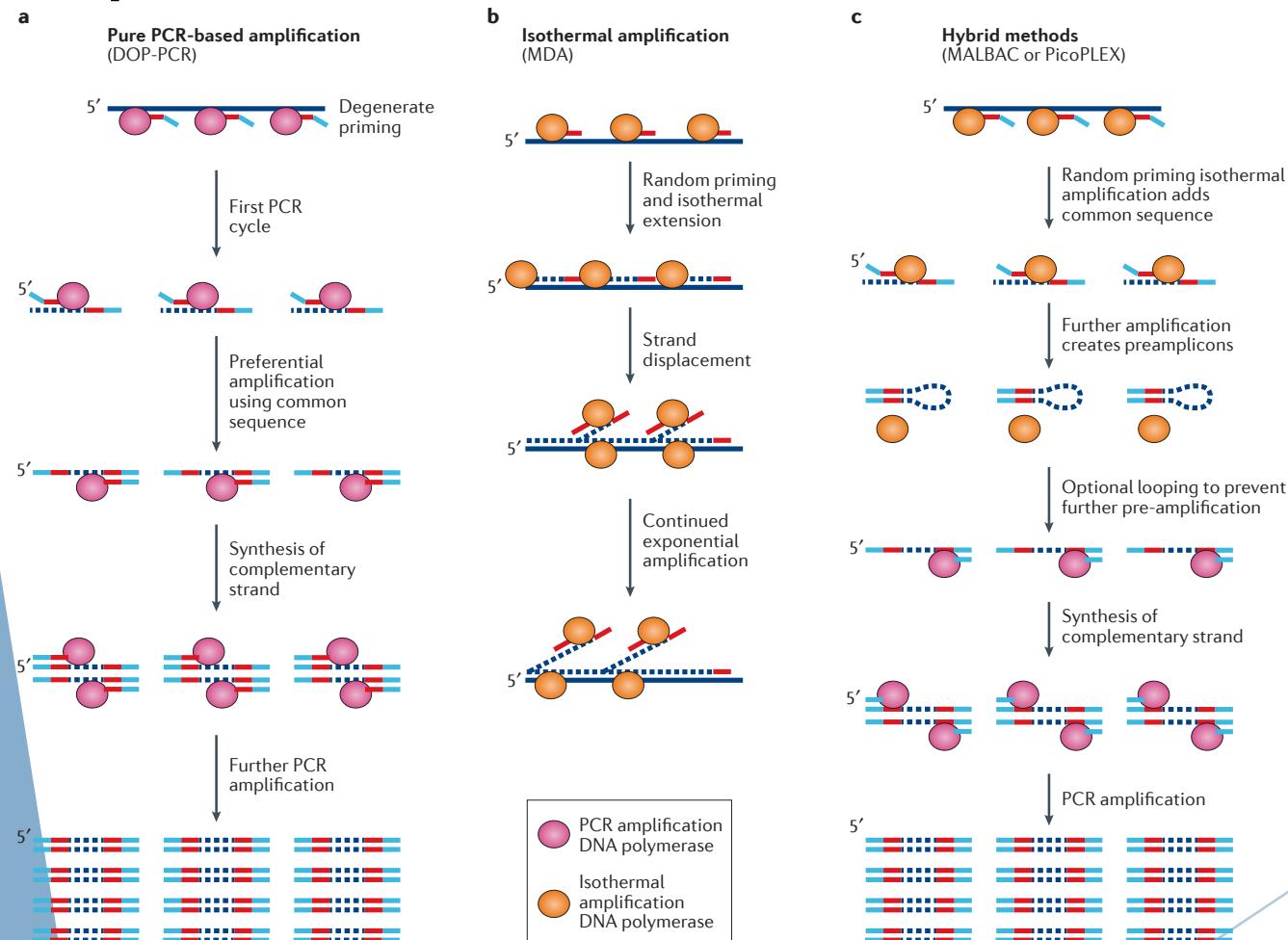
- (Quick) overview of different scSeq methods
- Different single cell -omics methods

Overview of single cell -omics

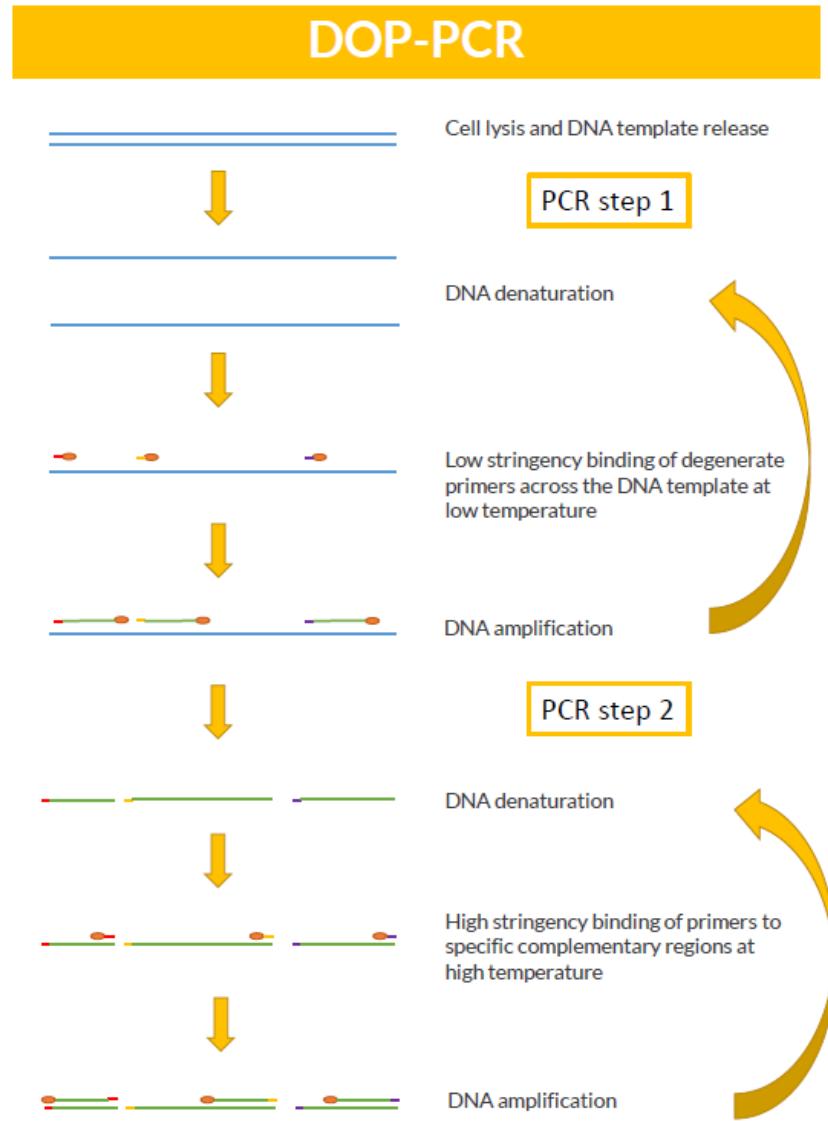


Single cell genomic sequencing

Overview of the three main whole-genome amplification methods

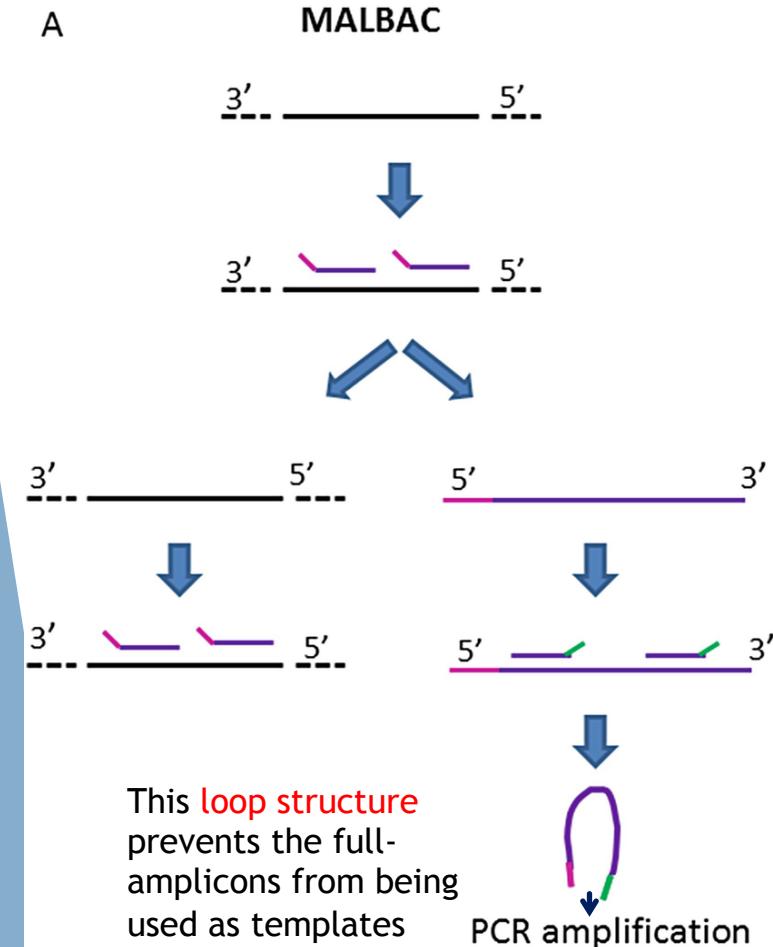


Degenerate Oligonucleotide - Primed PCR (DOP-PCR)



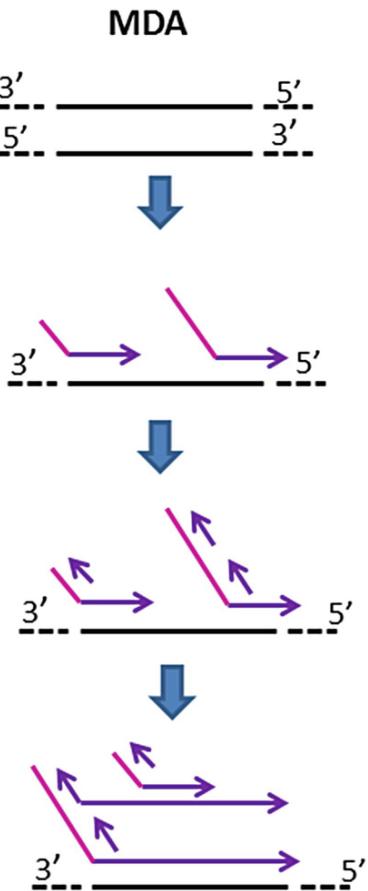
MALBAC: multiple annealing and looping based amplification cycles

A

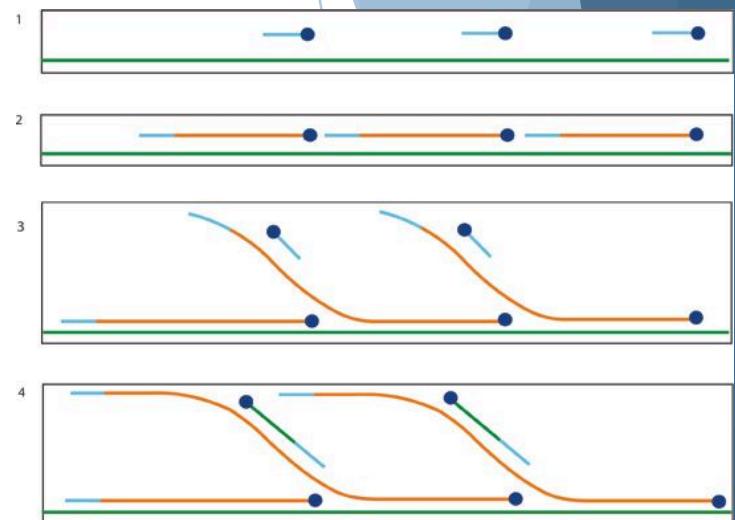


MDA: multiple displacement amplification

B

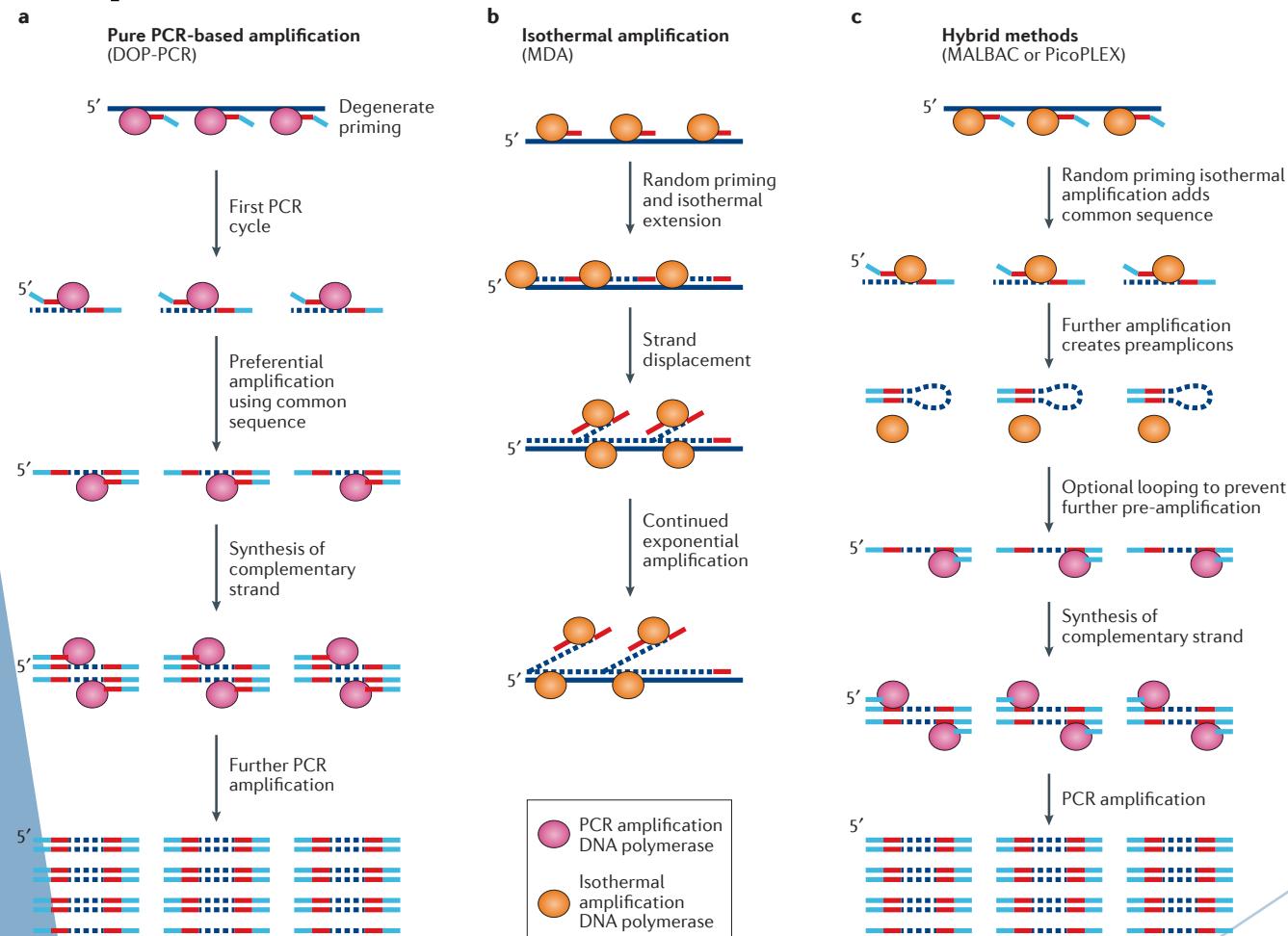


MALBAC vs MDA



The **phi29 DNA polymerase** enables the newly synthesized strand to displace the formerly synthesized one on the same template. Free primers will anneal to these displaced single-strands and continue such cycles of "displace and anneal".

Overview of the three main whole-genome amplification methods

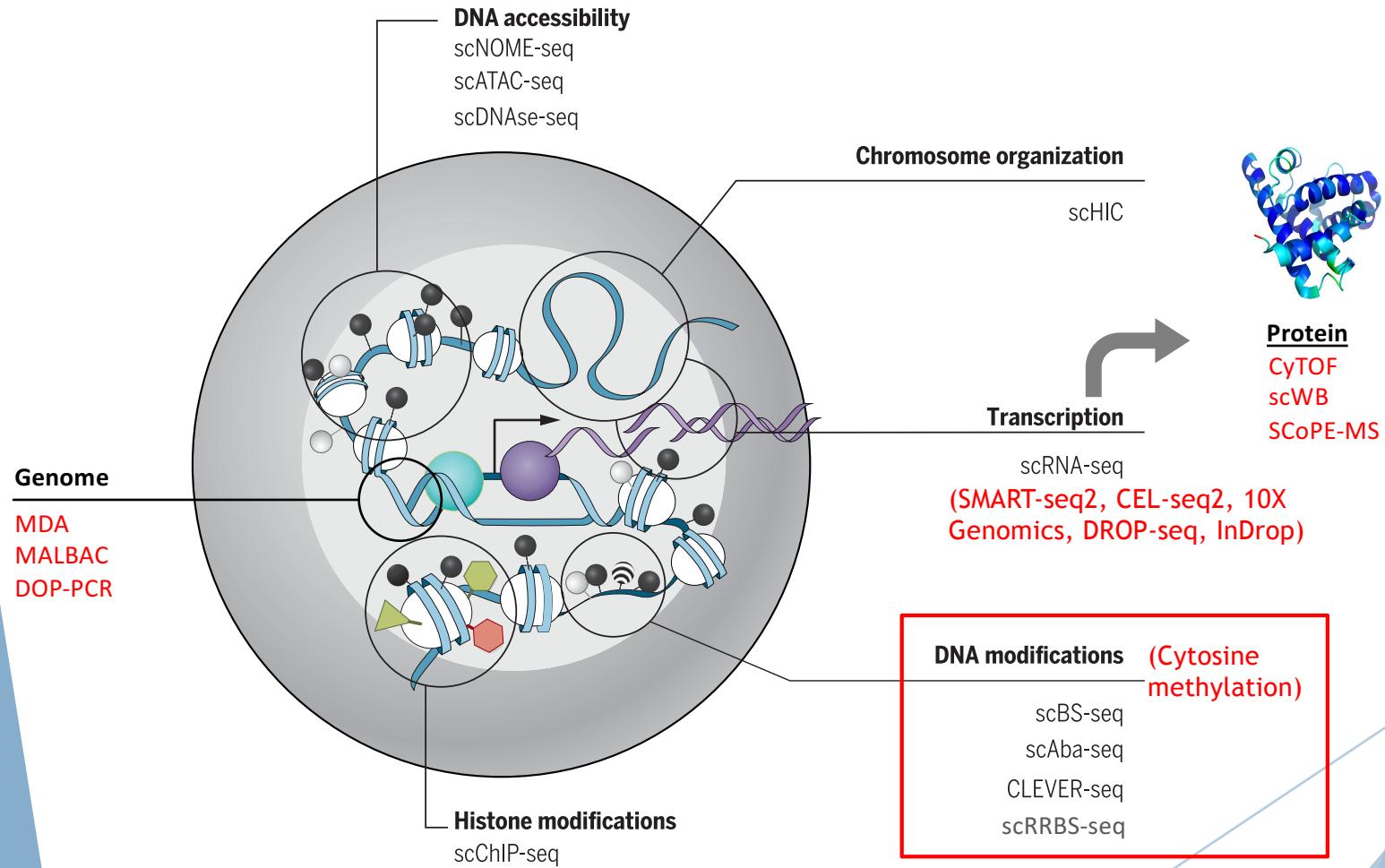


- Highest to lowest genome recovery rate (sequencing depth)?

MDA (~80%) > MALBAC (~50%) > DOP-PCR (~6%)
- Highest duplication ratio (duplicate the product more than the original template)?

DOP-PCR > MDA , MALBAC

Overview of single cell -omics



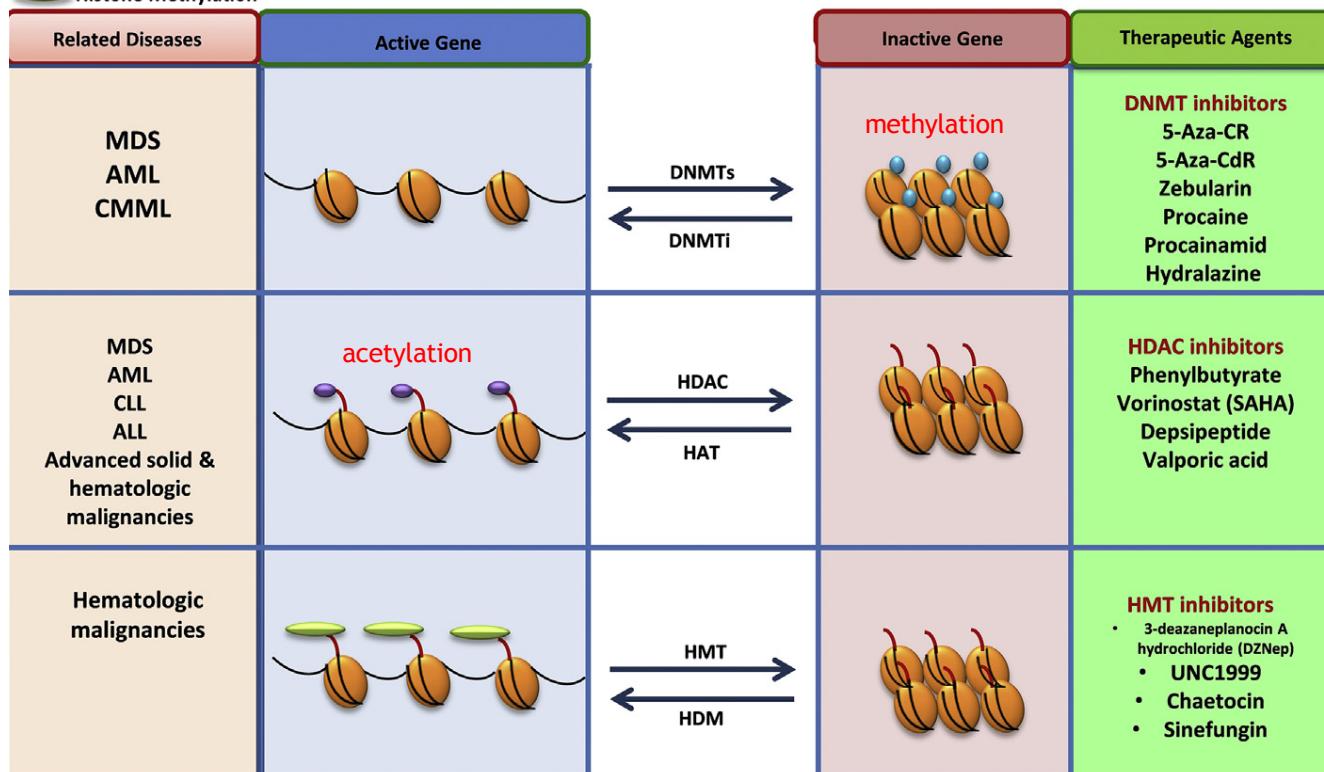
Single cell epigenomic sequencing

Why epigenomics?

DNA Methylation

Histone Acetylation

Histone Methylation



Histone acetylation:
opening of the chromatin mass & the onset of transcription,
DNA Methylation:
condenses chromatin & accompanies transcriptional inhibition

The cycle of DNA demethylation

Active **DNA demethylation** occurs by thymine DNA glycosylase (TDG) coupled with base excision repair (BER) or replication-dependent dilution of **5hmC**, **5fC** or **5caC**.

DNMT: de novo methyltransferase enzymes

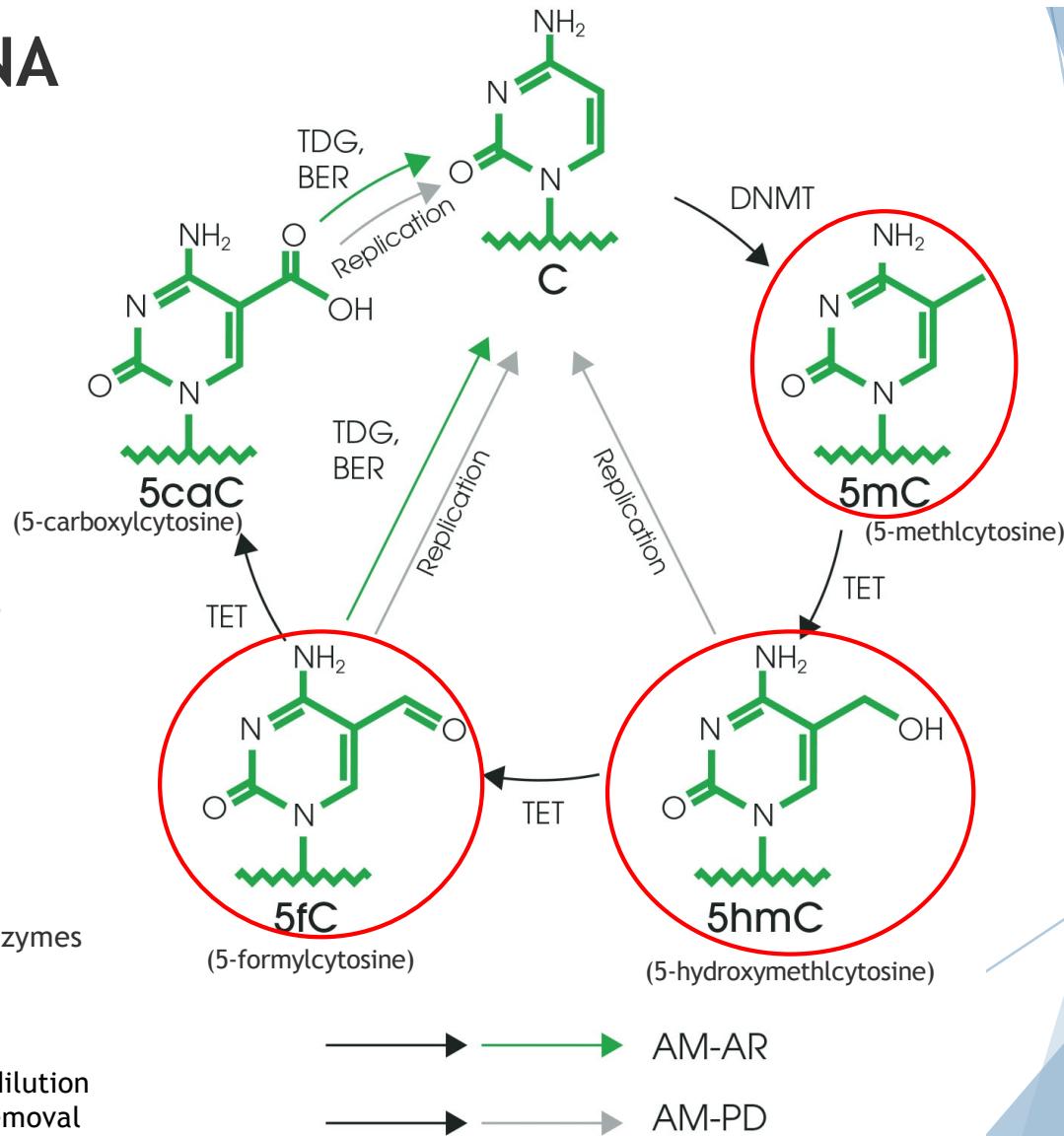
TET: ten-eleven translocation

TDG: thymine DNA glycosylase

BER: base excision repair

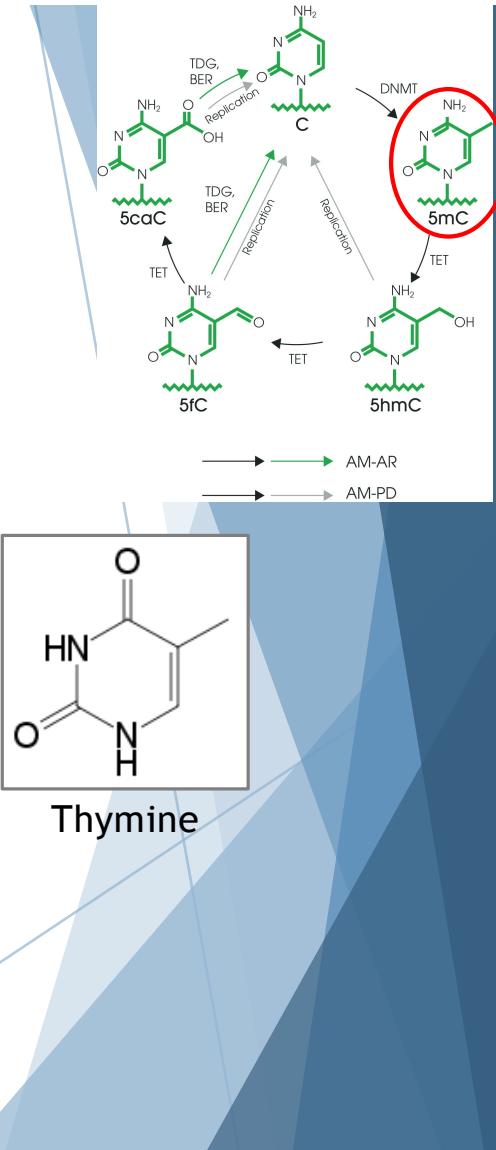
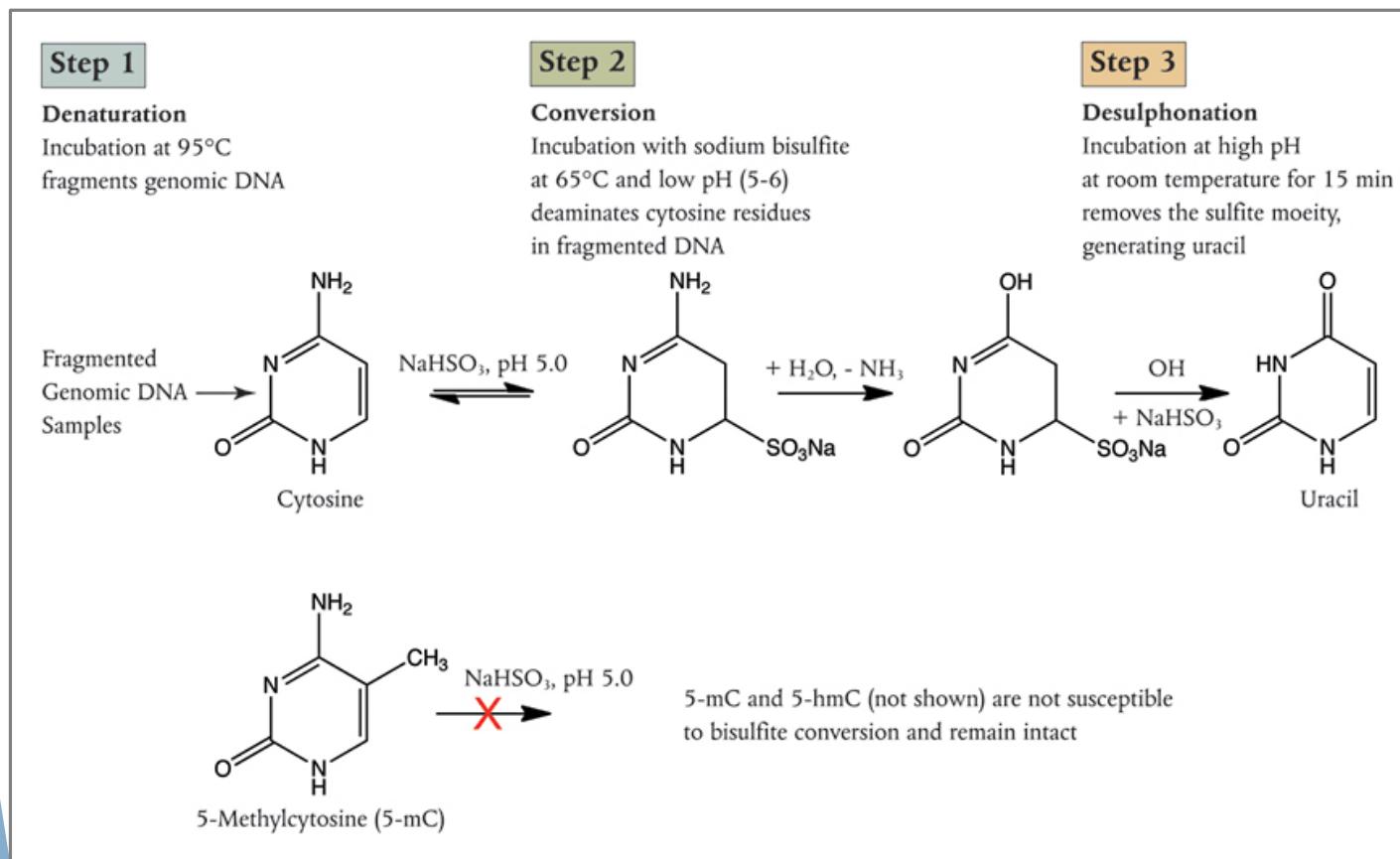
AM-PD: Active modification-passive dilution

AM-AR: active modification-active removal



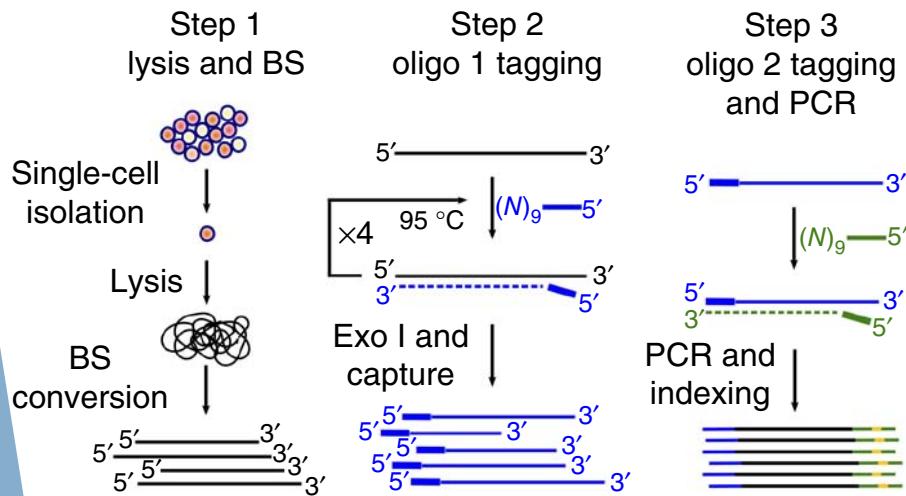
Bisulfite conversion

- C → U
- C* → C



Single cell bisulfite sequencing (scBS-seq)

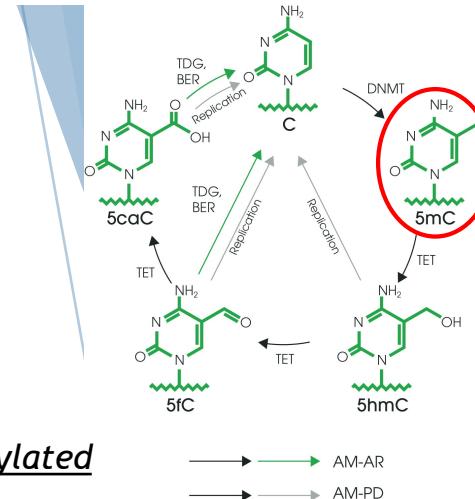
a



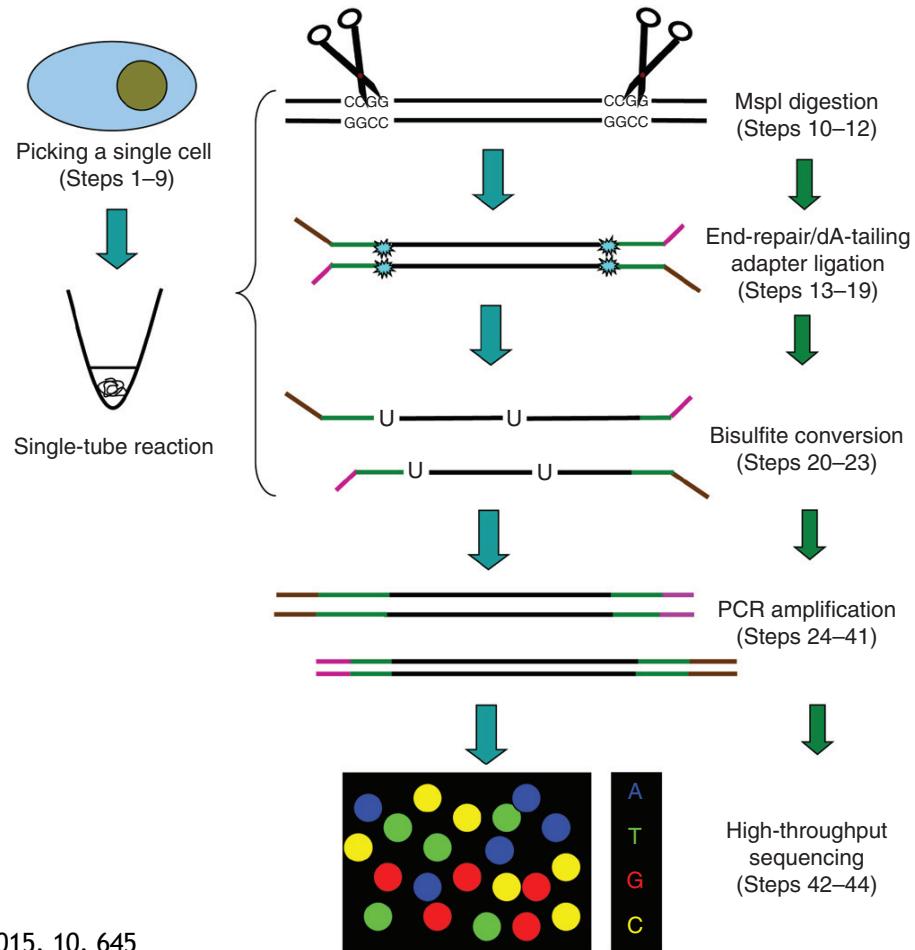
Step 1: bisulfite treatment \rightarrow DNA fragmentation & conversion of unmethylated cytosines to thymine

Step 2: synthesis of complementary strands is primed using oligonucleotides containing Illumina adaptor sequences and a 3' stretch of nine random nucleotides

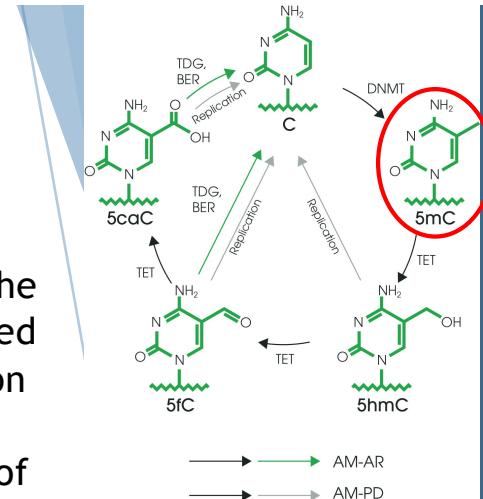
Step 3: After capturing the tagged strands, a second adaptor is similarly integrated, and PCR amplification is performed with indexed primers



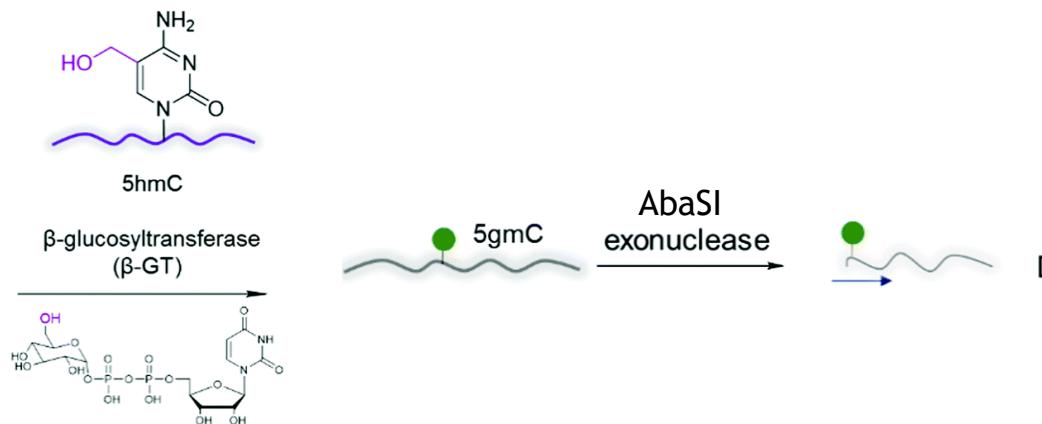
scRRBS-seq: single-cell reduced-representation bisulfite sequencing



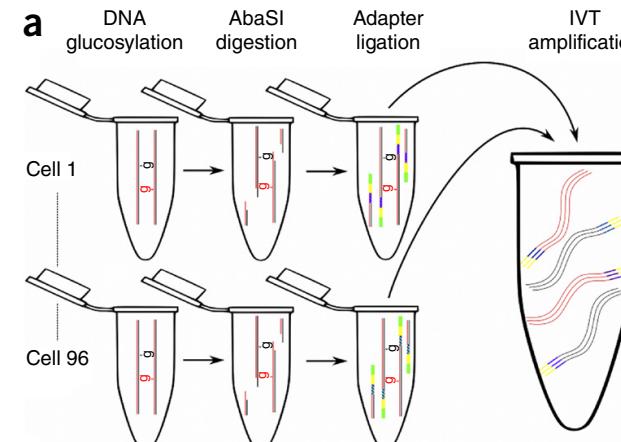
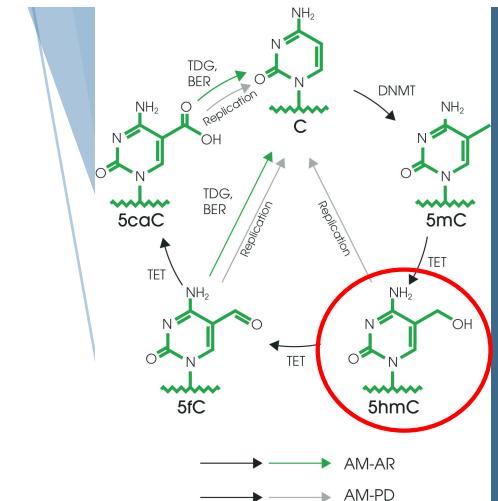
Mainly focus on the **GpC island** (related to gene expression regulation -> (de)methylation of “C” in CpG island)



scAba-seq: single-cell 5hmC sequencing



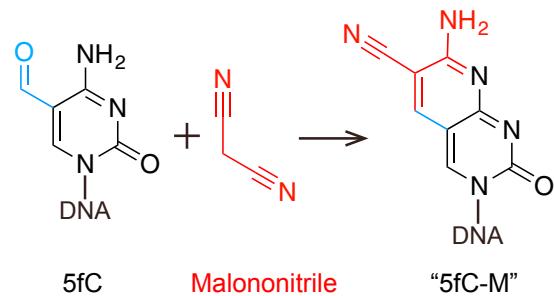
5hmC marks in DNA from individual cells are **glucosylated with T4 phage- β -glucosyltransferase (T4 β -GT)**, and the DNA is digested with the restriction endonuclease **AbaSI**. The digested DNA is ligated to an adapter containing a cell-specific barcode, an Illumina 5' adapter, and a T7 promoter. The ligated DNA from different cells is pooled and amplified using *in vitro* transcription mediated by T7 RNA polymerase.



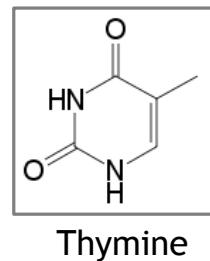
CLEVER-seq

(chemical-labeling-enabled C-to-T conversion sequencing)

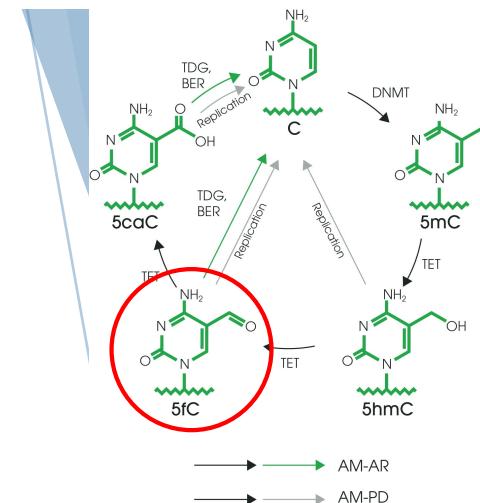
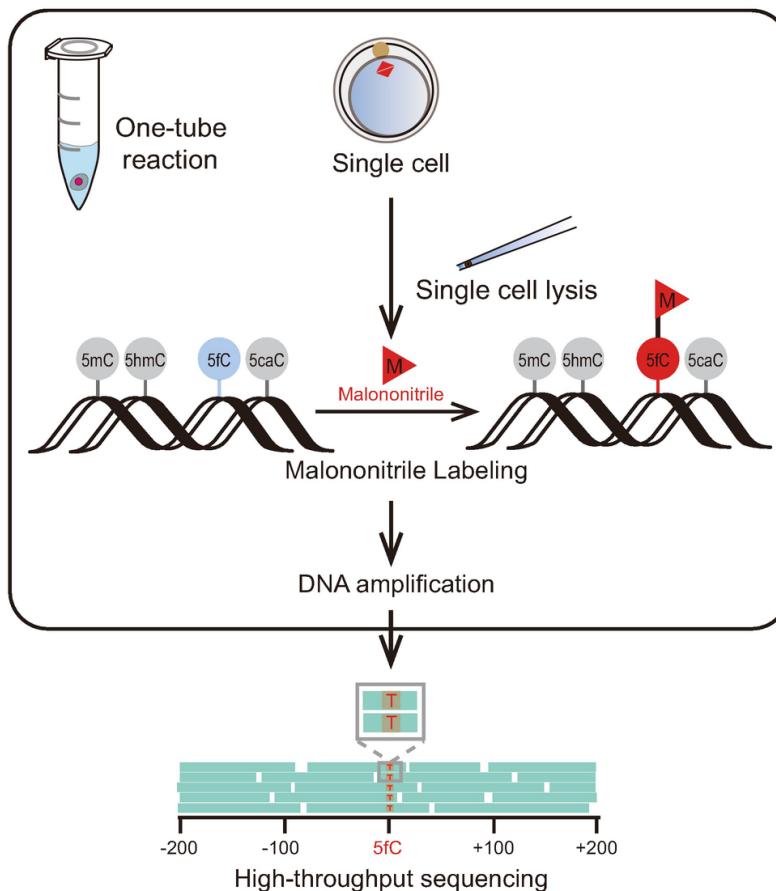
- A single-cell, single-base resolution whole-genome **5fC**-sequencing technology



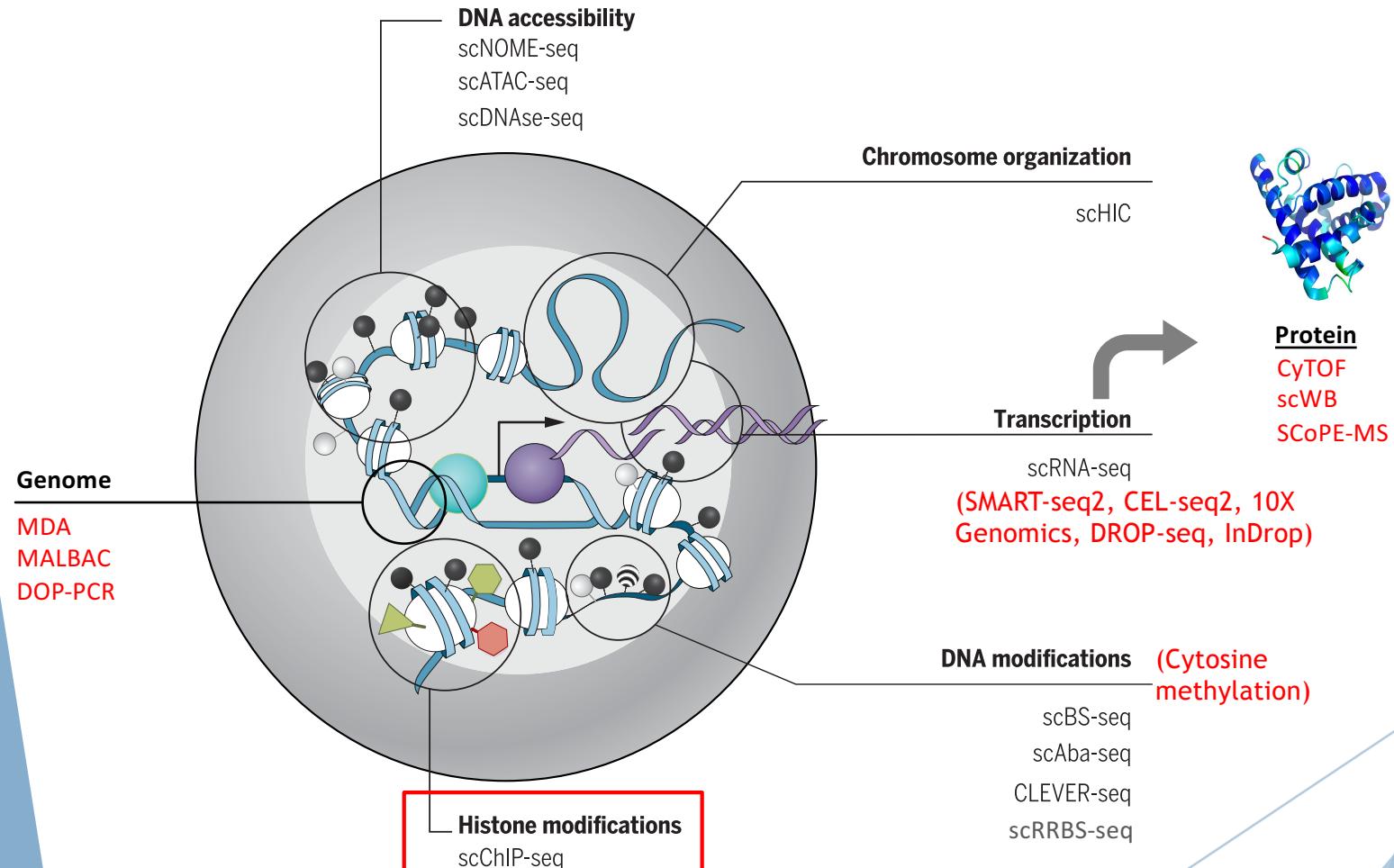
After chemical treatment, the 5fC-adduct ("5fC-M") is read as a **dT** during DNA amplification by various DNA polymerases



Cell Stem Cell, 2017, 20, 720-731



Overview of single cell -omics



Science, 2017, 358, 69-75 identify the binding sites of DNA-associated proteins (histone, TF)

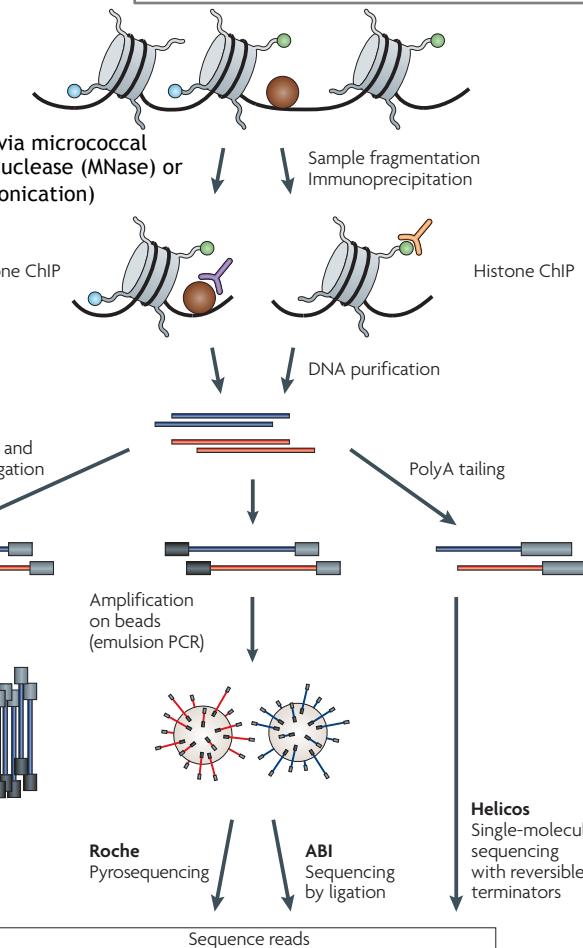
Single cell chromatin immunoprecipitation (CHIP) sequencing

Single cell chromatin immunoprecipitation (ChIP) sequencing

ChIP-seq is a widely used method for mapping histone modifications, transcription factors and other protein-DNA interactions genome-wide.

ChIP-seq

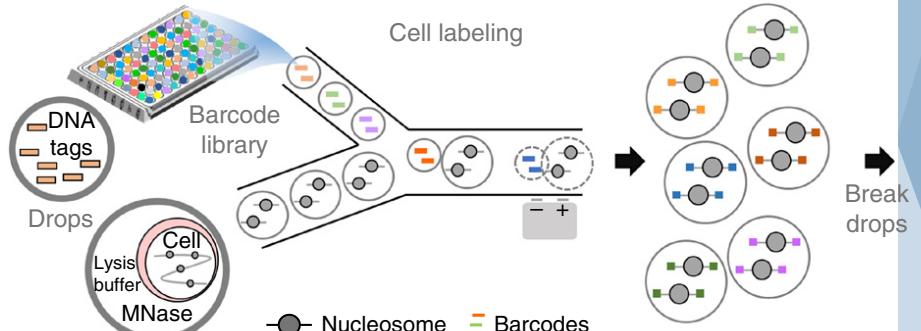
Nature reviews/
Genetics,
2009, 10,
669



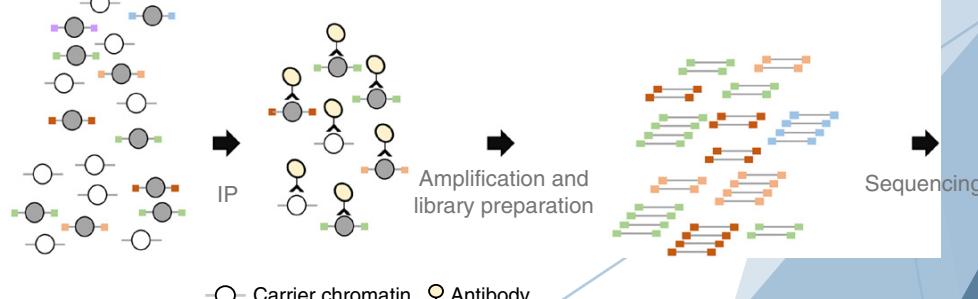
scChIP-seq

Chromatin-bearing drops and barcode drops are merged in a microfluidic device, and DNA barcodes are ligated to the chromatin fragments, thus indexing them to originating cell.

a

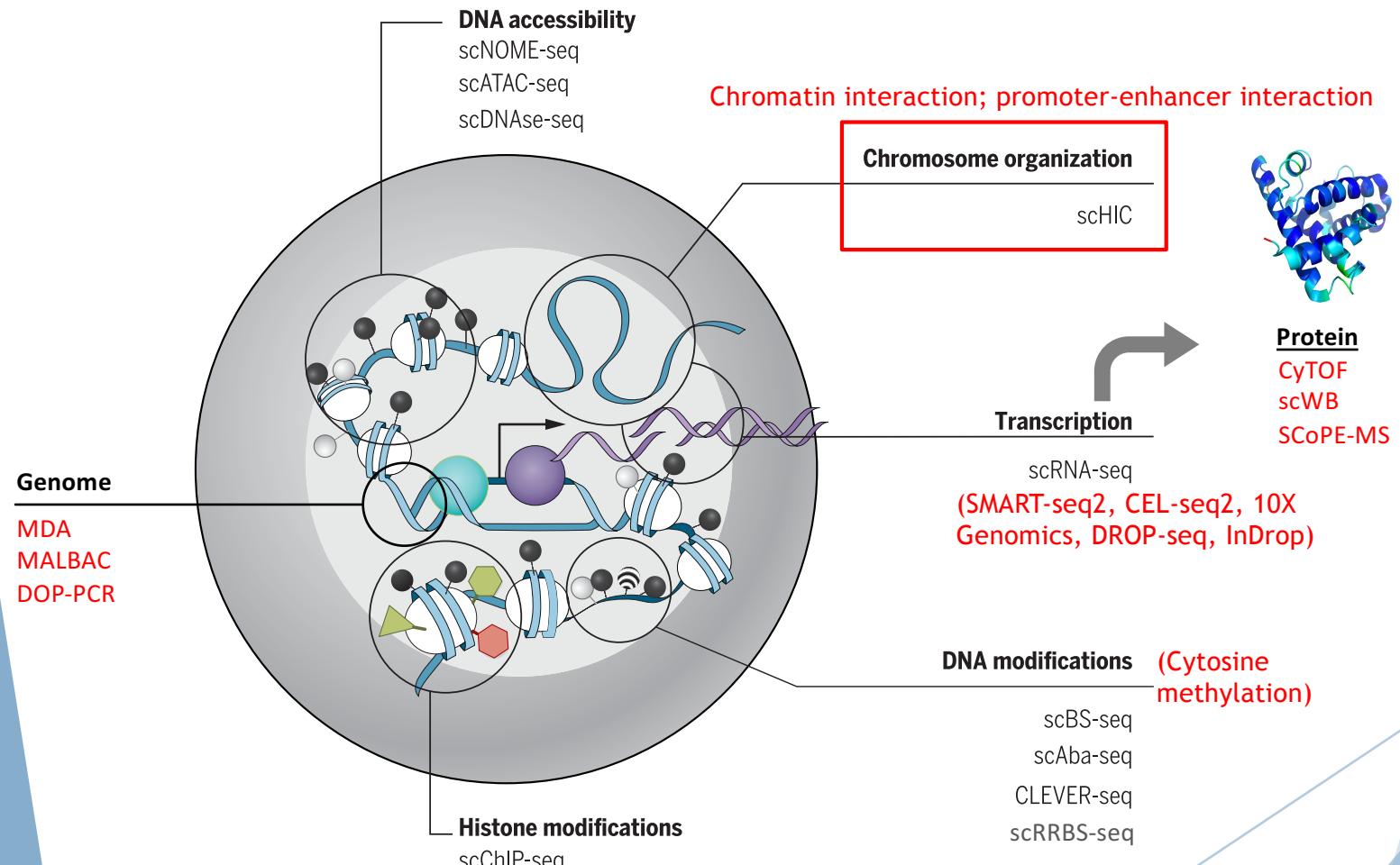


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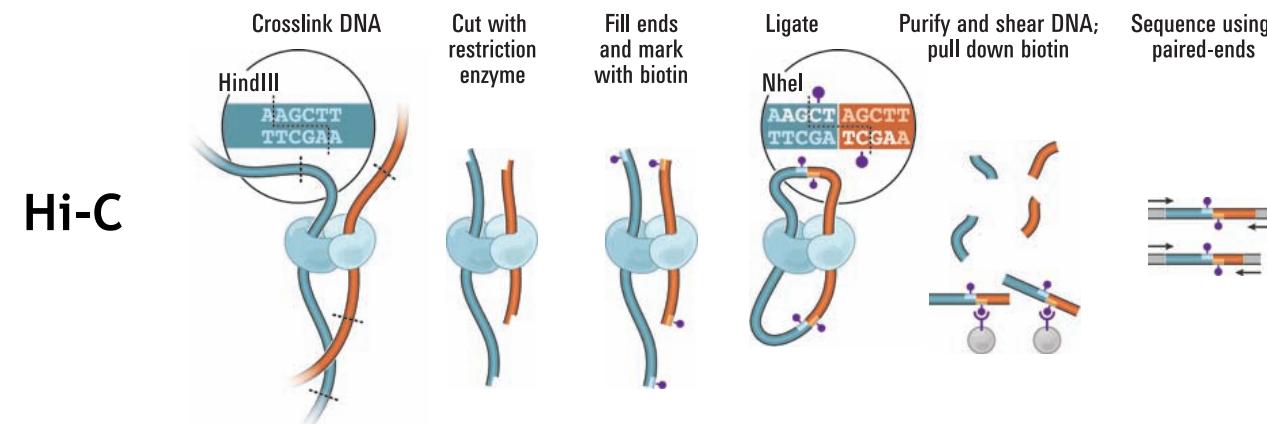


Nature Biotechnology, 2015, 33, 1165

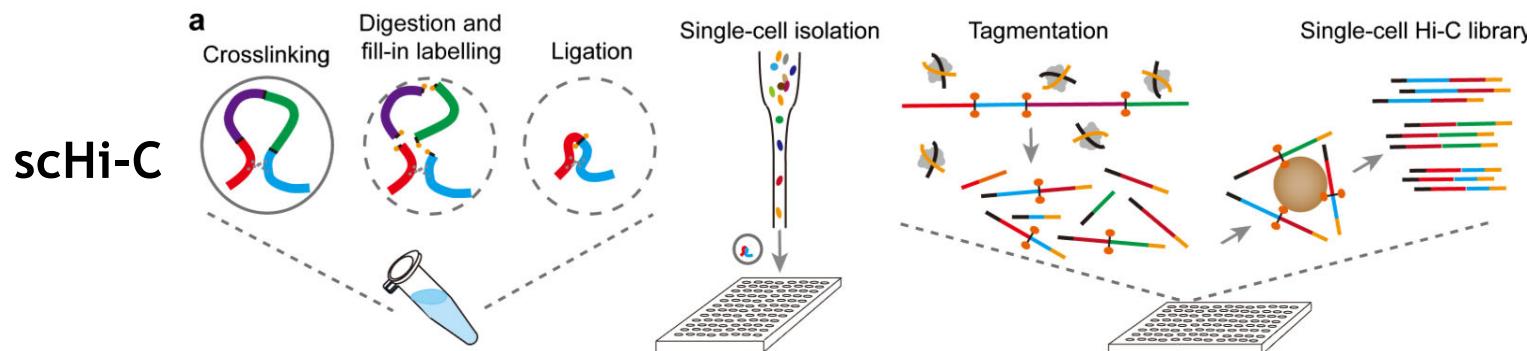
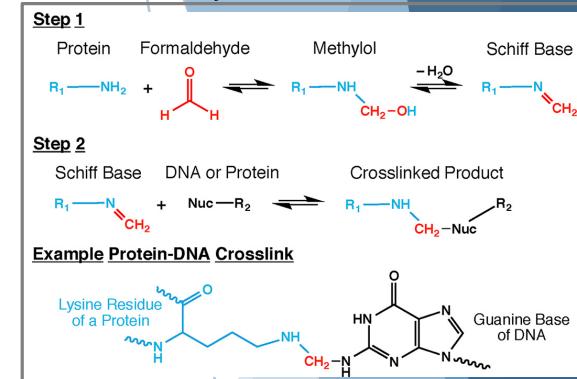
Overview of single cell -omics



Single cell Hi-C (high-resolution chromosome conformation capture)

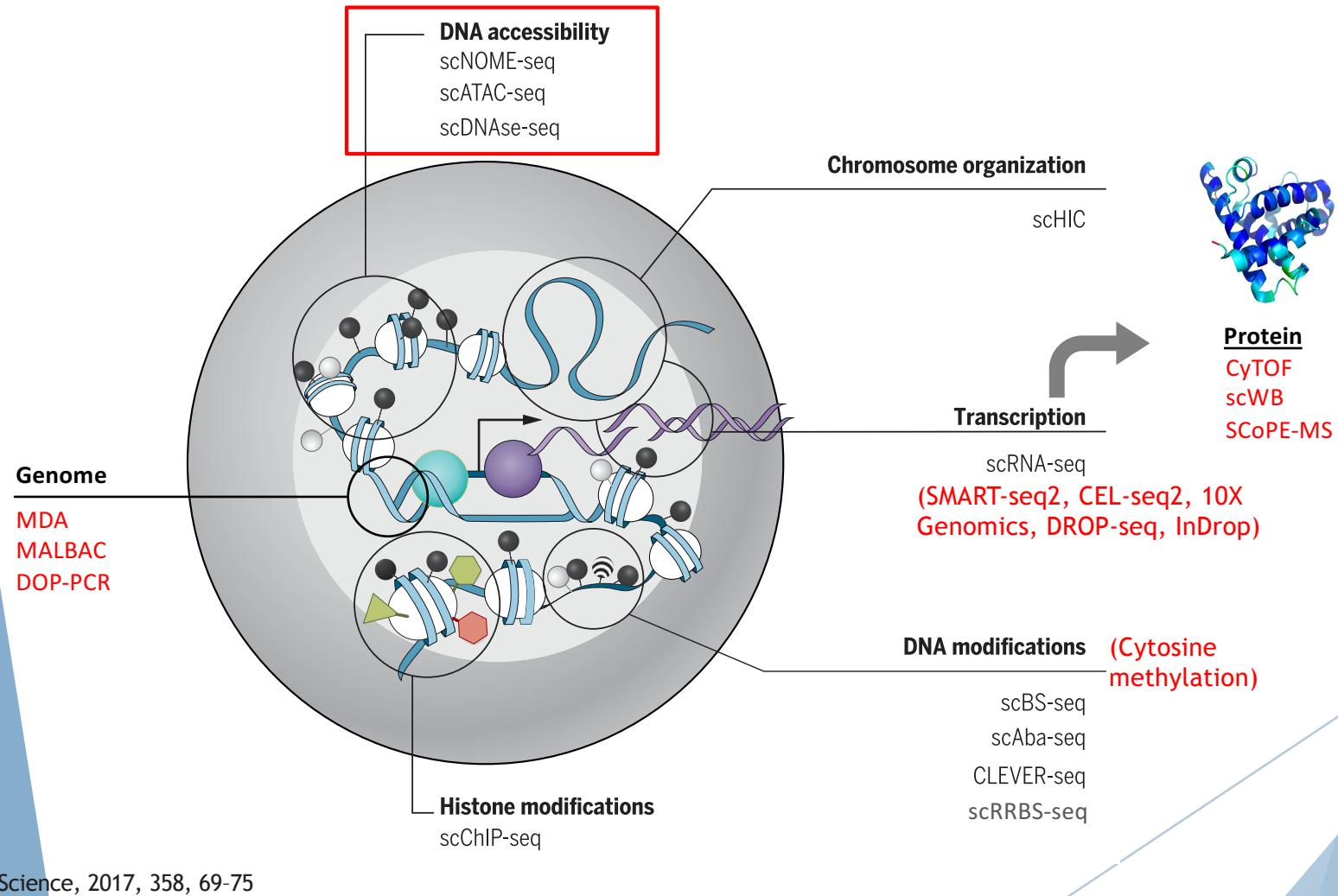


Formaldehyde fixation mechanism

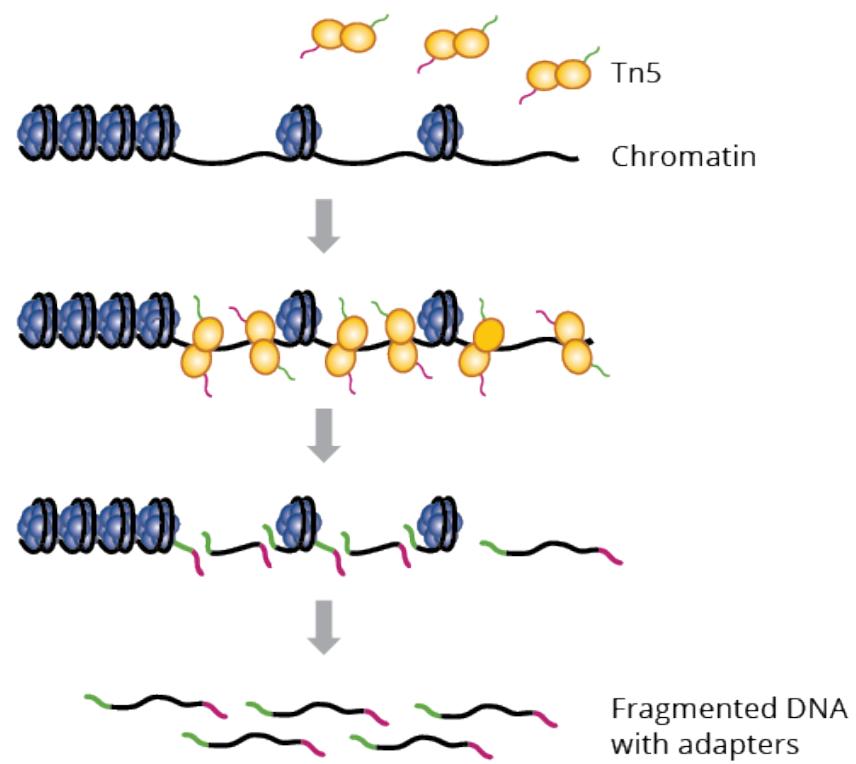


Science, 2009, 326, 289; Nature, 2017, 547, 61

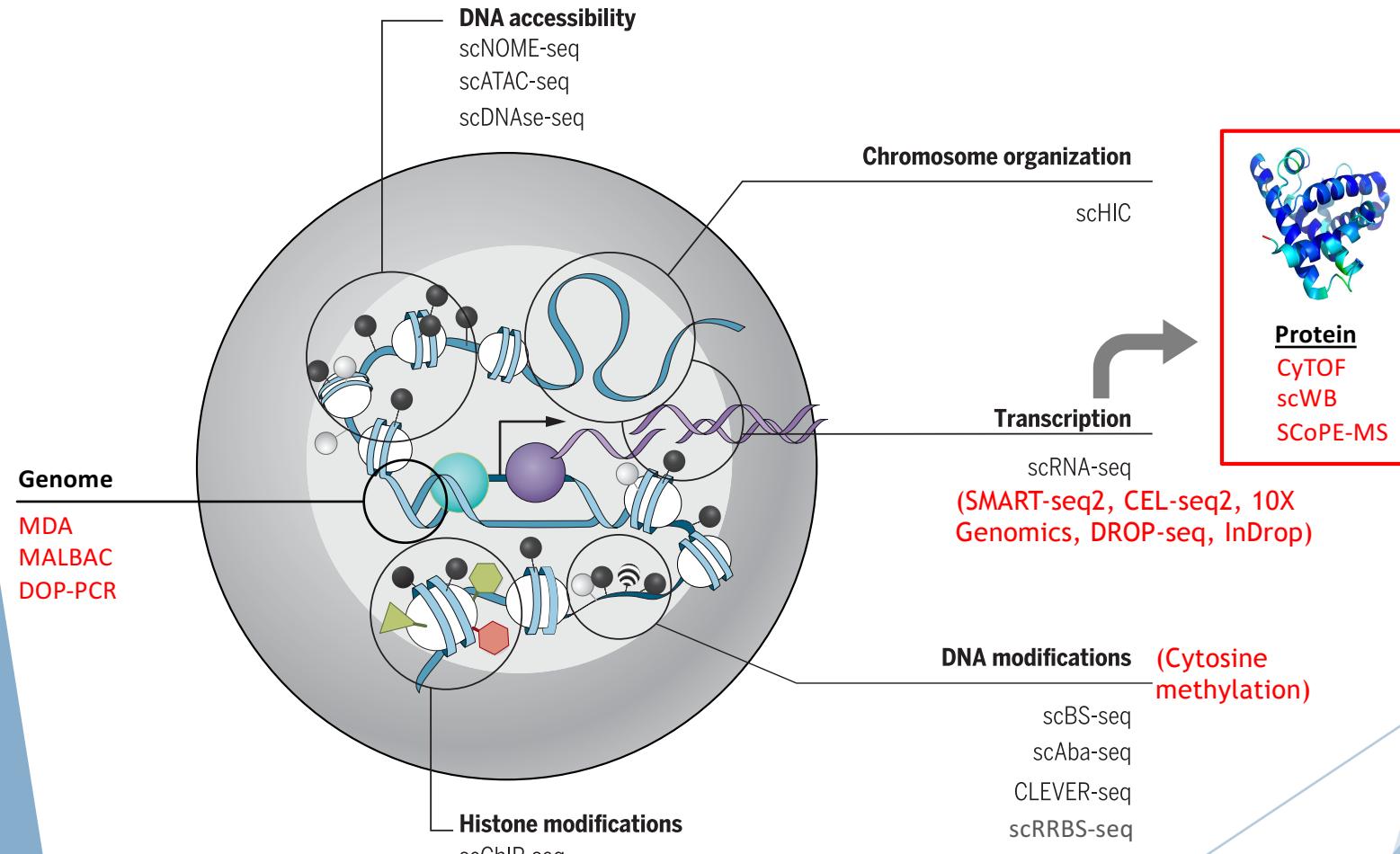
Overview of single cell -omics



ATAC-seq: Assay for transposase-accessible chromatin

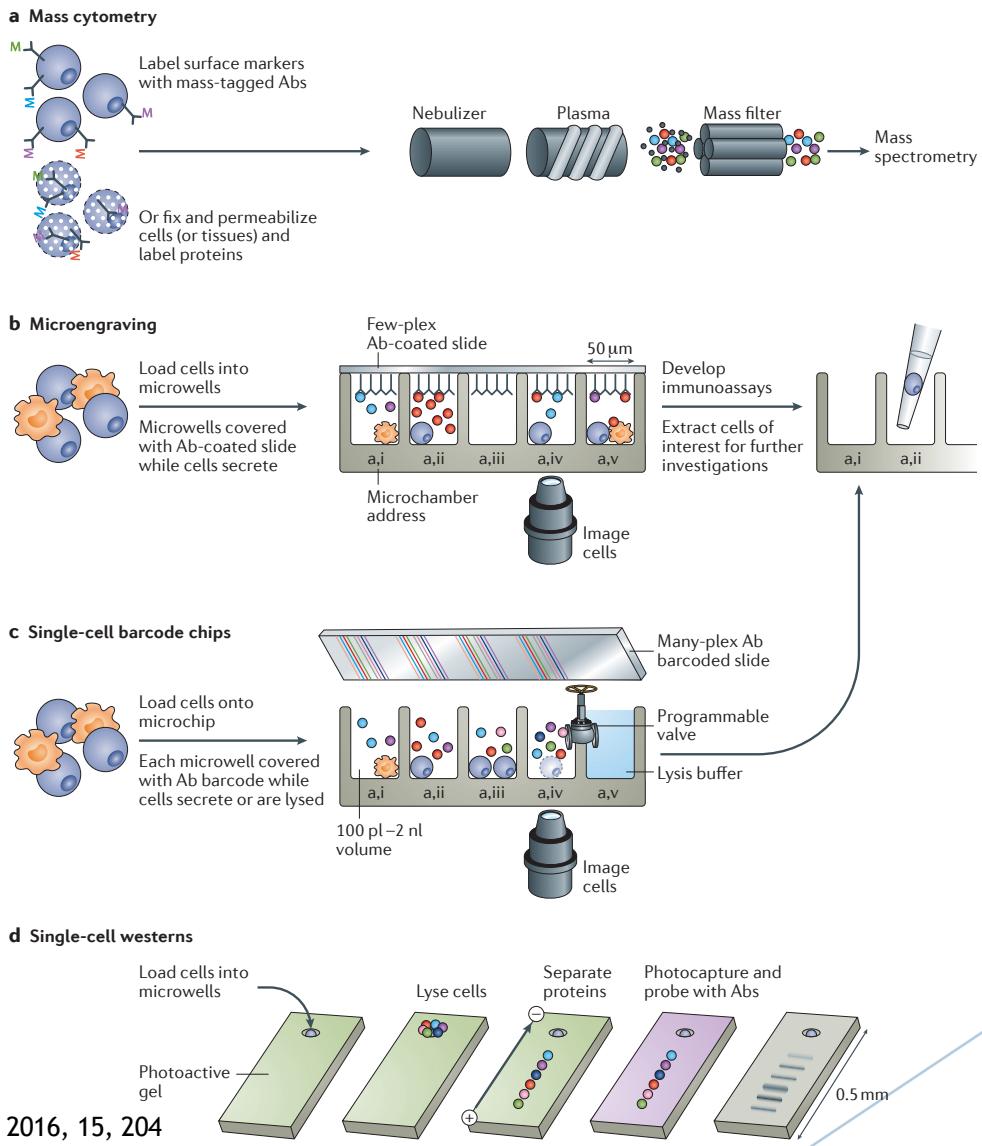


Overview of single cell -omics



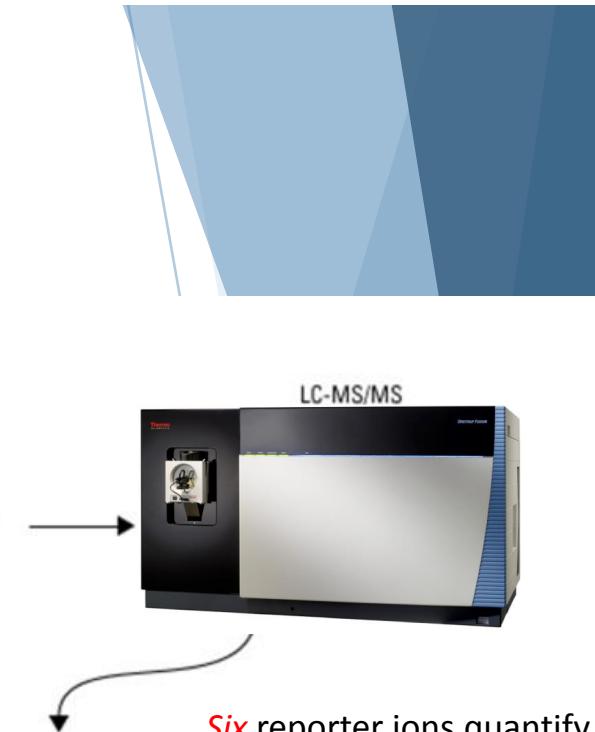
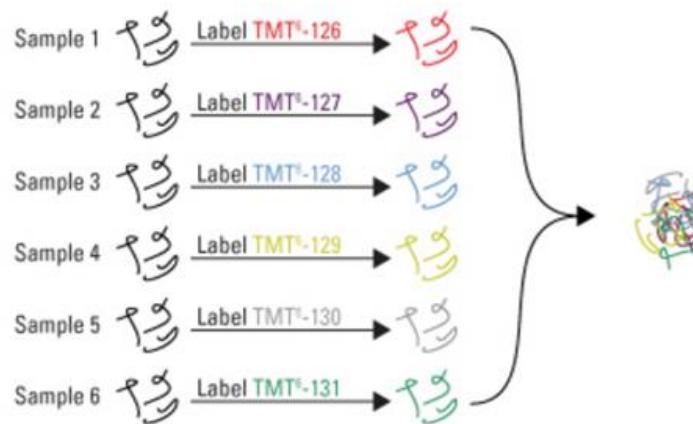
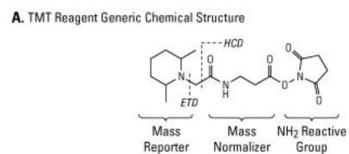
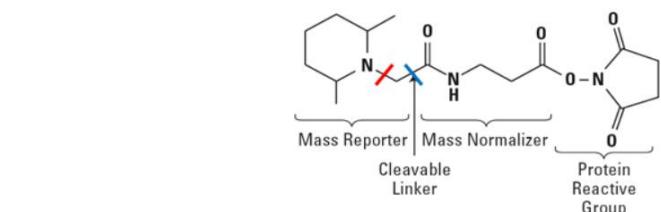
Single cell proteomics

Emerging single-cell proteomics methods

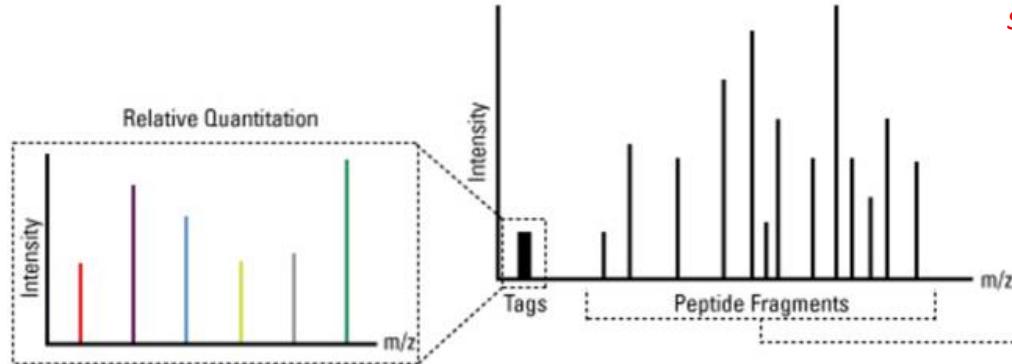


SCoPE-MS (Single Cell ProtEomics by Mass Spectrometry)

TMT⁰ (tandem mass tags) Method Development & SRM

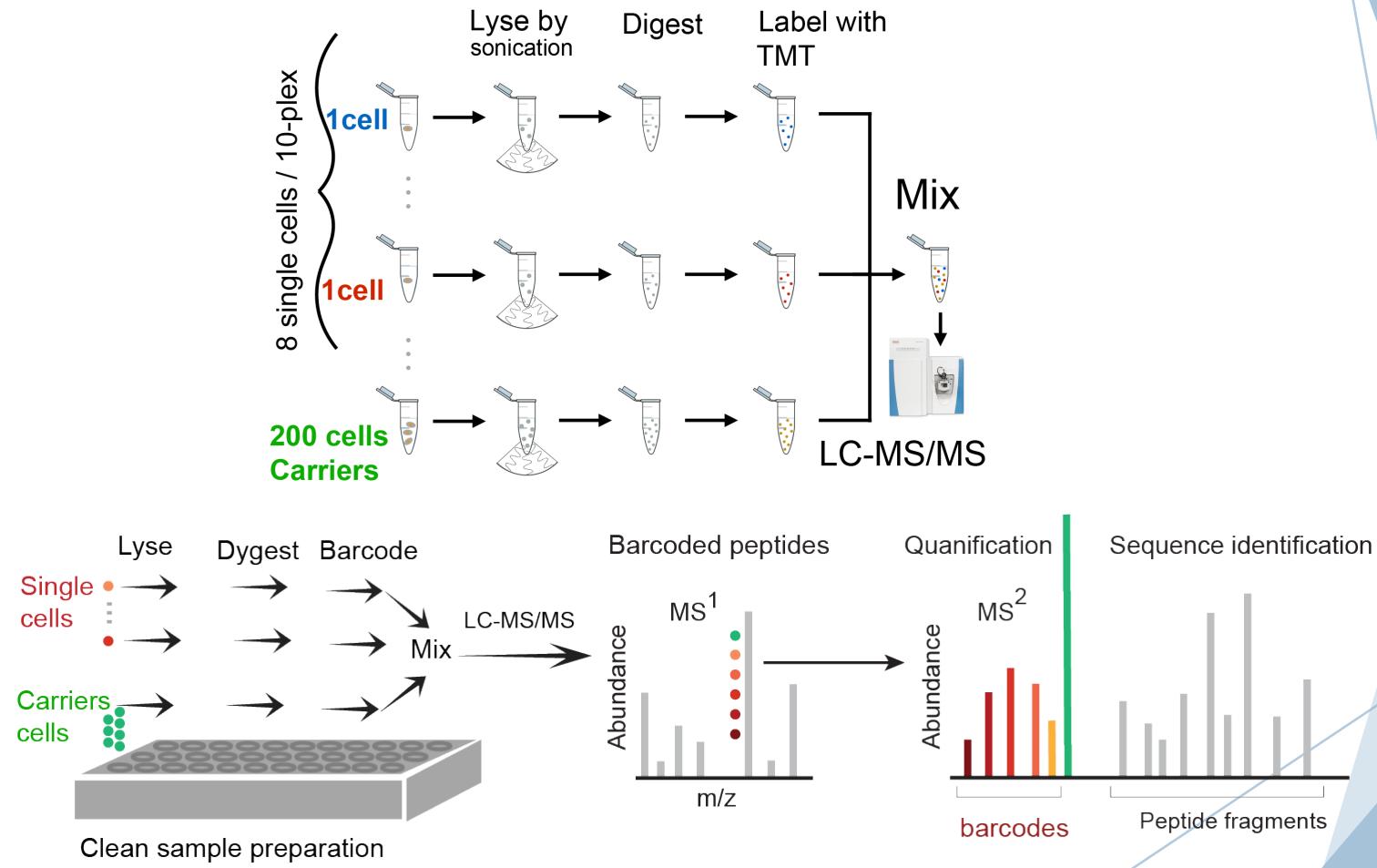


Six reporter ions quantify
same peptide from
six samples in
one spectrum



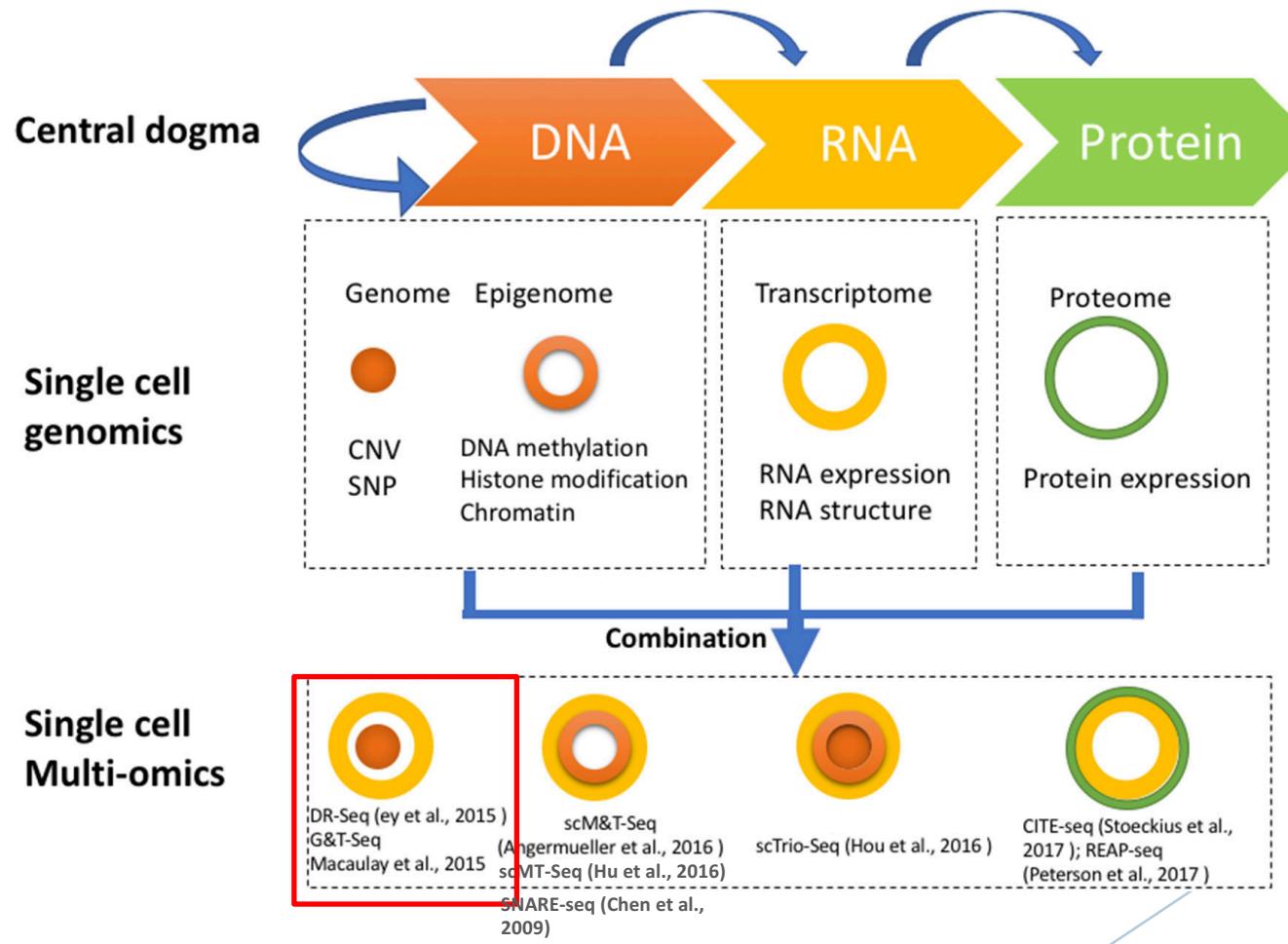
Sequence Assignment
and
Protein Identification

SCoPE-MS (Single Cell ProtEomics by Mass Spectrometry)



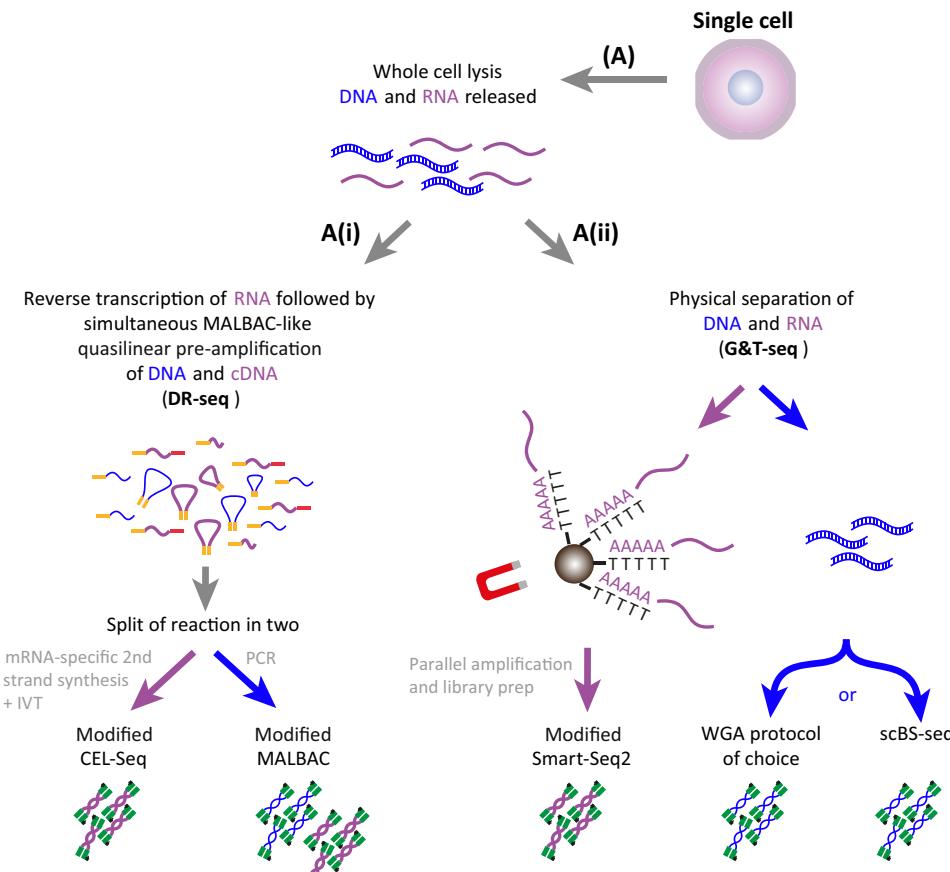
Single cell multi-omics

Strategies for multi-omics profiling of single cells



Single cell DNA- and RNA- Sequencing

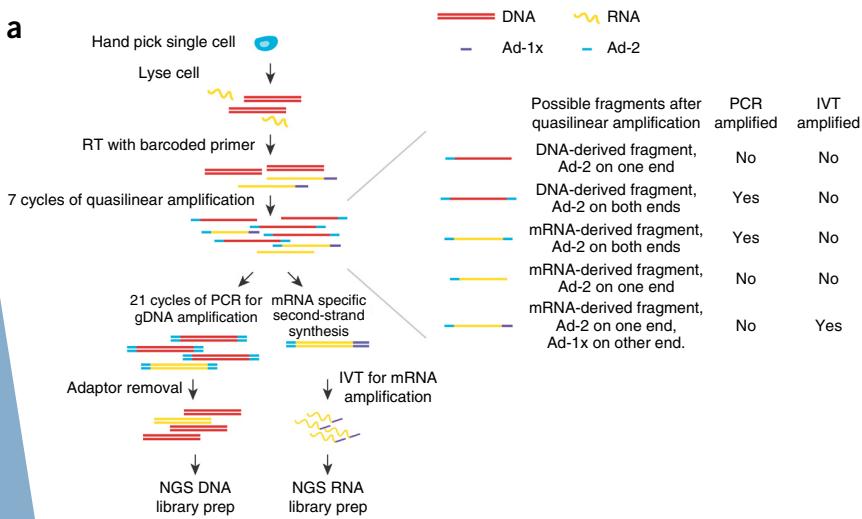
Single cell DNA- and RNA-Sequencing



DR-seq vs G&T-seq

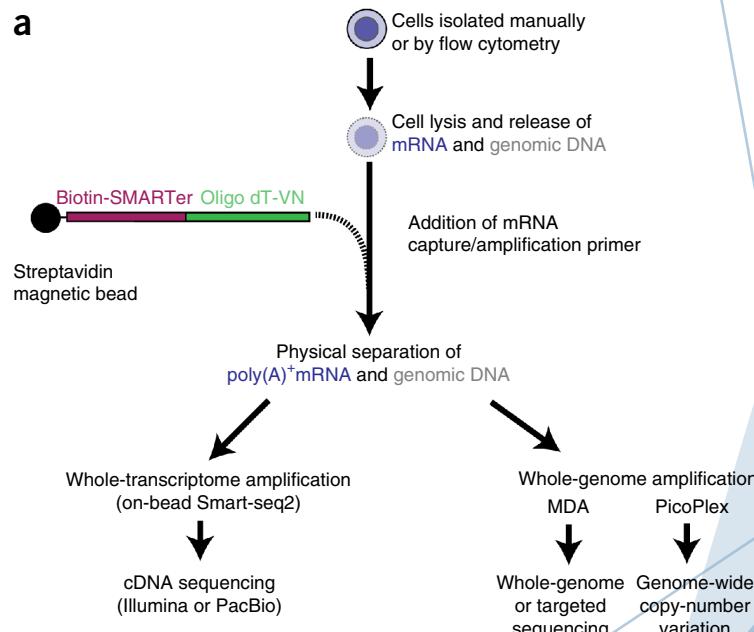
DR-seq

a



G&T-seq

a



Single cell DNA- and RNA-Sequencing

(C)

DR-seq

Loss of nucleic acids

Minimal risk of loss

Nature of RNA-seq

3'end tag transcript seq

Nature of gDNA-seq

MALBAC-like amplified gDNA,
contaminated
with co-amplified cDNA

**Shown amenable to
bisulphite-sequencing**

no

G&T-seq (like)

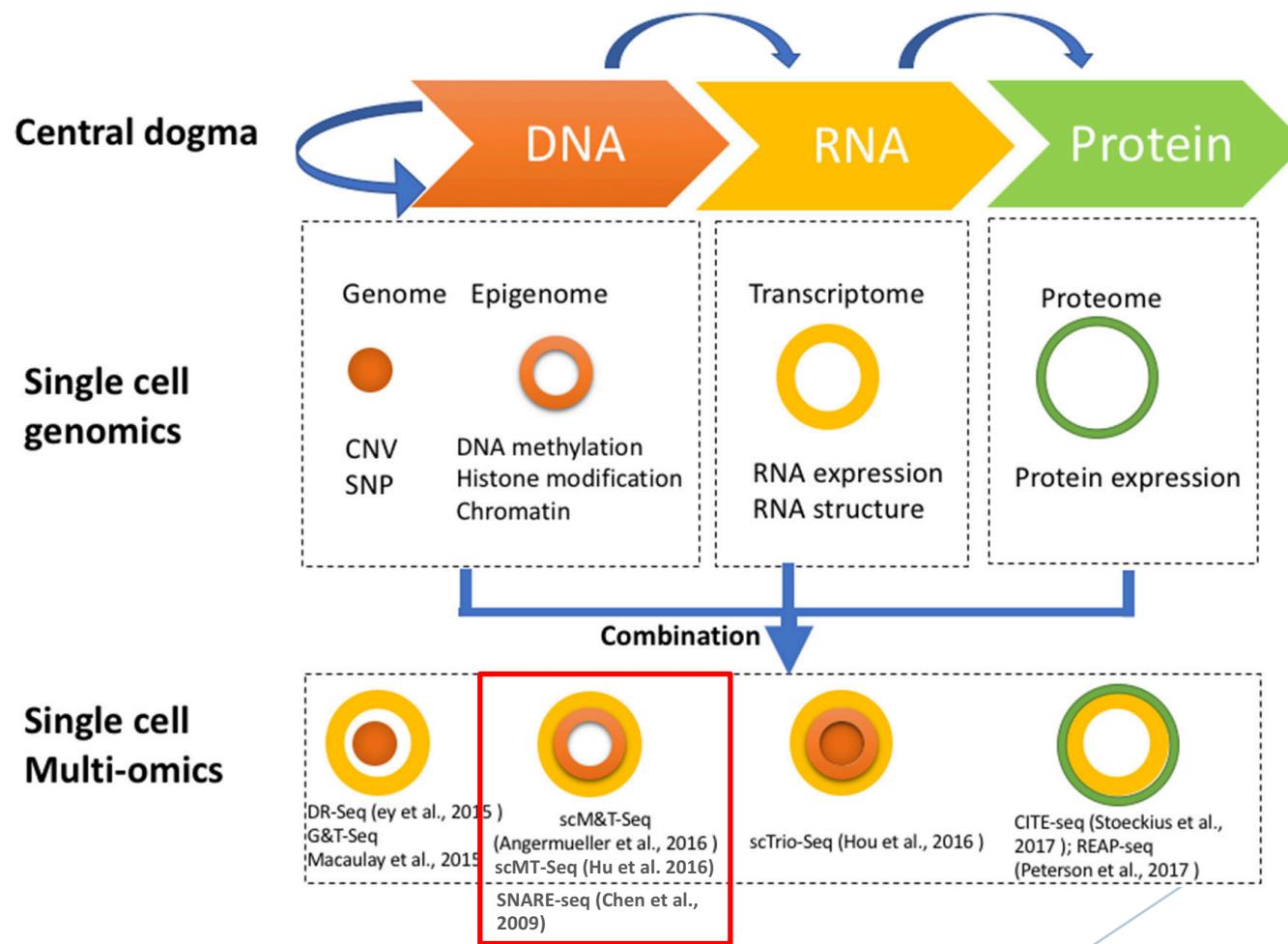
Potential loss of mRNA and DNA
molecules

Full-length transcript seq

In line with chosen WGA

yes

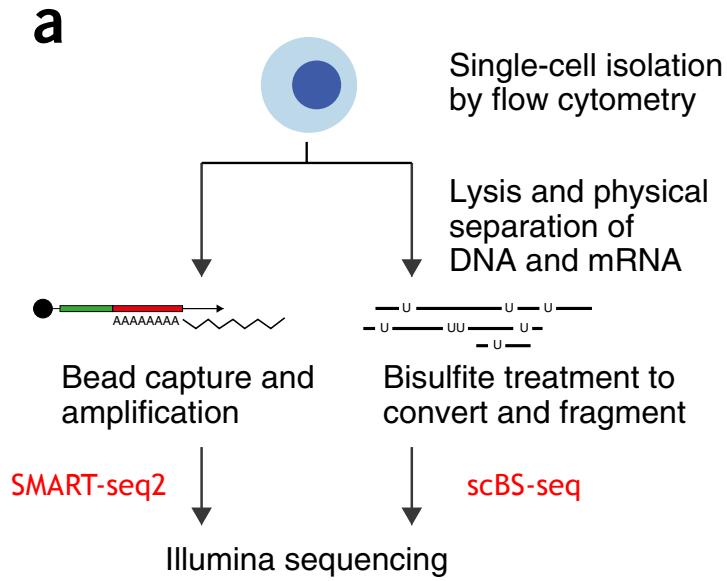
Strategies for multi-omics profiling of single cells



Single cell RNA- and methylation- Sequencing

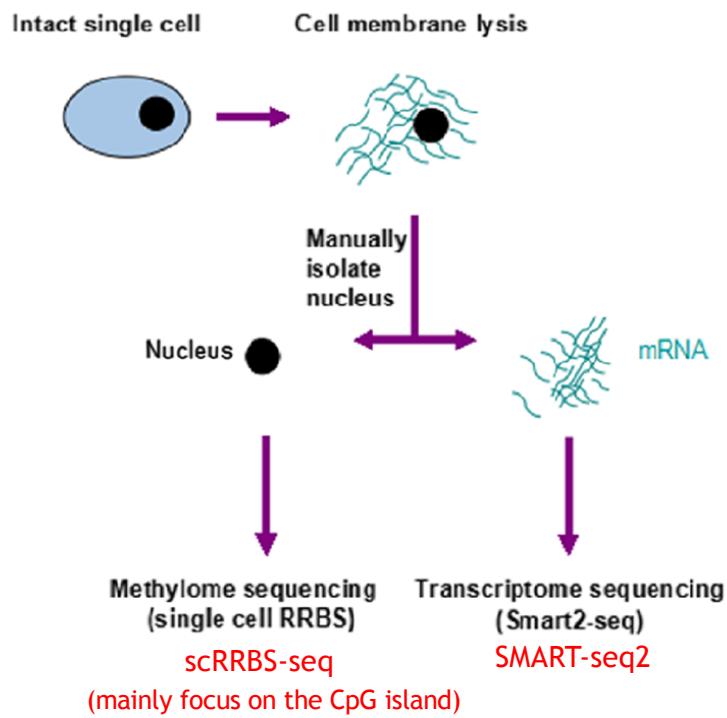
scM&T-seq

A parallel single-cell genome-wide **Methylome** and **Transcriptome** sequencing that allows for the discovery of associations between transcriptional and epigenetic variation



scMT-seq

(single cell Methylome and Transcriptome sequencing)



Single cell RNA- and ATAC- Sequencing



SNARE-seq

(droplet-based single-nucleus chromatin accessibility and mRNA expression sequencing)

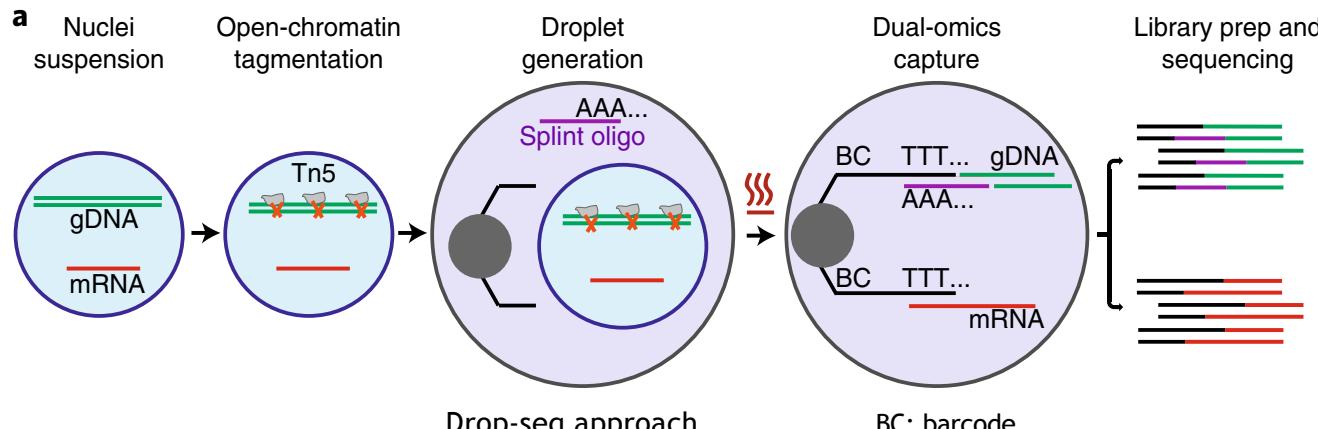
nature
biotechnology

LETTERS

<https://doi.org/10.1038/s41587-019-0290-0>

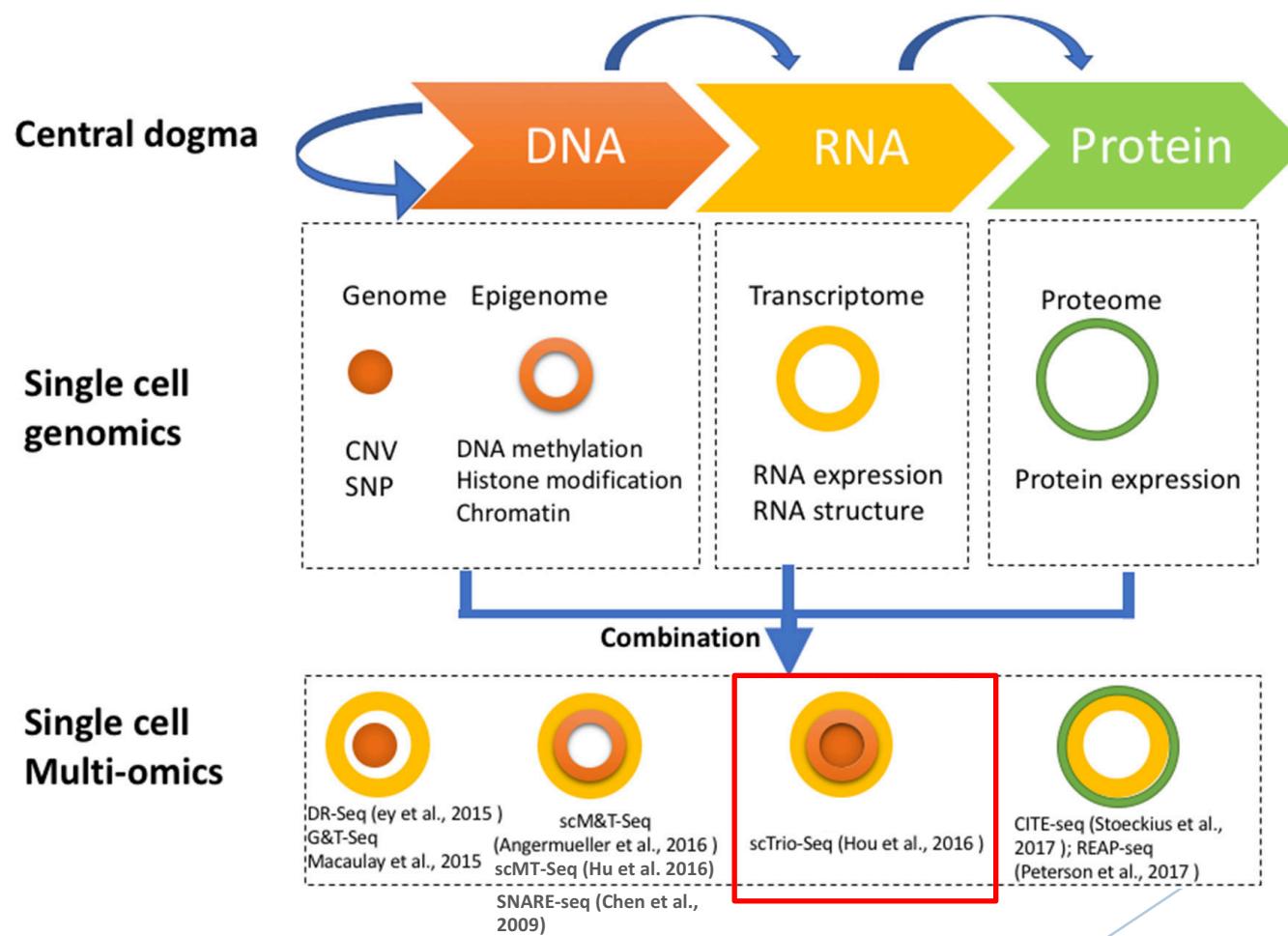
High-throughput sequencing of the transcriptome and chromatin accessibility in the same cell

Song Chen , Blue B. Lake  and Kun Zhang *



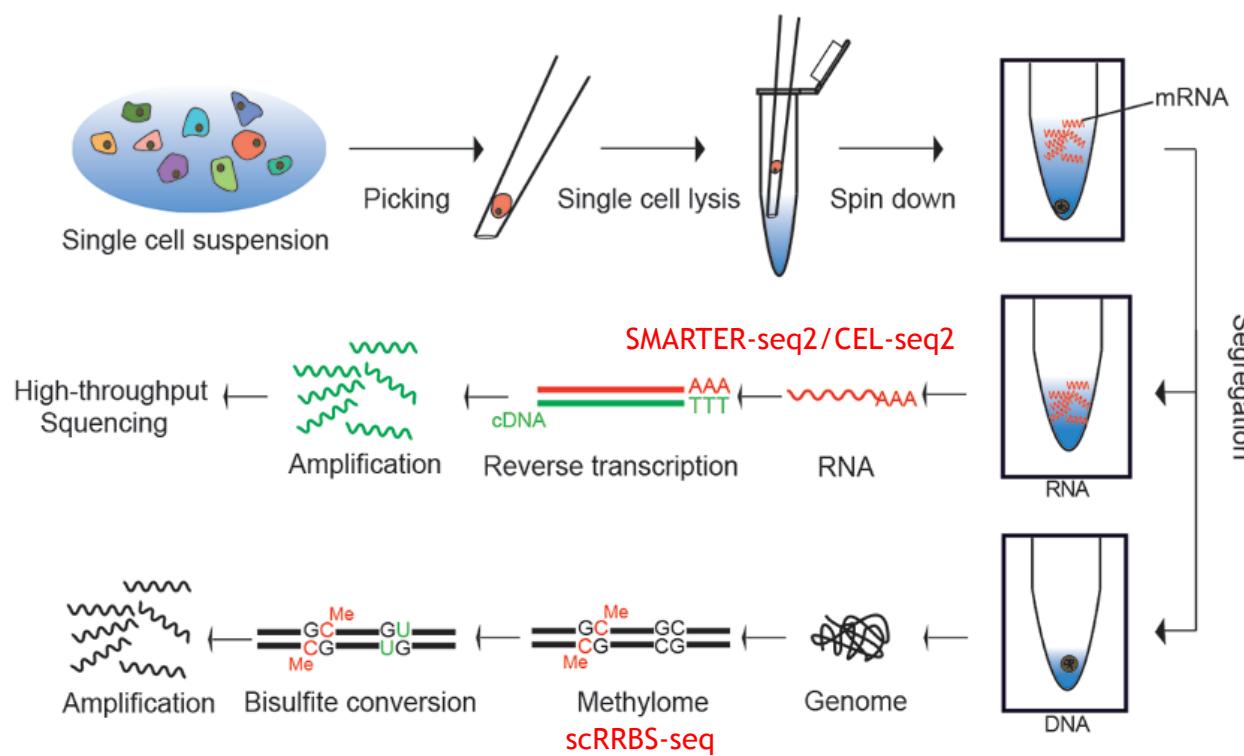
- a **splint oligonucleotide** with sequence complementary to the adaptor sequence inserted by transposition (5' end) and the poly(A) bases (3' end) allowing capture by oligo(dT)-bearing barcoded beads

Strategies for multi-omics profiling of single cells



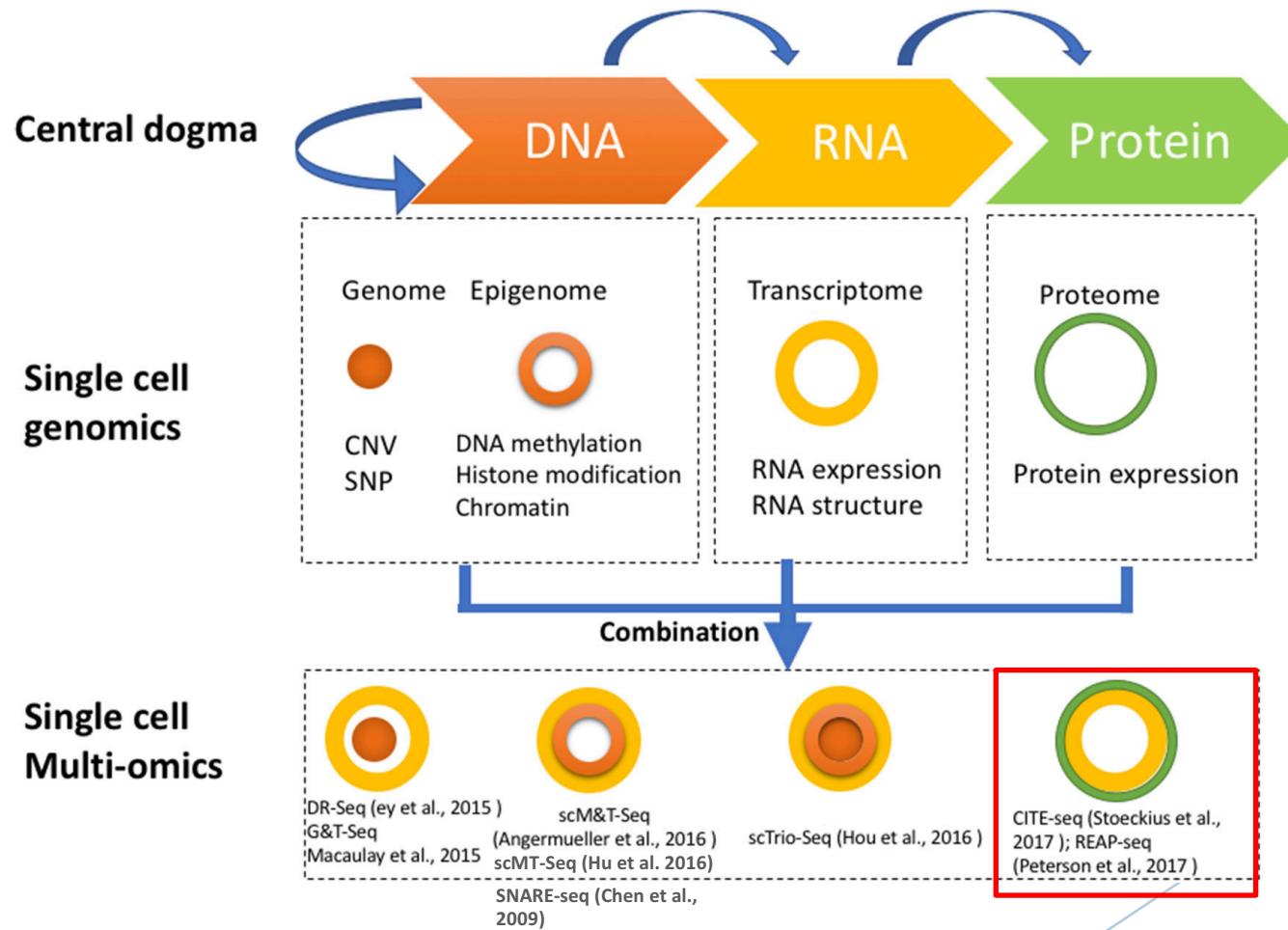
scTrio-seq

scTrio-seq simultaneously analyzes the **genomic copy-number variations (CNVs)**, **DNA methylome**, and **transcriptome** of an individual mammalian cell

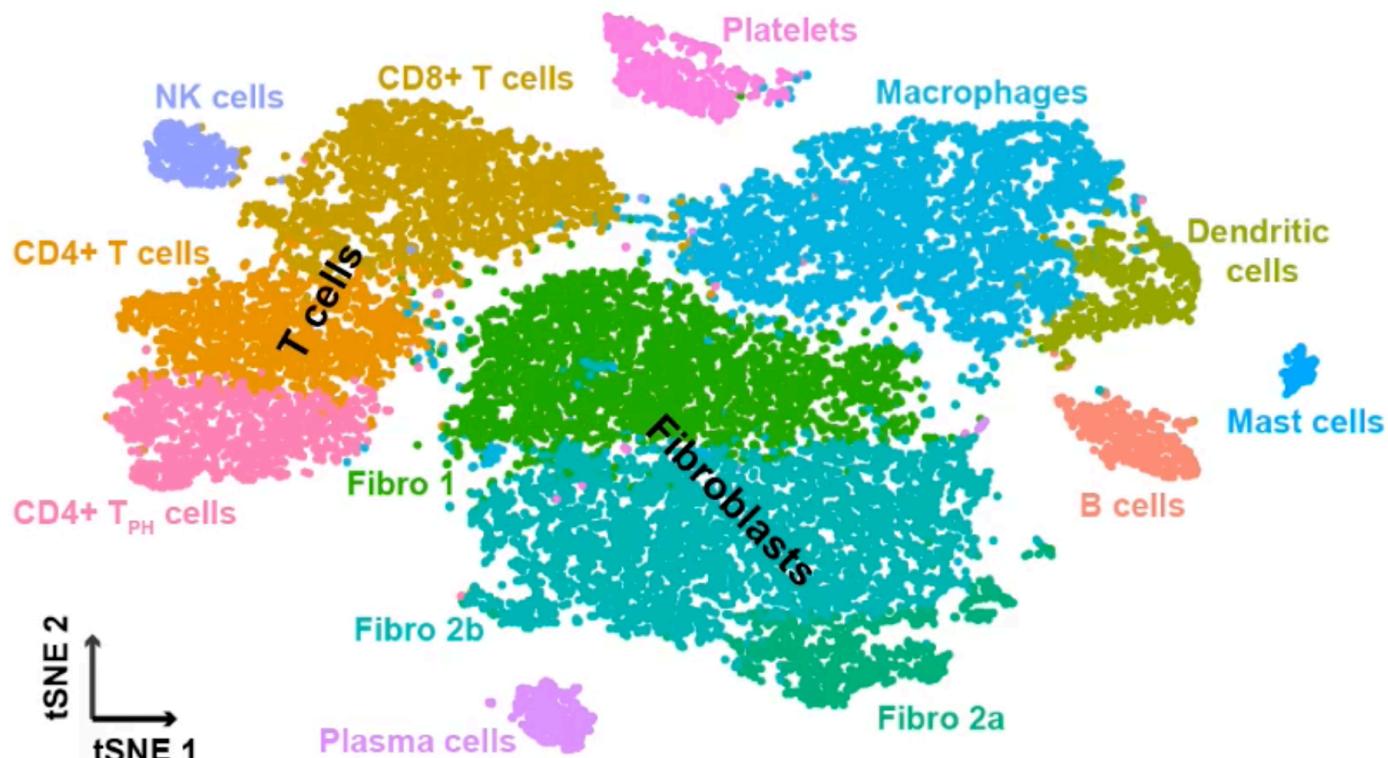


A flow chart illustrating the scTrio-seq technique. After a single cell was lysed with mild lysis buffer, the lysis product was centrifuged. The supernatant was transferred to a new tube for transcriptome sequencing analyses, while the pellet (containing the nucleus) was bisulfite-converted for genome (CNVs) and epigenome sequencing analyses.

Strategies for multi-omics profiling of single cells

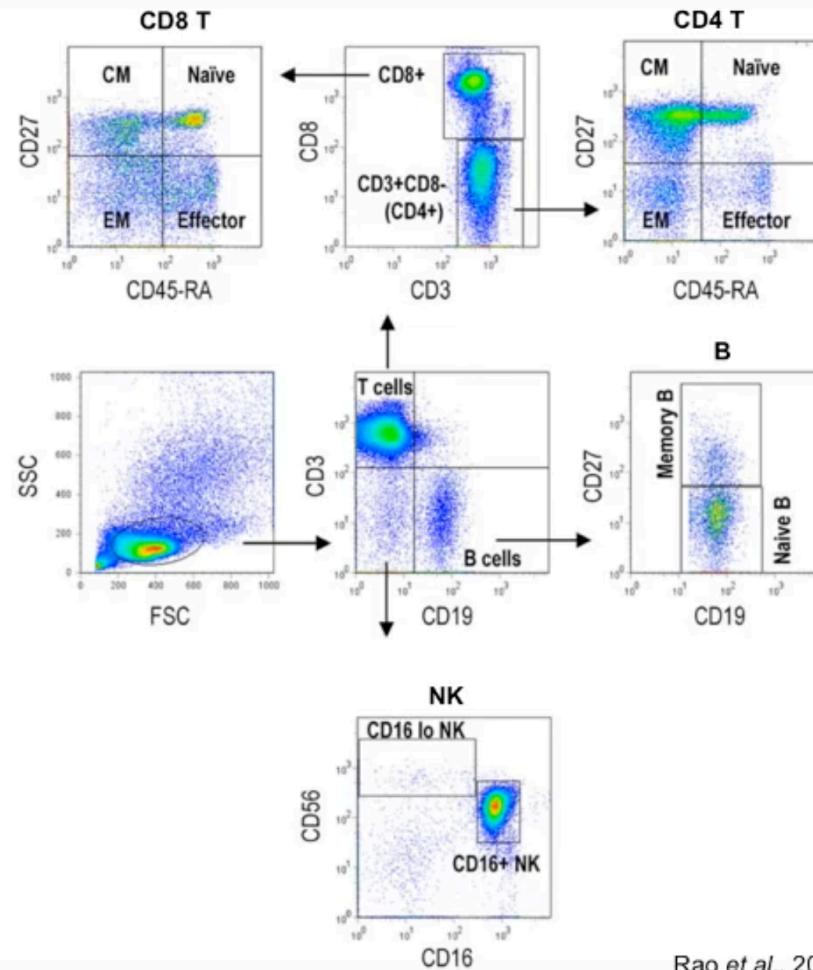


Distinguishing cells/states that are transcriptomically similar is difficult in scRNA-seq

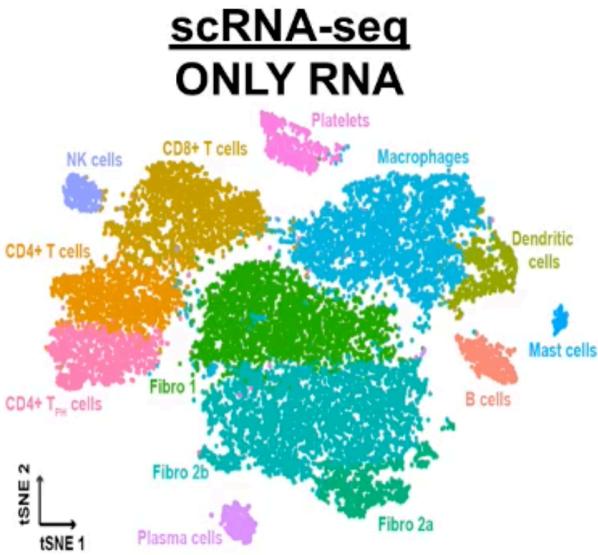


~21K cells synovial fluid in rheumatoid arthritis - Stephenson et al., 2018
(Satija & Innovation labs)

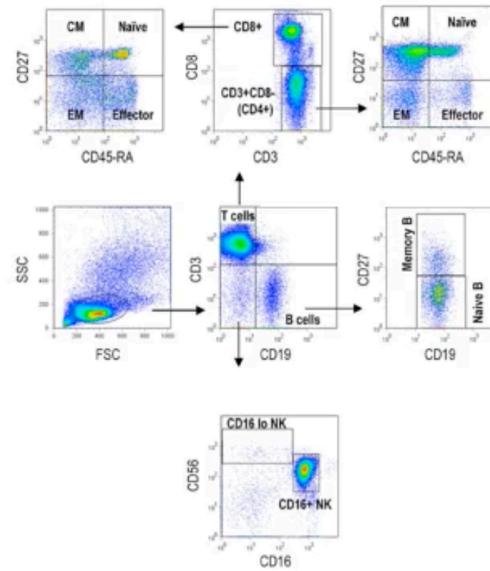
Cell **cytometry** is routinely used for detailed characterization of cell types & states



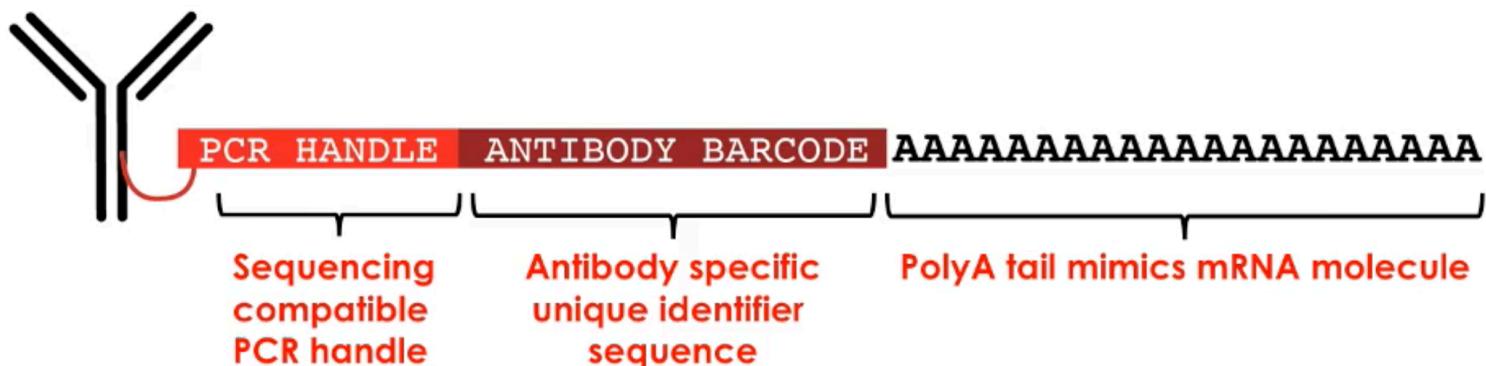
Can we merge these two methods to measure proteins and mRNA from single cells at large scale?



Flow cytometry / CyTOF
ONLY 'few' PROTEINS

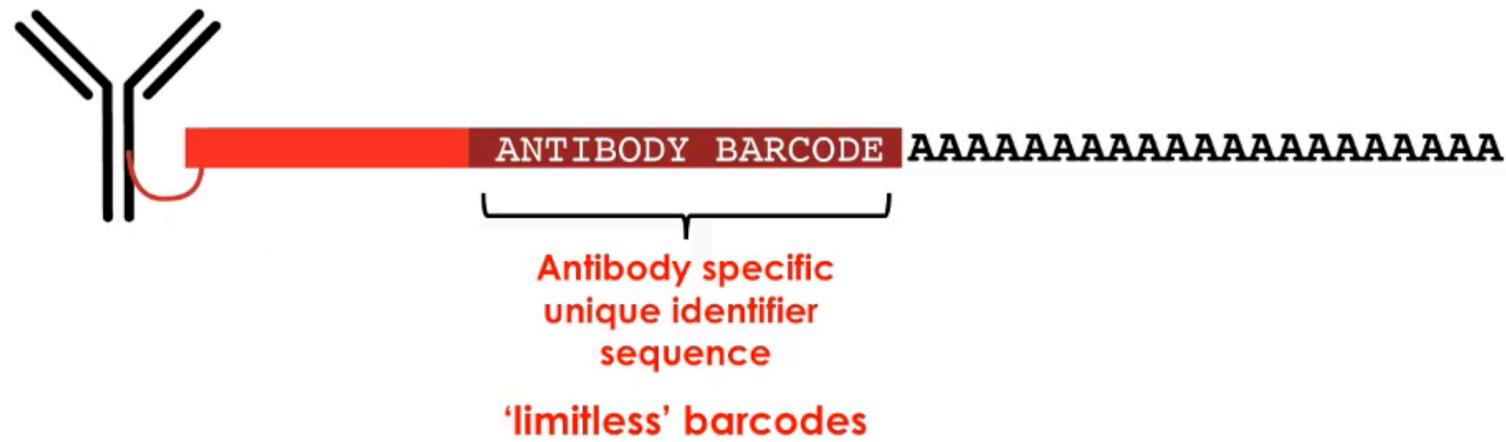


How can we get a sequenceable readout from an antibody?

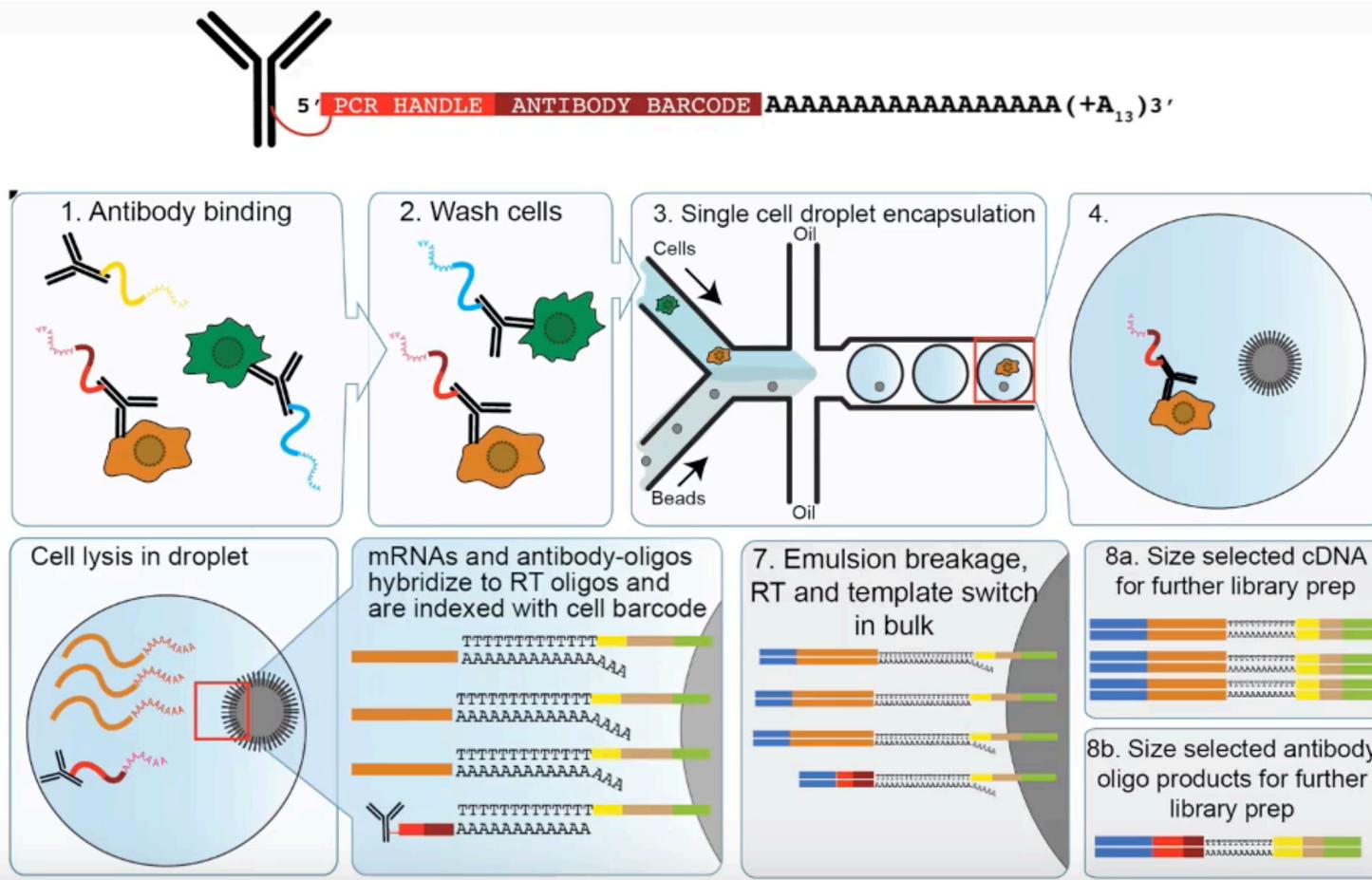


Protein abundance readout using DNA-barcoded polyadenylated oligos

DNA-barcoding = “limitless” multiplexing



CITE-seq: Cellular indexing of transcriptomes and epitopes in single cells



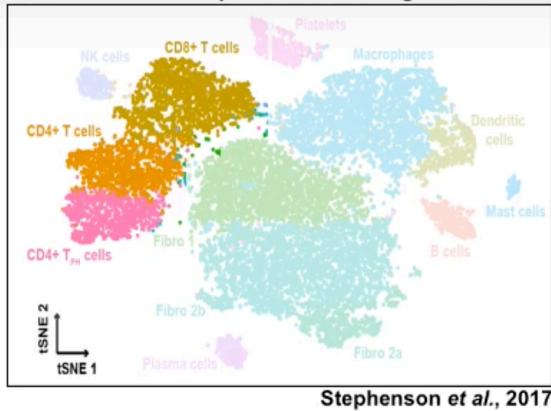


The ideal model to test CITE-seq:
immune system

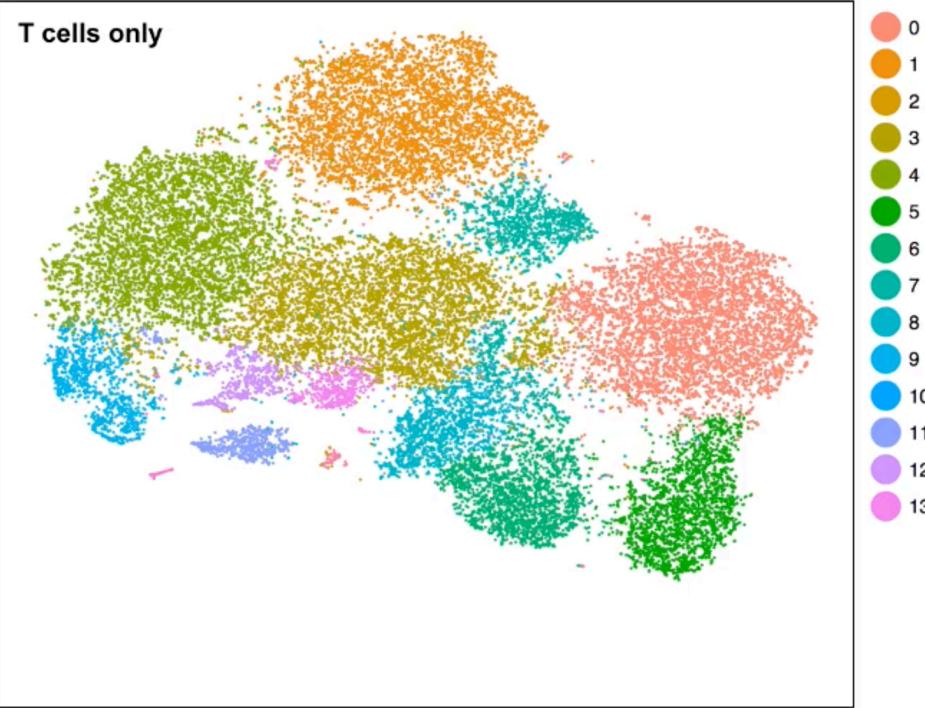
Multimodal clustering resolves more T cell clusters

13 T cell clusters with distinct protein and RNA markers

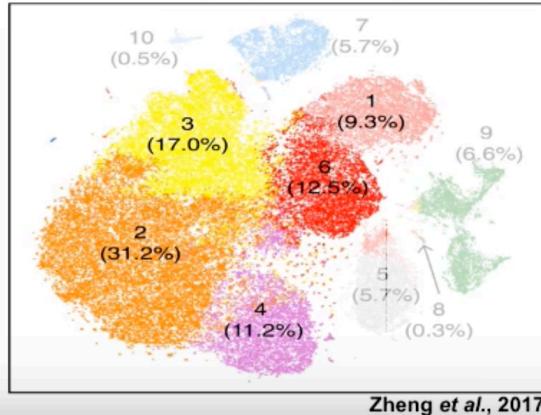
~3 T cell clusters (RNA clustering of 21K cells)



70K PBMCs with 83 CITE-seq markers
Multimodal clustering (RNA and protein)



~5 T cell clusters (RNA clustering of 68K PBMCs)



Conclusion

- **(Quick) overview of different scSeq methods**
 - scDNA, scRNA, scChIP, scEpi
- **Different single cell -omics methods**
 - scDNA/RNA, scRNA/epi, scDNA/RNA/epi,
scRNA/protein, scRNA/ATAC

