

# Single cell multi-omics

**Miao-Ping Chien**

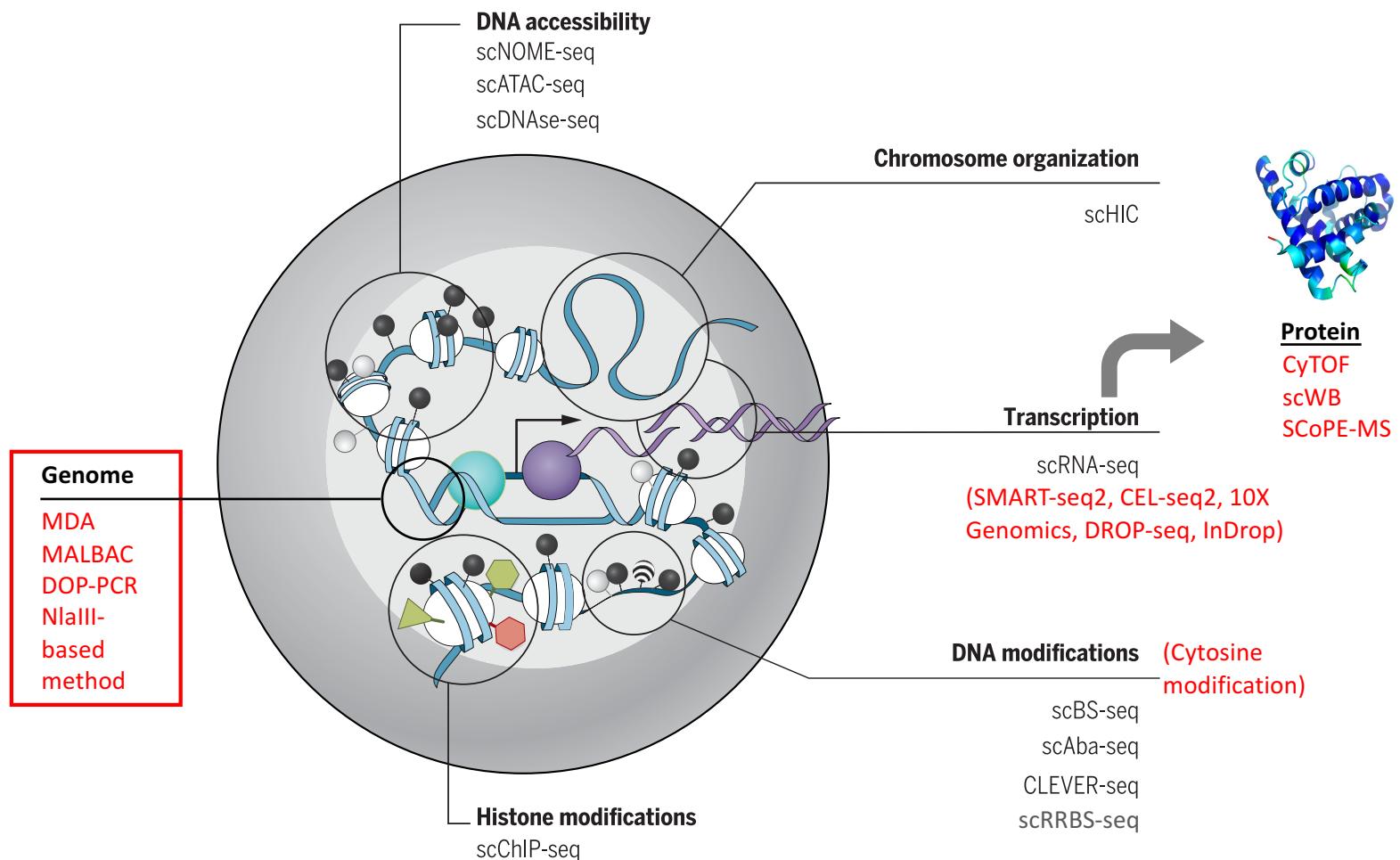
Erasmus MC, Group leader

2021 Single Cell Analysis Workshop, 2020/10/19

# **Outline**

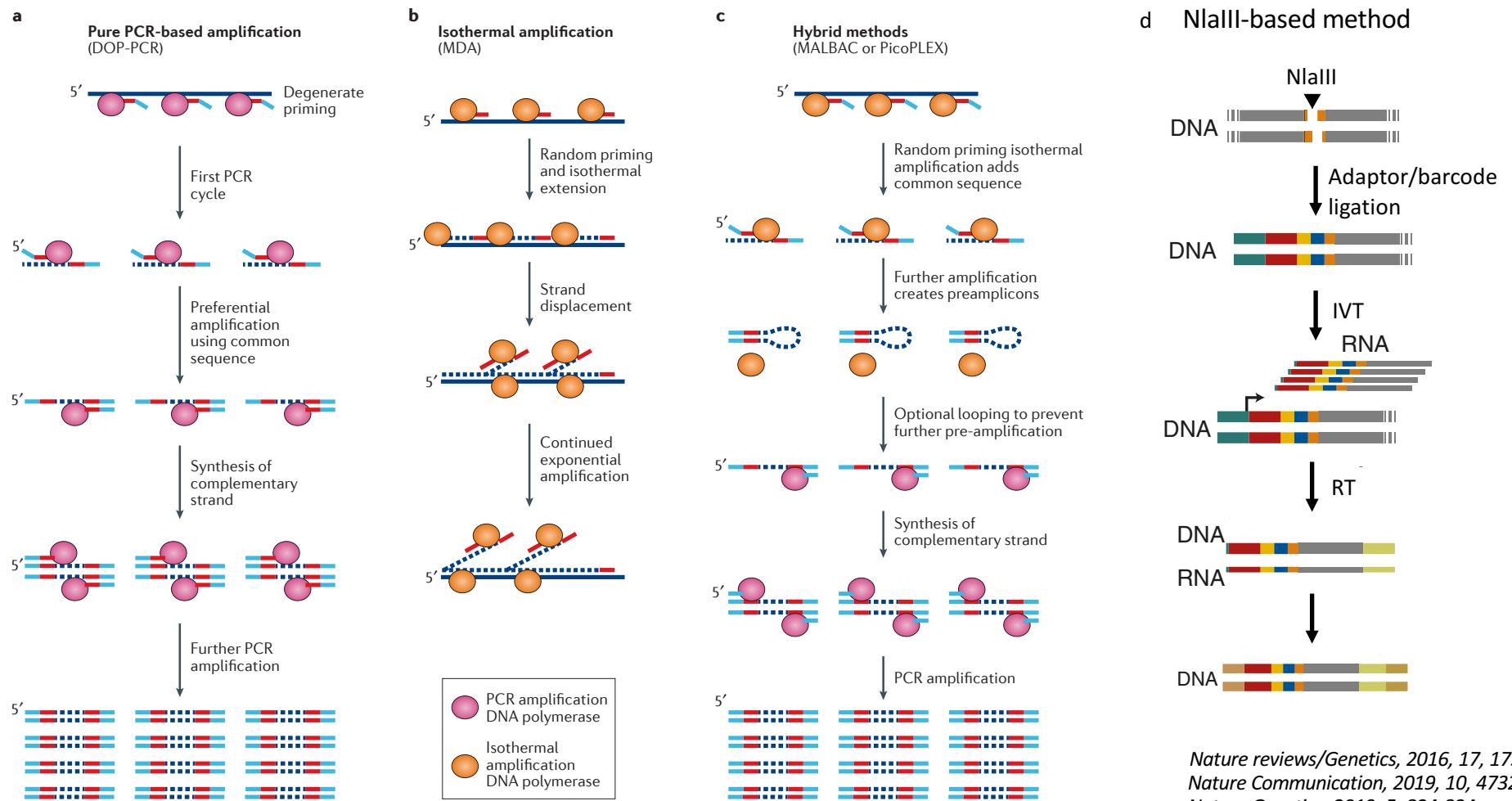
- (Quick) overview of different single cell –omics methods
- Different single cell multi–omics methods

# Overview of single cell -omics



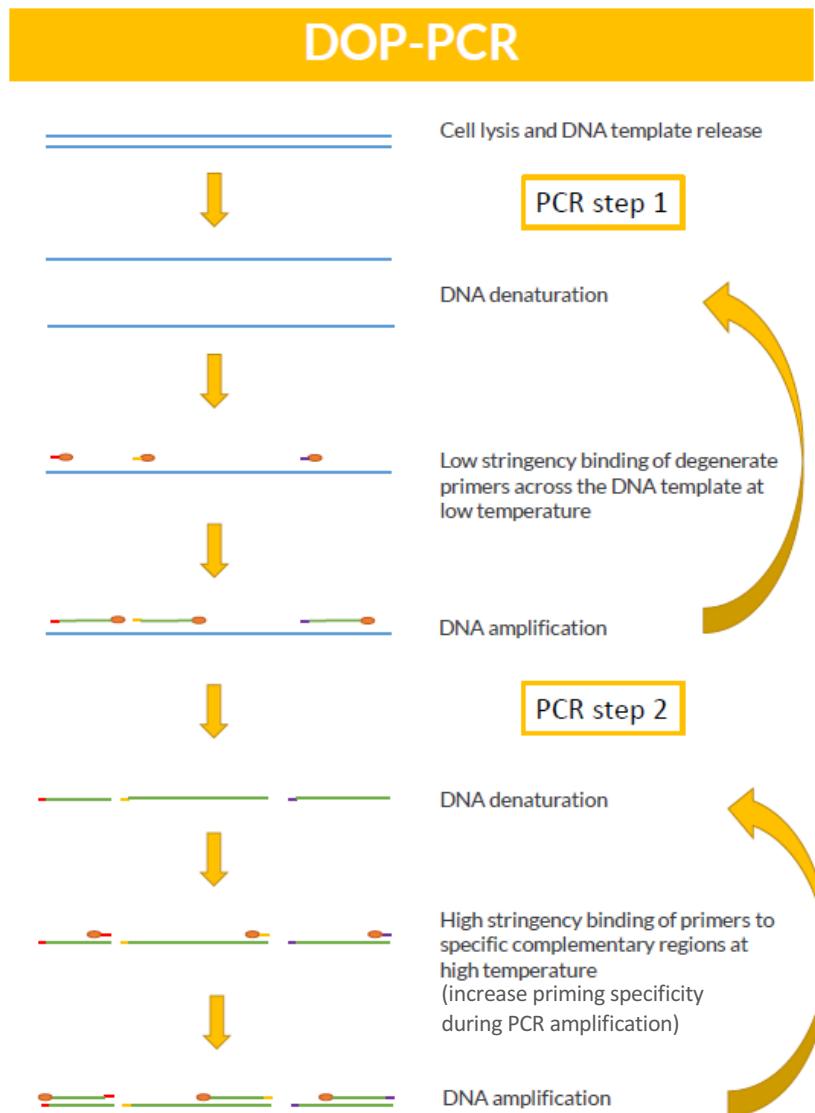
# **Single cell genomic sequencing**

# Overview of the main whole-genome amplification methods



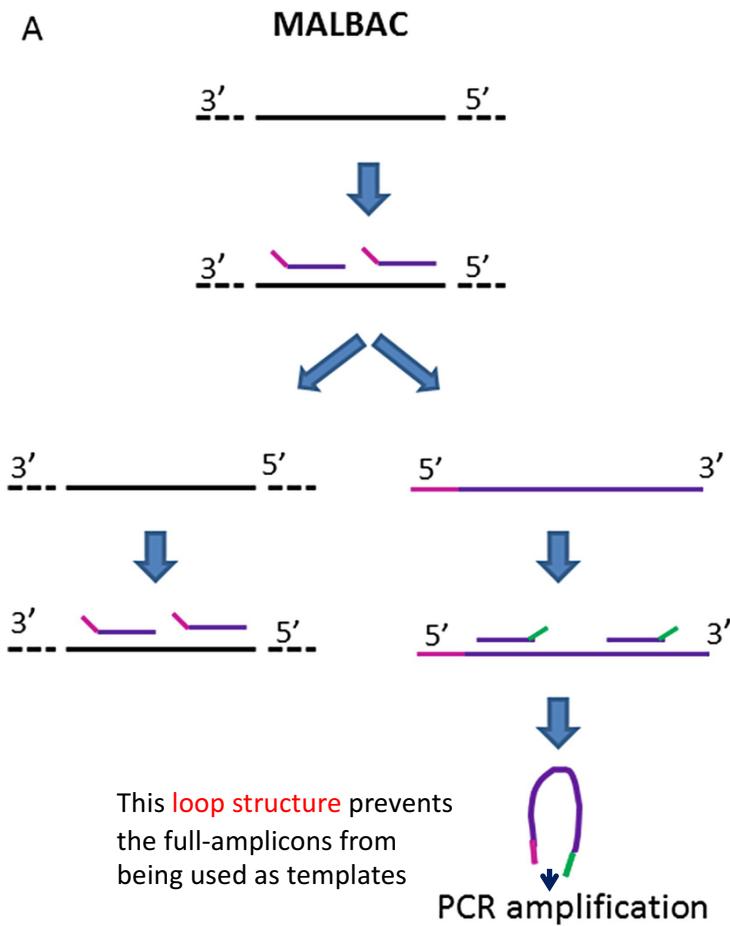
*Nature reviews/Genetics, 2016, 17, 175  
Nature Communication, 2019, 10, 4732;  
Nature Genetics, 2019, 5, 824-834*

# Degenerate Oligonucleotide-Primed PCR (DOP-PCR)

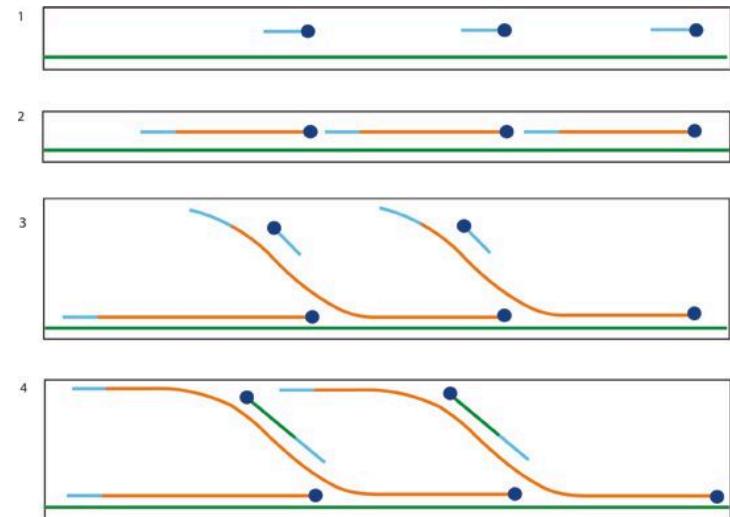
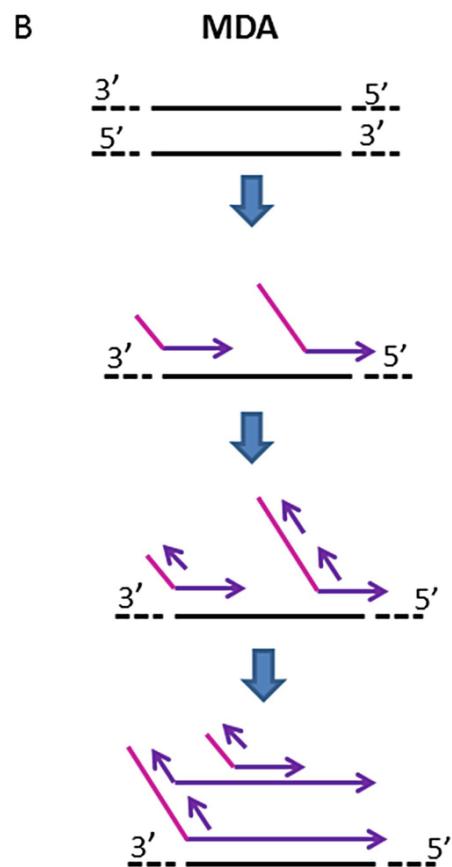


# MALBAC vs MDA

**MALBAC:** multiple annealing and looping based amplification cycles

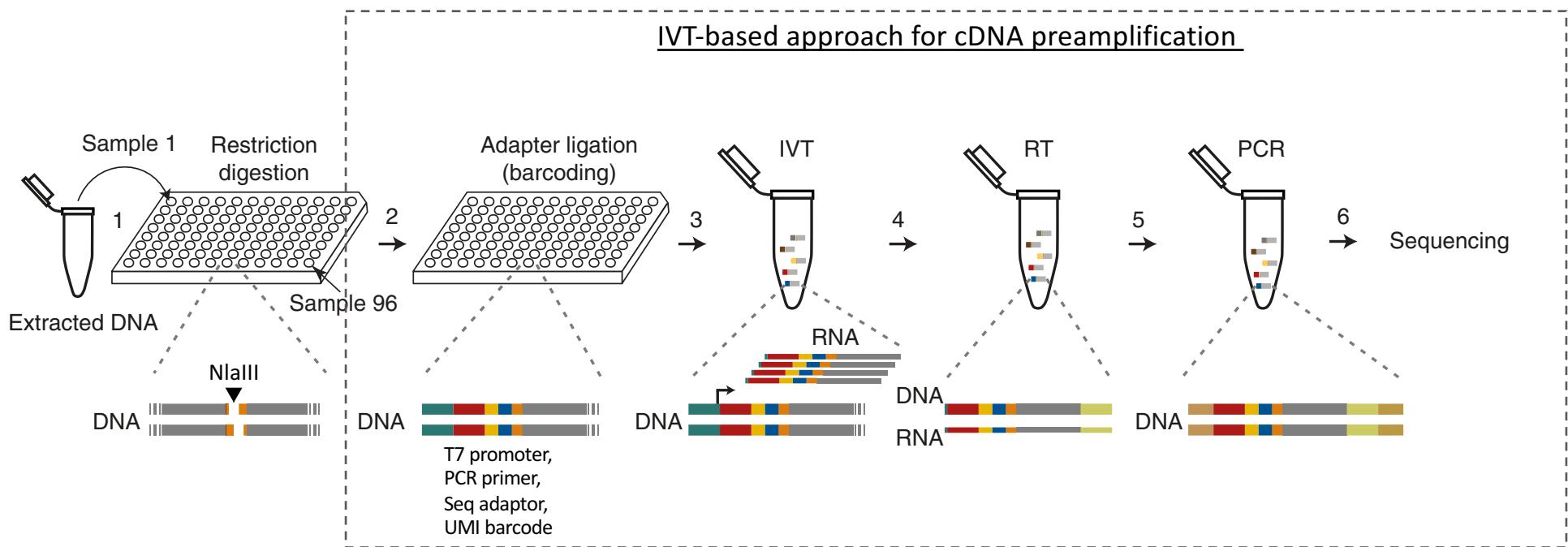


**MDA:** multiple displacement amplification



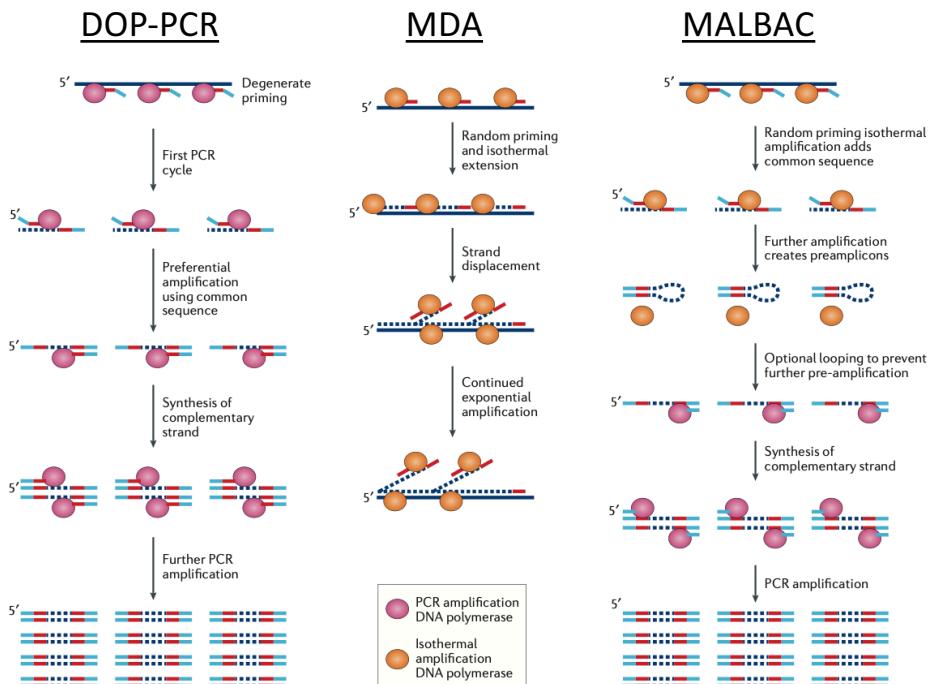
The **phi29 DNA polymerase** enables the newly synthesized strand to displace the formerly synthesized one on the same template. Free primers will anneal to these displaced single-strands and continue such cycles of "displace and anneal".

# NlaIII-based scDNAseq method



*Nature Communication*, 2019, 10, 4732;  
*Nature Genetics*, 2019, 5, 824-834

# Overview of the main whole-genome amplification methods



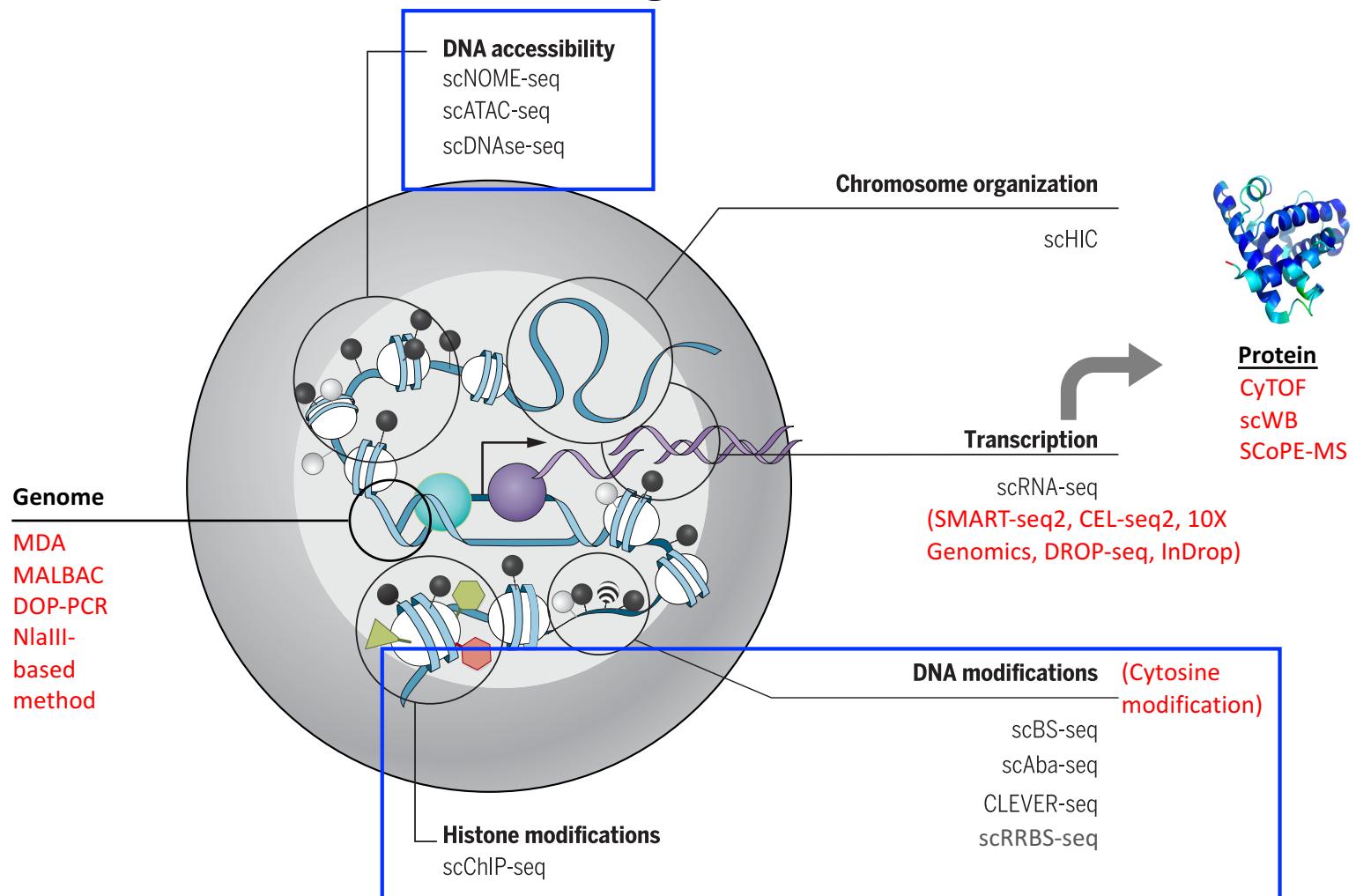
- Highest to lowest genome recovery rate?  
MDA (~80%) > MALBAC (~50%) > DOP-PCR (~6%) / NlalII-based method (~5-10%)
- Highest to lowest duplication ratio (duplicate the product more than the original template)?  
DOP-PCR > MDA , MALBAC, NlalII-based method
- Homogeneous amplification?  
MALBAC, NlalII-based method > MDA, DOP-PCR

*Nature reviews/Genetics, 2016, 17, 175  
Nature Communication, 2019, 10, 4732;  
Nature Genetics, 2019, 5, 824-834*

# Comparison

Method	Amplification	Application	Coverage	Refs
<b>Genomic Analysis</b>				
GenomePlex PCR	Multiplexed PCR	Copy number	Low coverage	[32]
MDA	MDA	Genome/exome	High coverage	SNP detection [33,34]
MALBAC	MALBAC	Copy number/genome	High coverage and uniform amplification	[41,42] SNP detection
NlaIII-based method	IVT	Copy number	Low coverage; uniform amplification	(SNP detection)

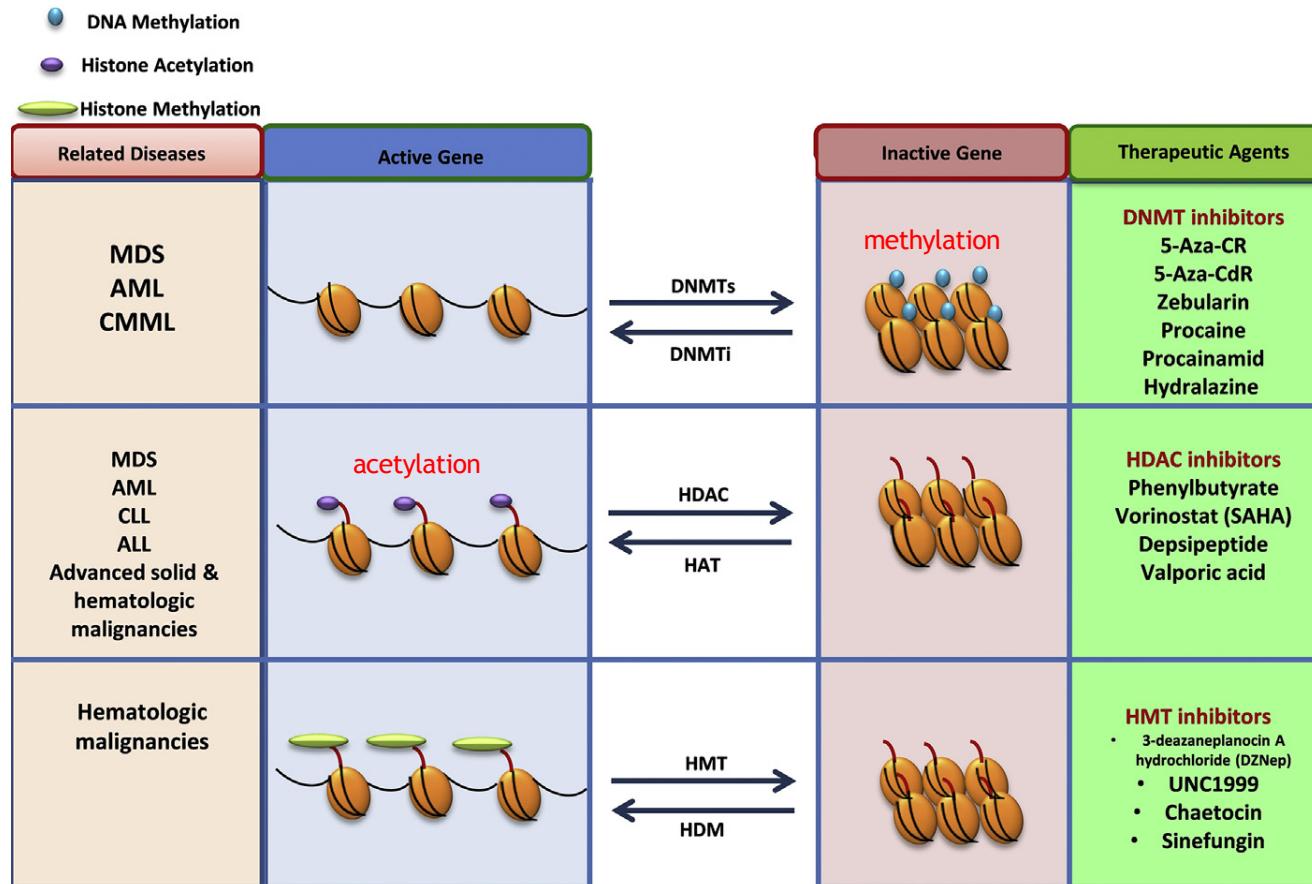
# Overview of single cell -omics



Science, 2017, 358, 69–75

# **Single cell epigenomic sequencing**

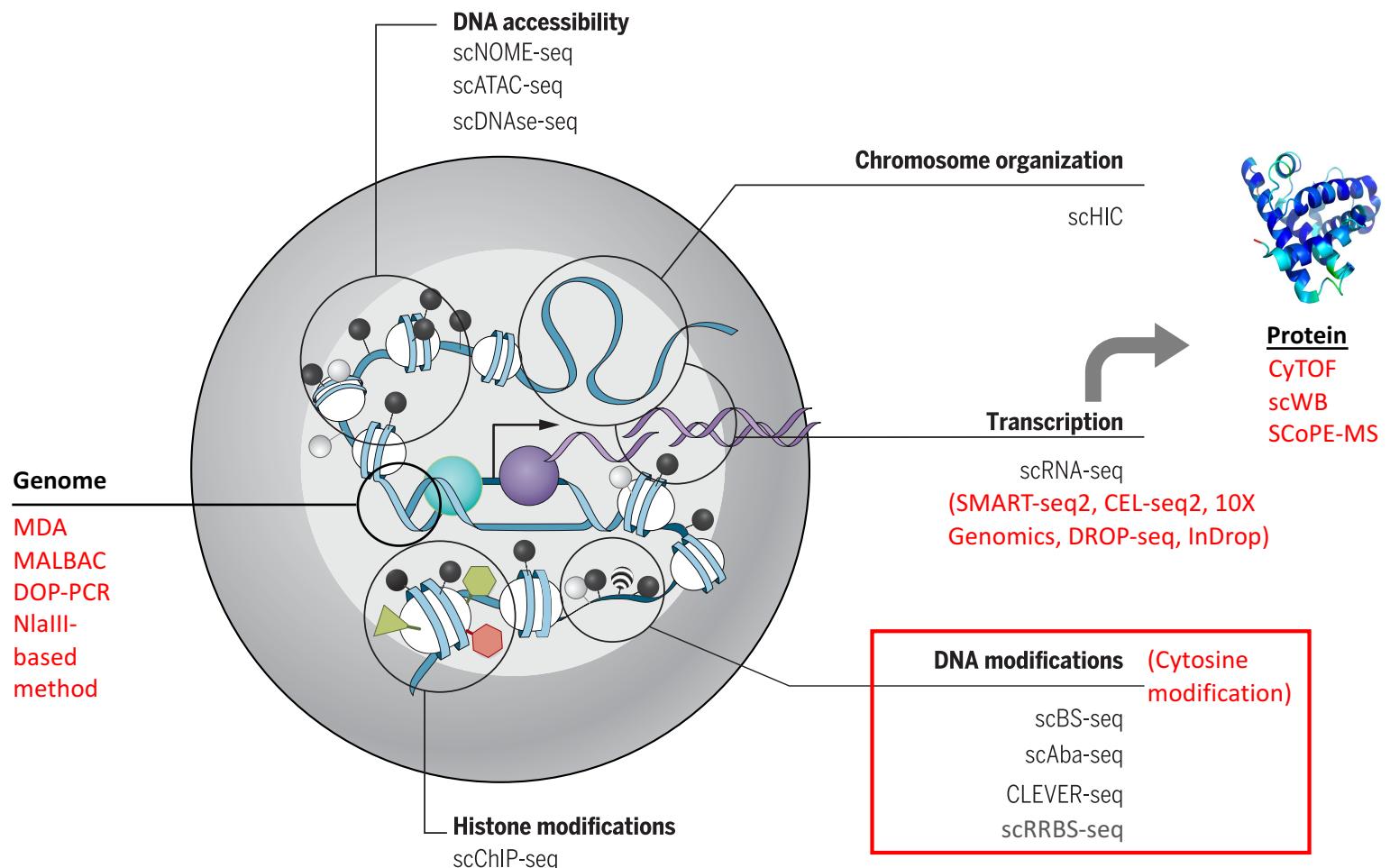
# Why epigenomics?



**Histone acetylation:** opening of the chromatin mass & the onset of transcription

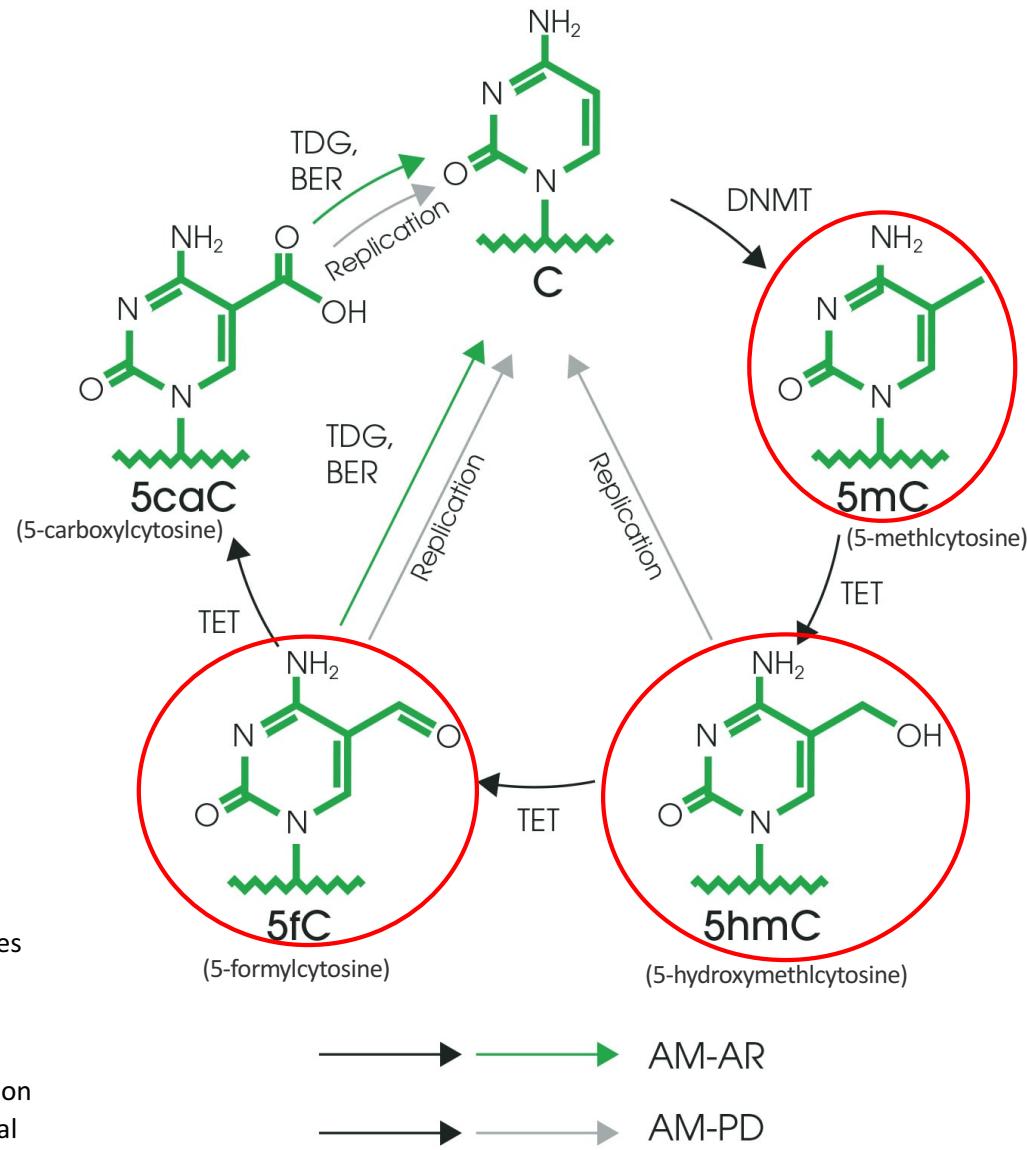
**DNA Methylation:** condenses chromatin & accompanies transcriptional inhibition

# Overview of single cell -omics



Science, 2017, 358, 69–75

# The cycle of DNA (de)methylation



# Bisulfite conversion

- C → U
- C\* → C

**Step 1**

**Denaturation**  
Incubation at 95°C  
fragments genomic DNA

**Step 2**

**Conversion**  
Incubation with sodium bisulfite  
at 65°C and low pH (5-6)  
deaminates cytosine residues  
in fragmented DNA

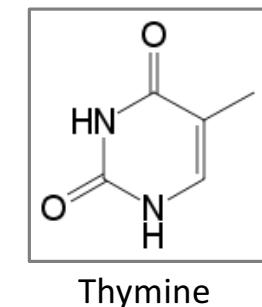
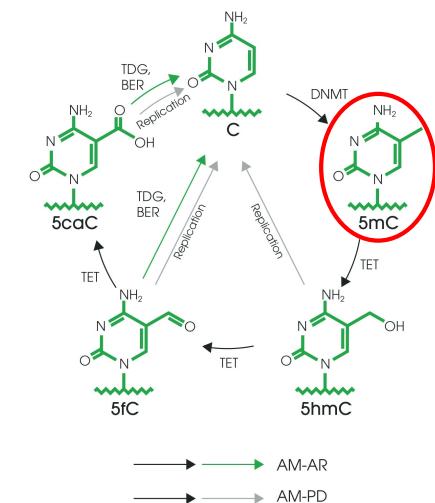
**Step 3**

**Desulphonation**  
Incubation at high pH  
at room temperature for 15 min  
removes the sulfite moiety,  
generating uracil

Fragmented Genomic DNA Samples → Cytosine → Uracil

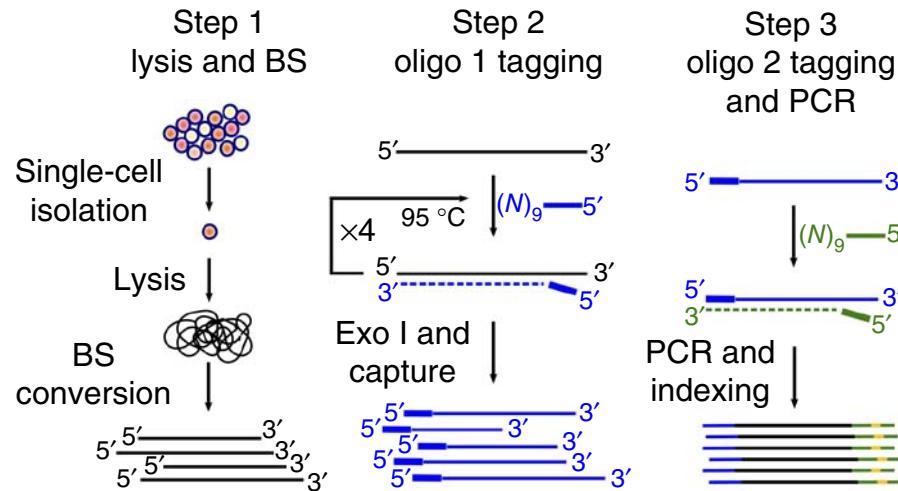
5-Methylcytosine (5-mC) → ~~NaHSO<sub>3</sub>, pH 5.0~~

5-mC and 5-hmC (not shown) are not susceptible to bisulfite conversion and remain intact



# Single cell bisulfite sequencing (scBS-seq)

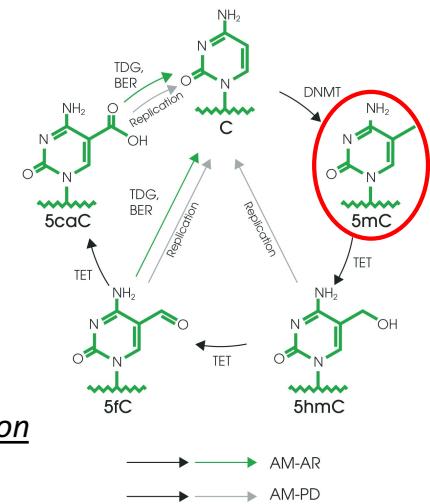
a



**Step 1: bisulfite treatment -> DNA fragmentation & conversion of unmethylated cytosines to thymine**

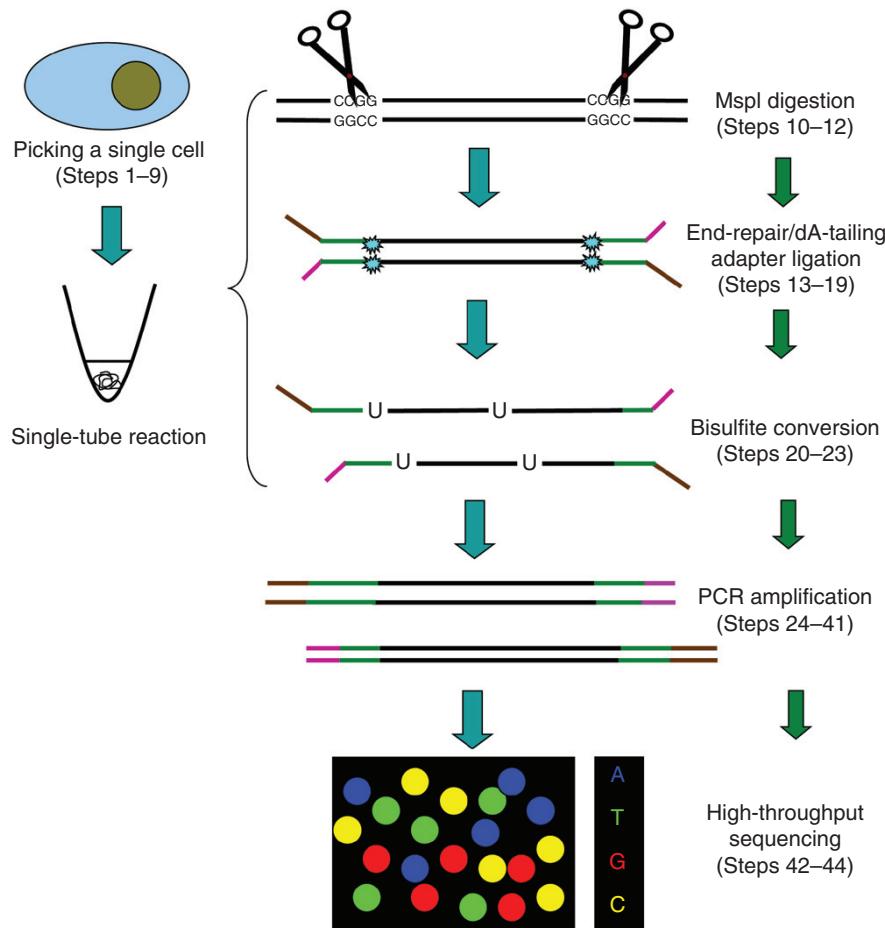
**Step 2:** synthesis of complementary strands is primed using oligonucleotides containing Illumina adaptor sequences and a 3' stretch of nine random nucleotides

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

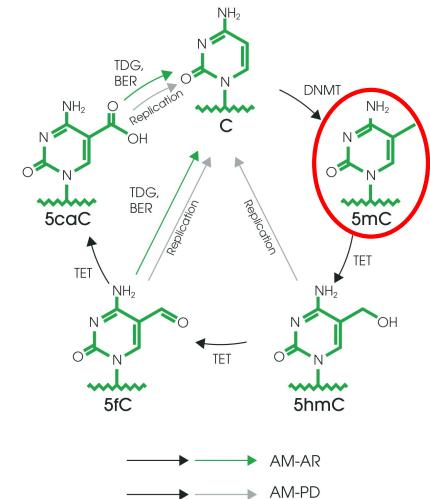


- C → U
- C\* → C

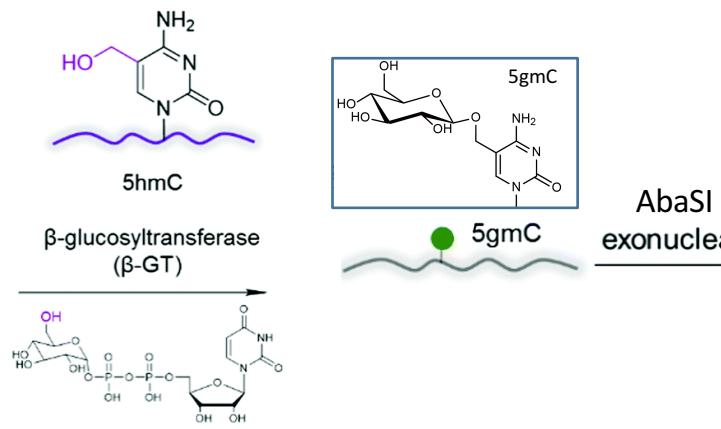
# scRRBS-seq: single-cell reduced-representation bisulfite sequencing



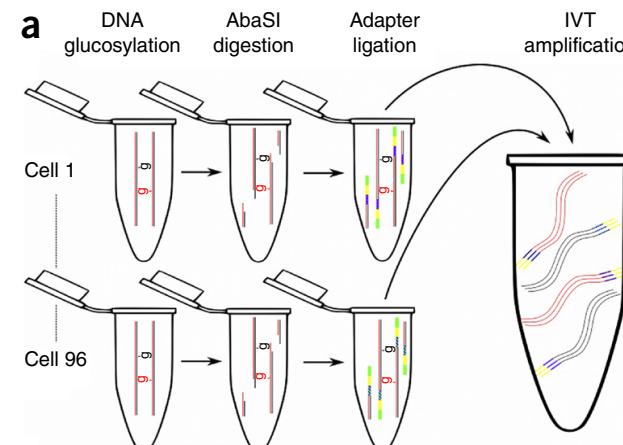
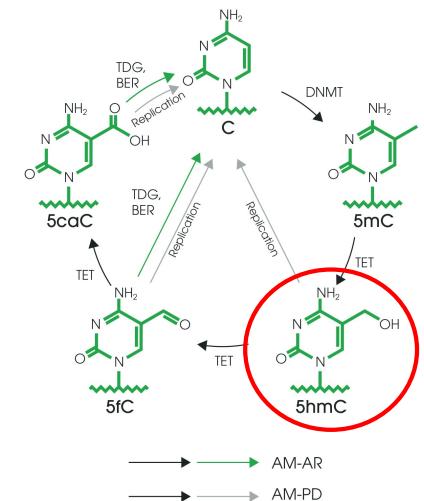
Mainly focus on the **GpC island** (related to gene expression regulation -> methylation of “C” in CpG island silences gene expression)



# scAba-seq : single-cell 5hmC sequencing



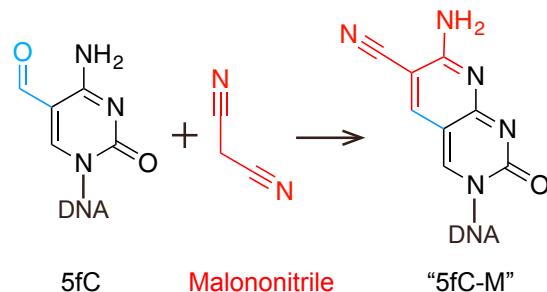
5hmC marks in DNA from individual cells are **glucosylated** with **T4 phage- $\beta$ -glucosyltransferase (T4  $\beta$ -GT)**, and the DNA is digested with the restriction endonuclease **AbaSI**. The digested DNA is ligated to an adapter containing a cell-specific barcode, an Illumina 5' adapter, and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



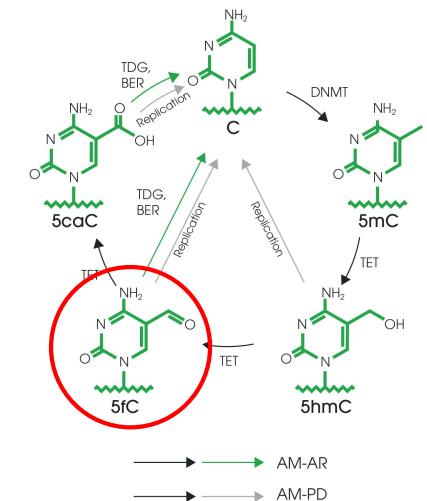
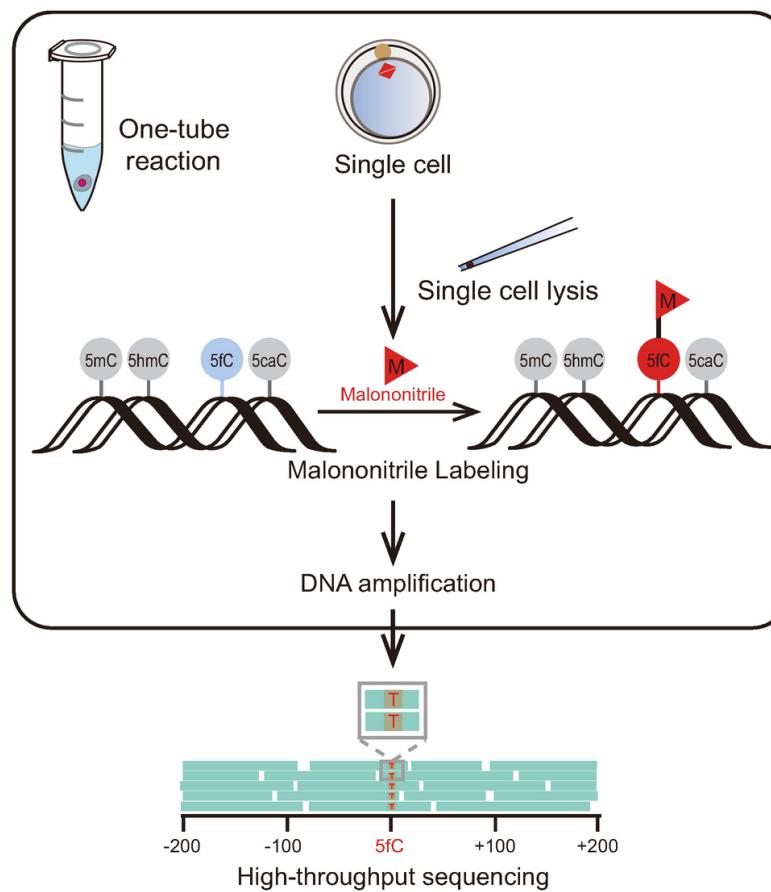
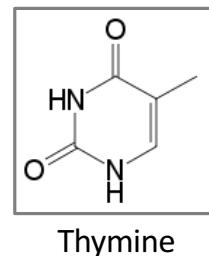
# CLEVER-seq

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

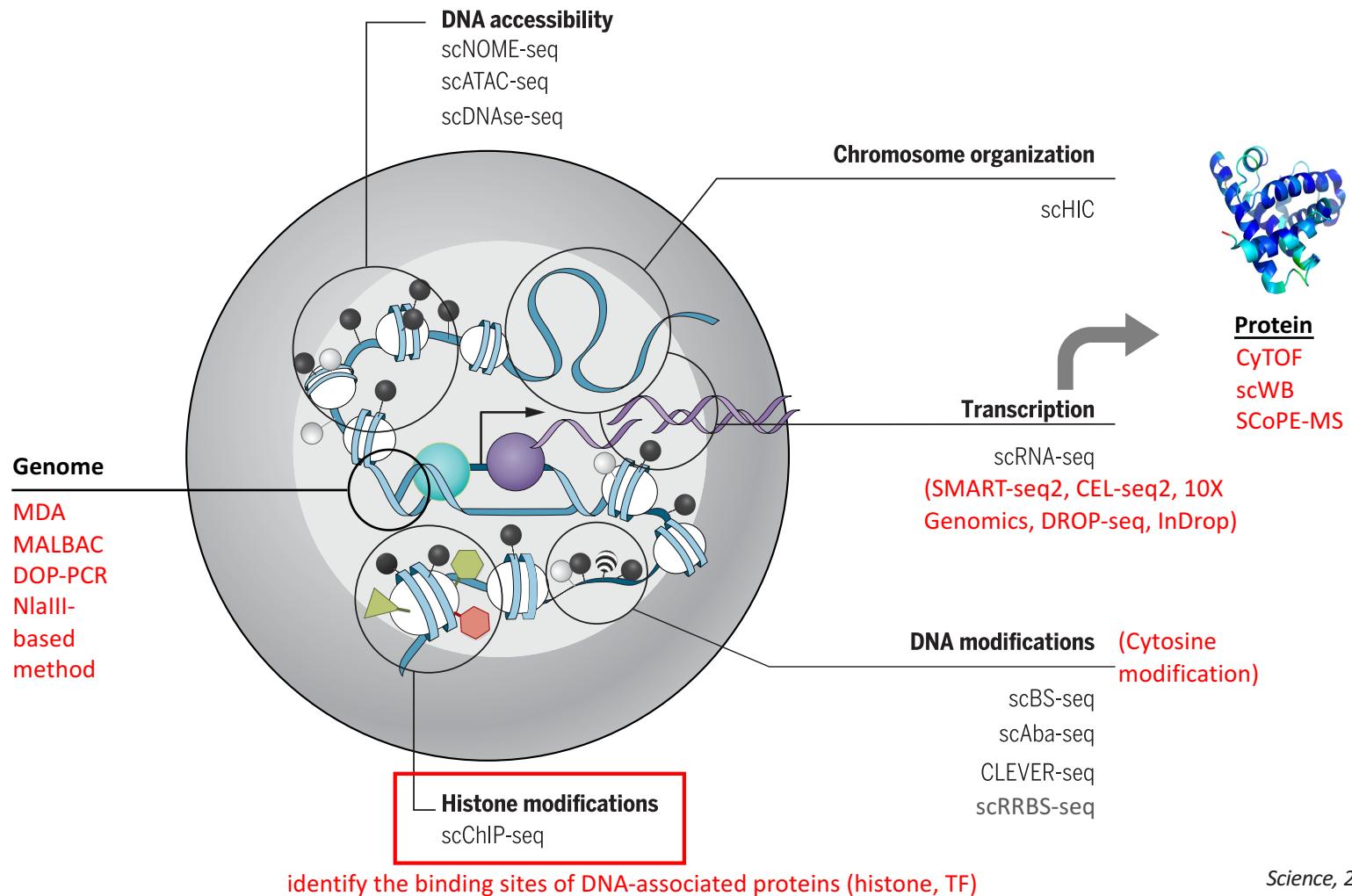
- A single-cell, single-base resolution whole-genome **5fc**-sequencing technology



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



# Overview of single cell -omics

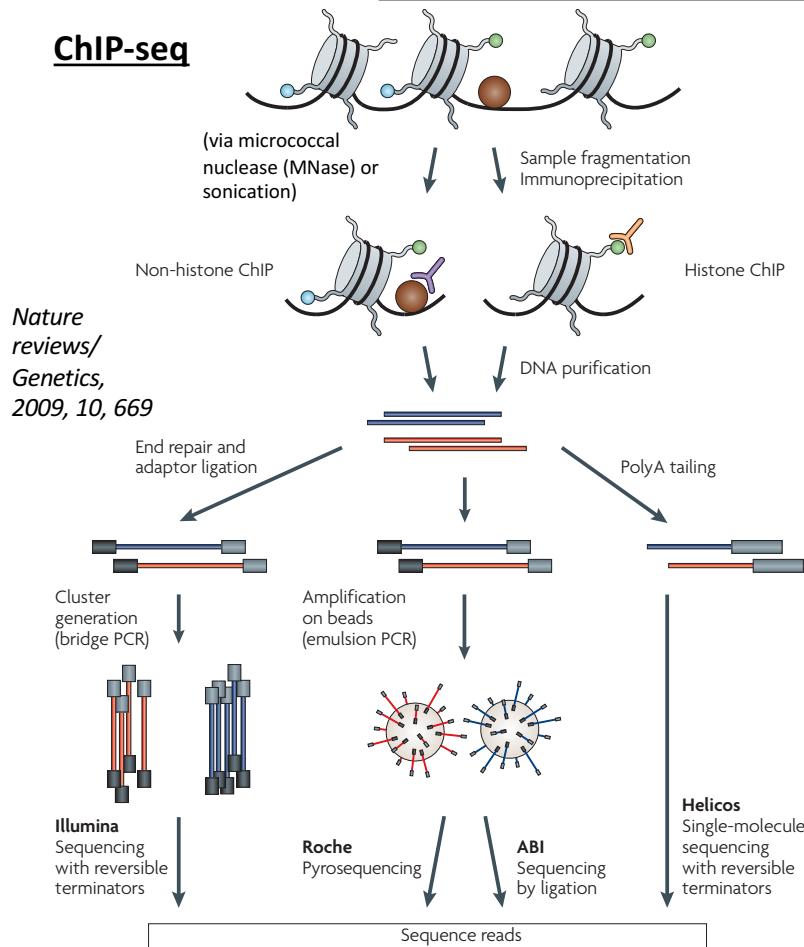


Science, 2017, 358, 69–75

# **Single cell chromatin immunoprecipitation (CHIP) sequencing**

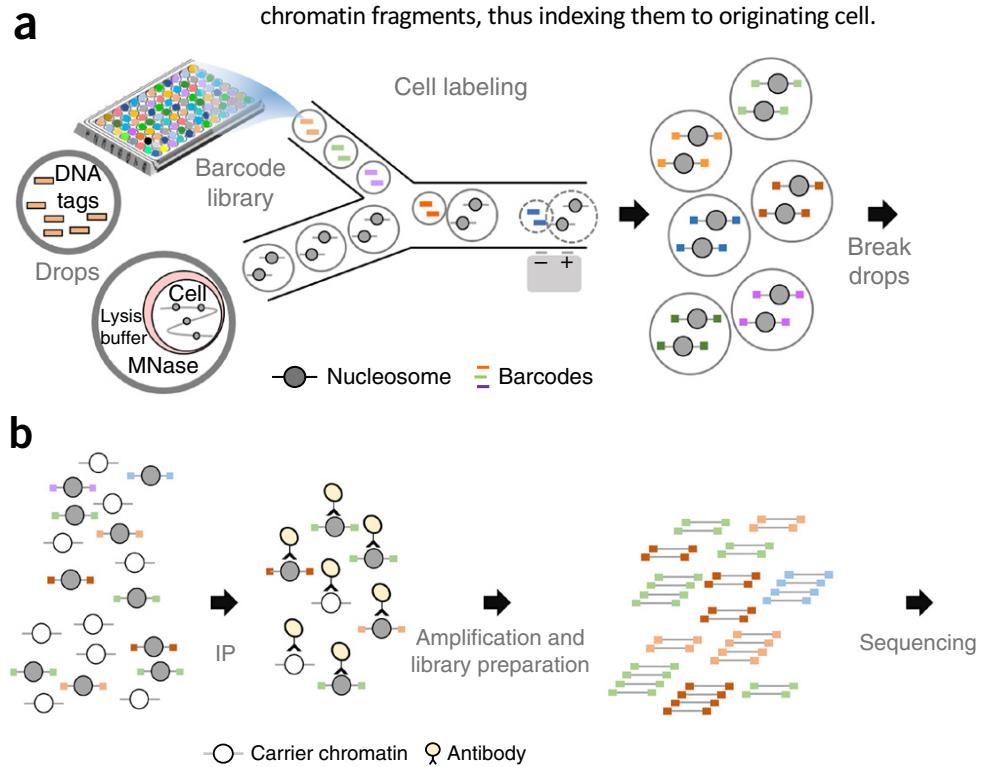
# Single cell chromatin immunoprecipitation (ChIP) sequencing

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



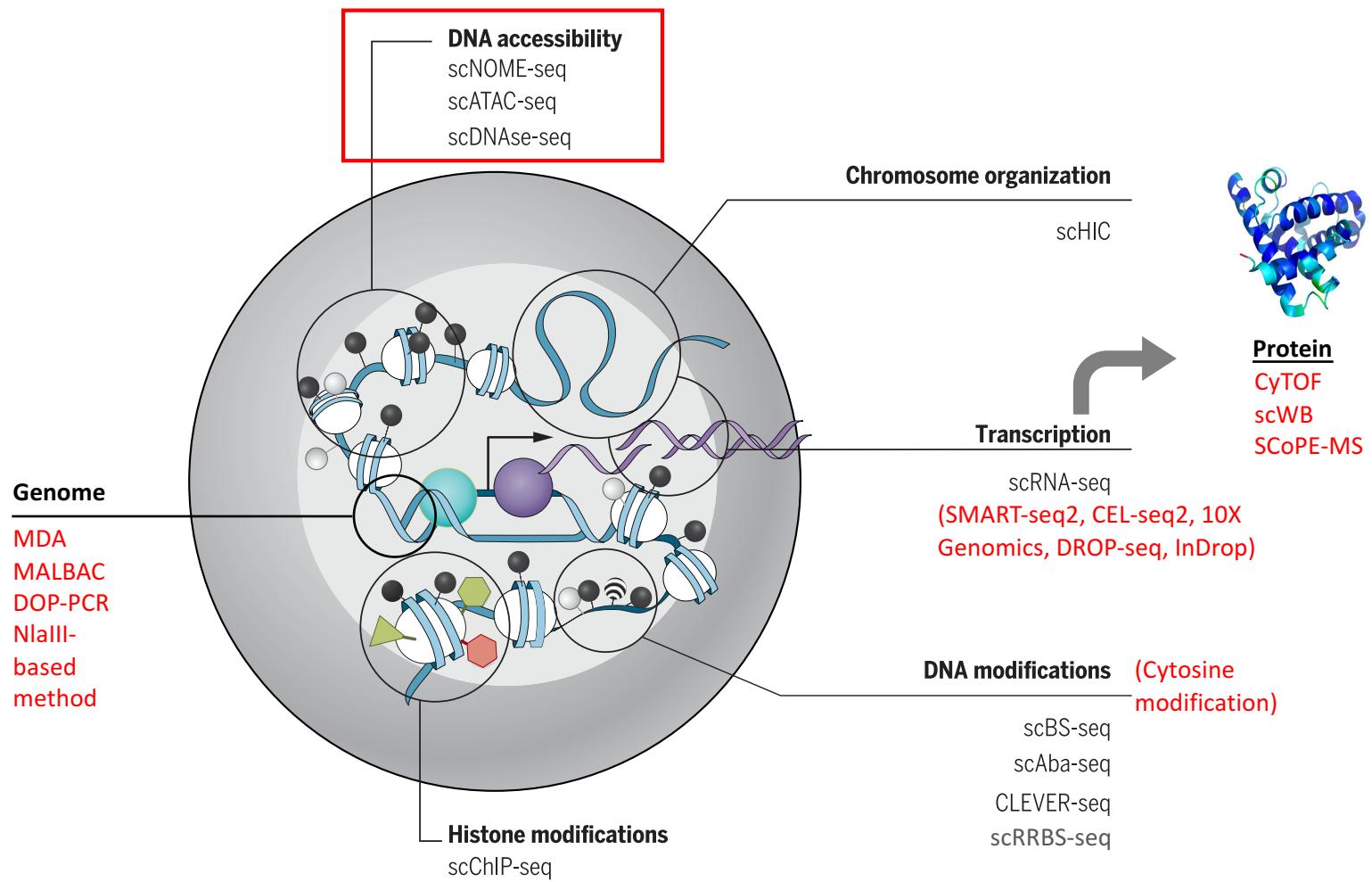
**scChIP-seq**

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.



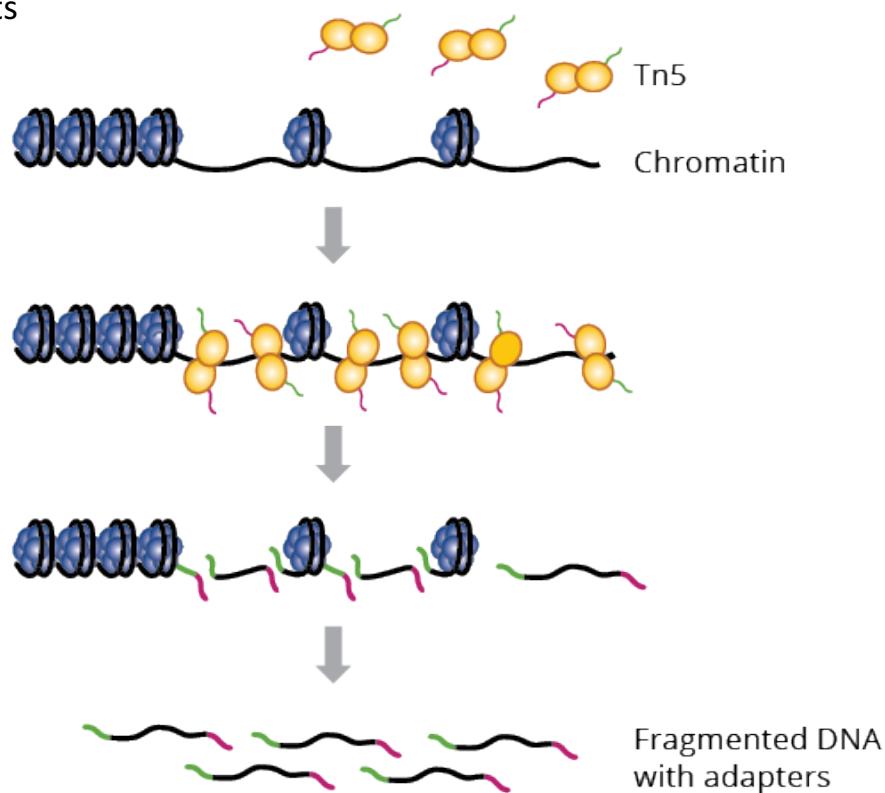
*Nature Biotechnology, 2015, 33, 1165*

# Overview of single cell -omics



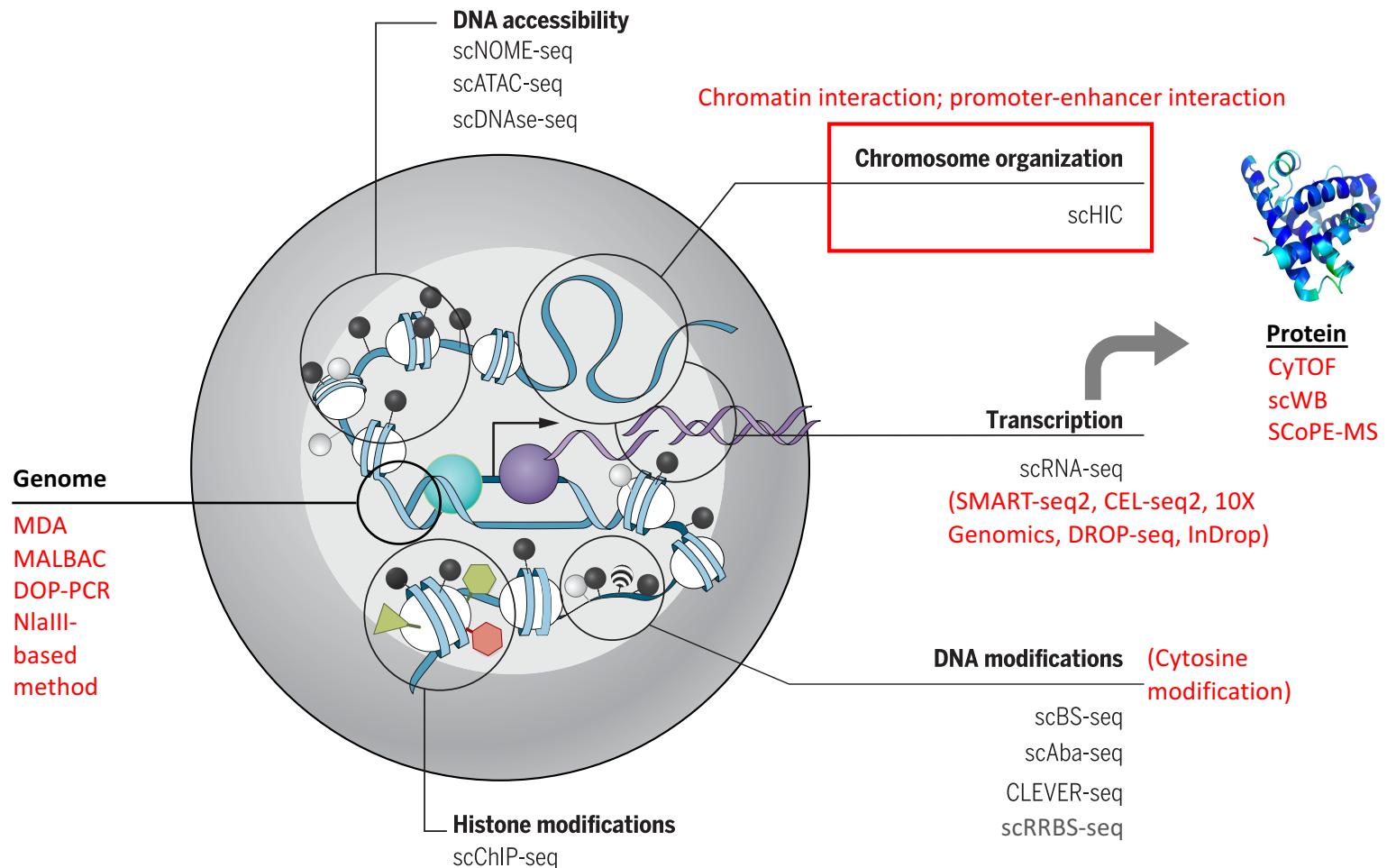
# scATAC-seq: Assay for transposase-accessible chromatin

- scATACseq can be applied to
  - nucleosome mapping experiments
  - map transcription factor binding sites
  - map DNA methylation sites

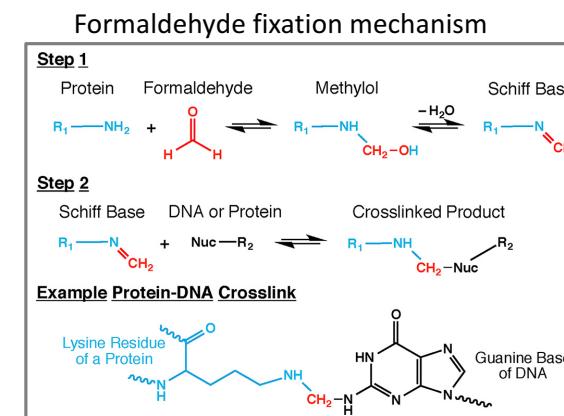
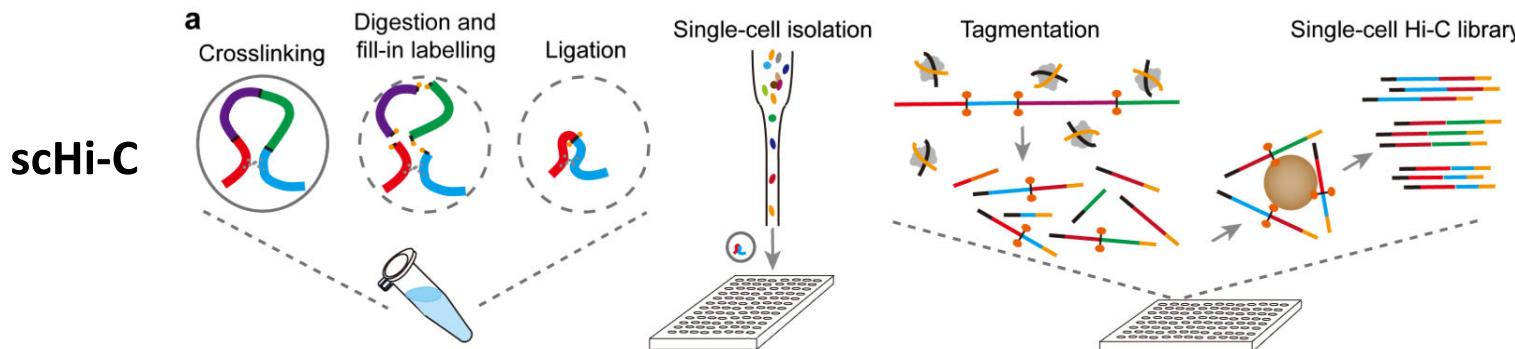
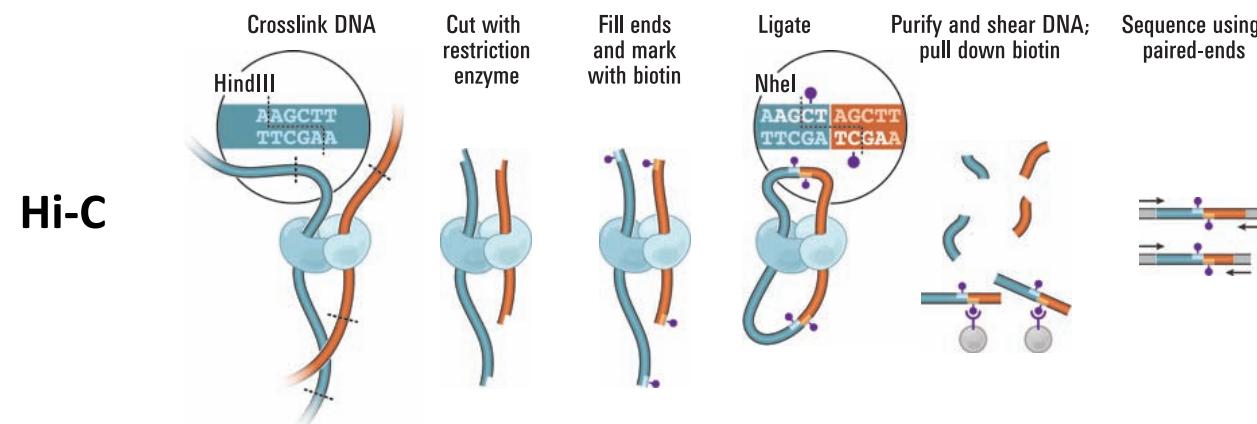


Buenrostro et al *Nat. Meth.* 2013

# Overview of single cell -omics

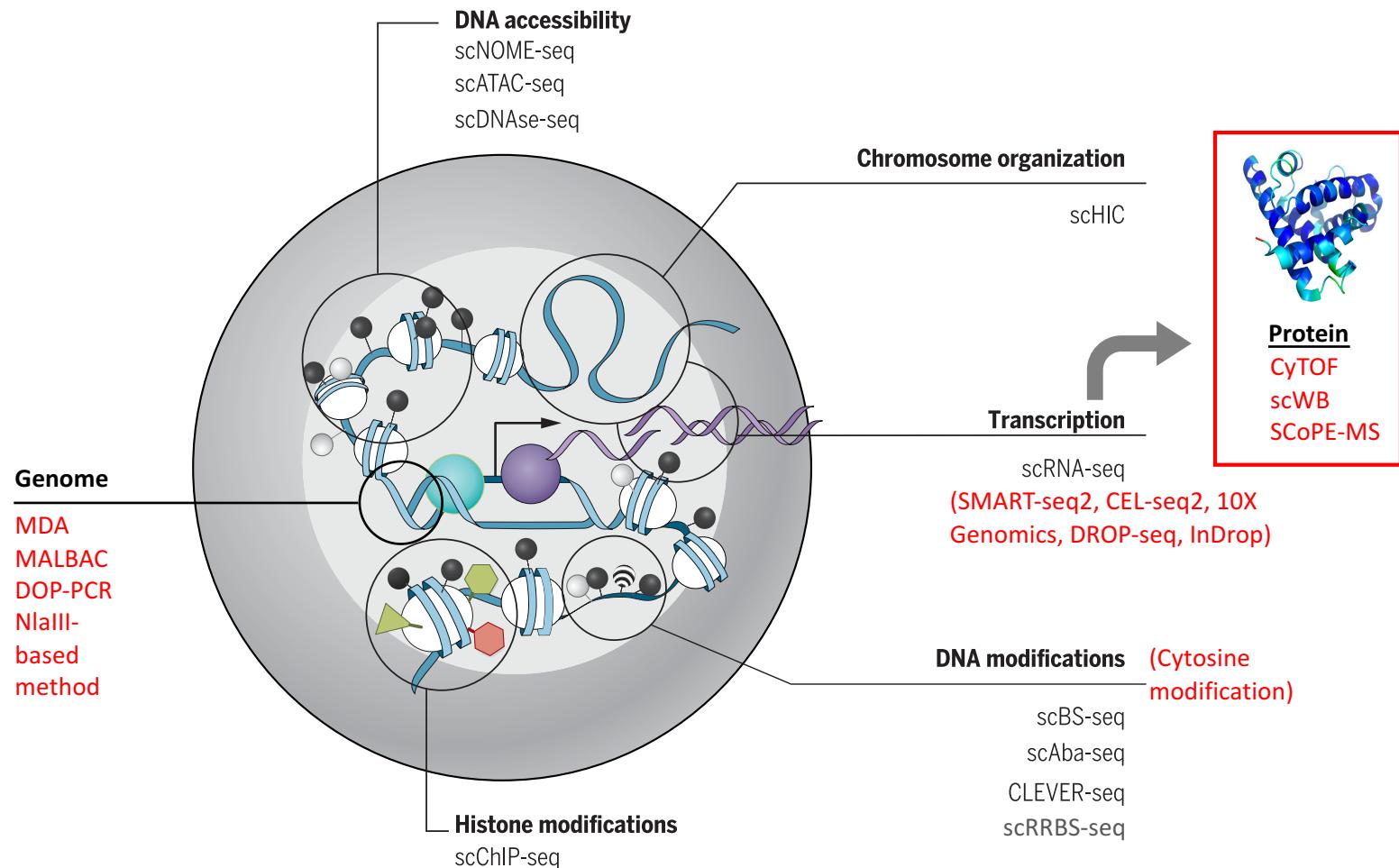


# Single cell Hi-C (high-resolution chromosome conformation capture)



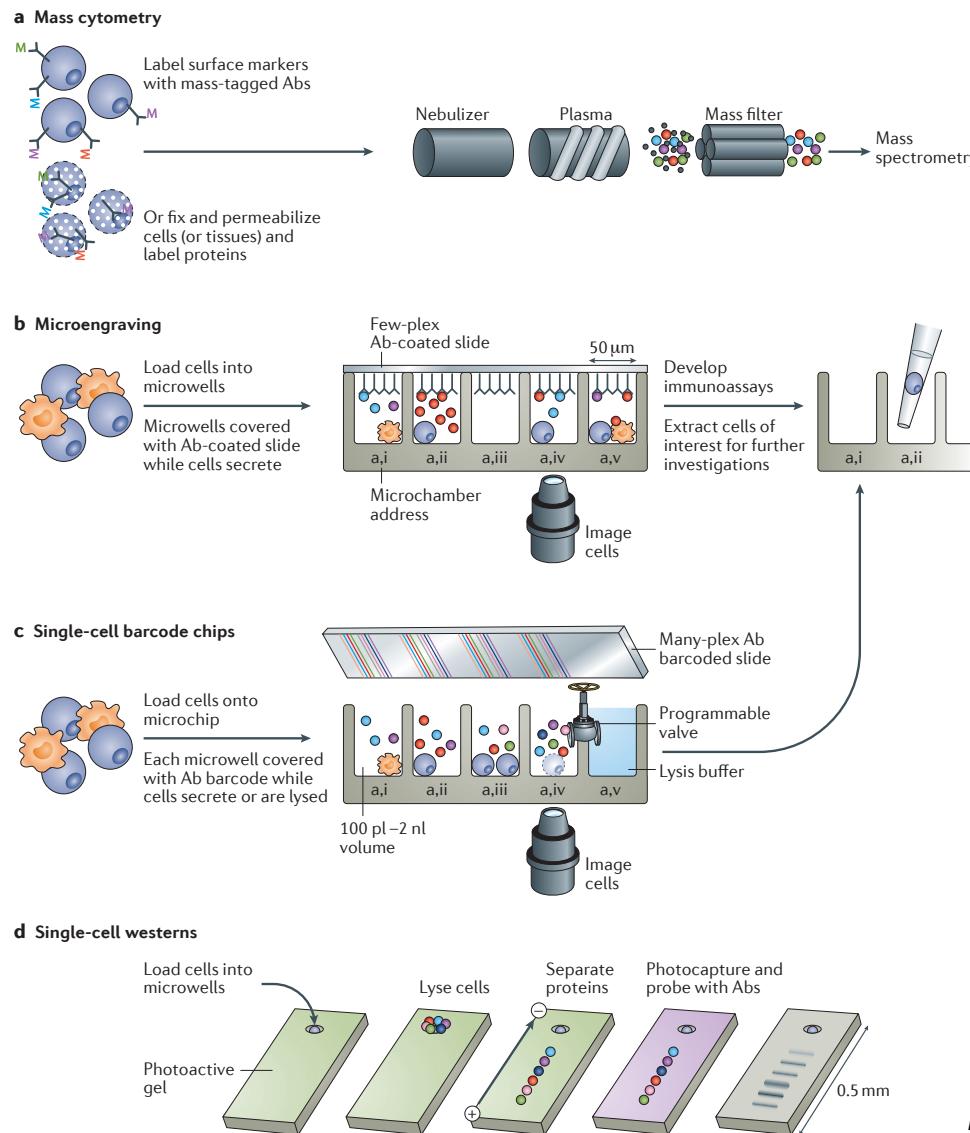
Science, 2009, 326, 289; Nature, 2017, 547, 61

# Overview of single cell -omics



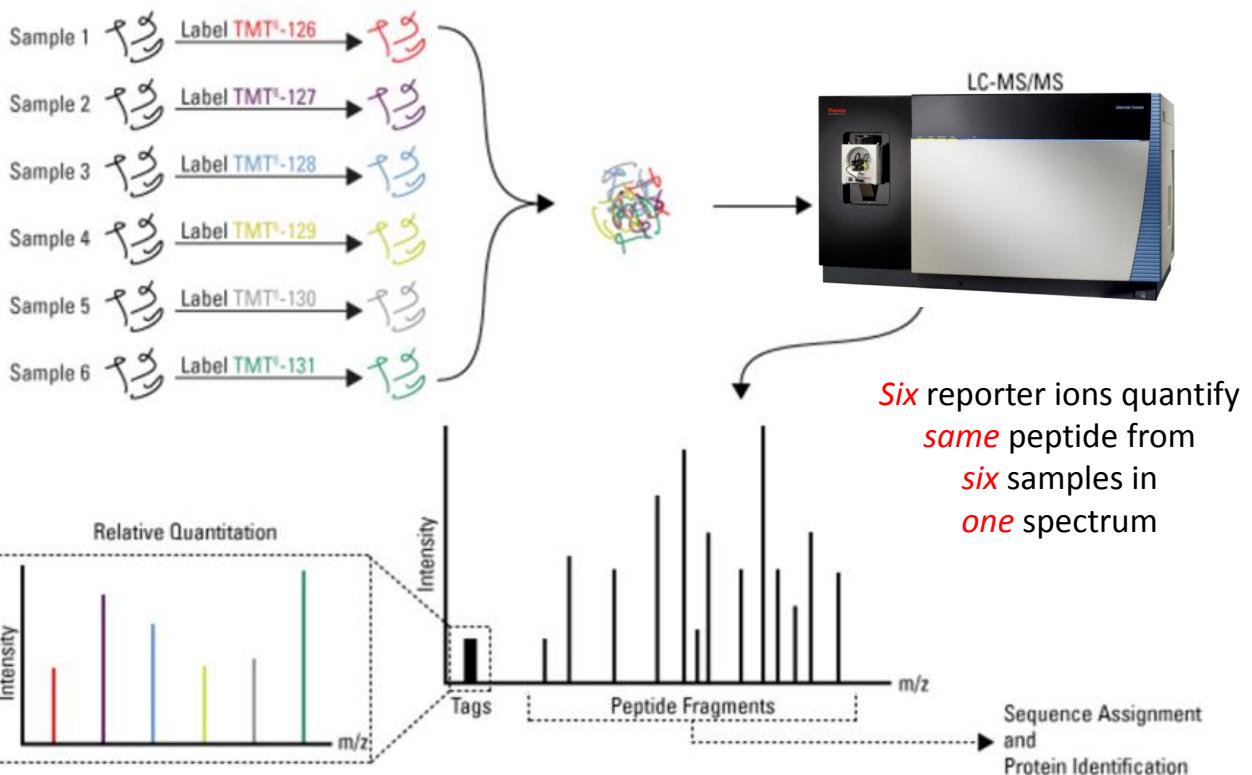
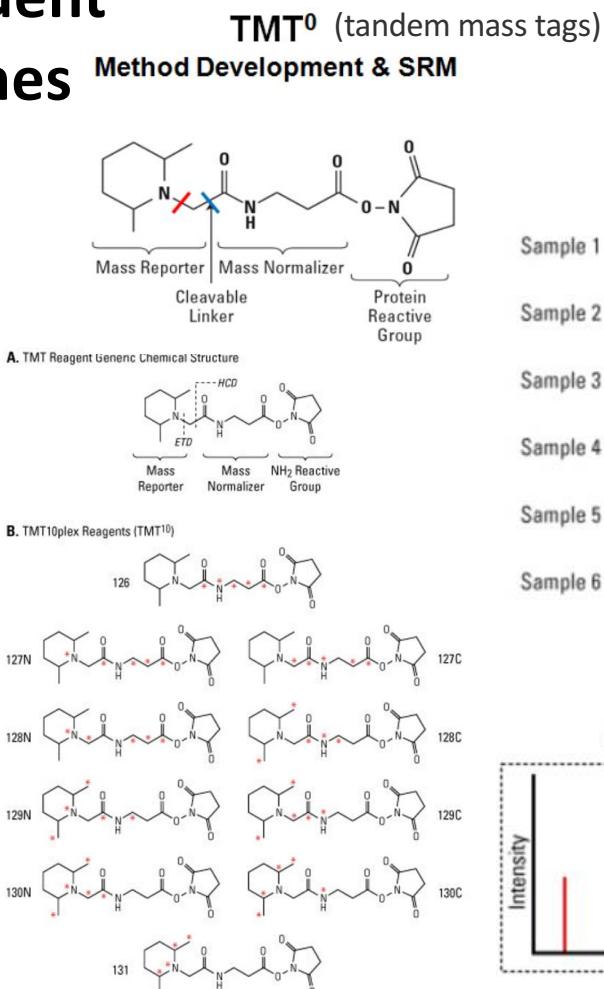
# **Single cell proteomics**

# Emerging single-cell proteomics methods (antibody-dependent approaches)



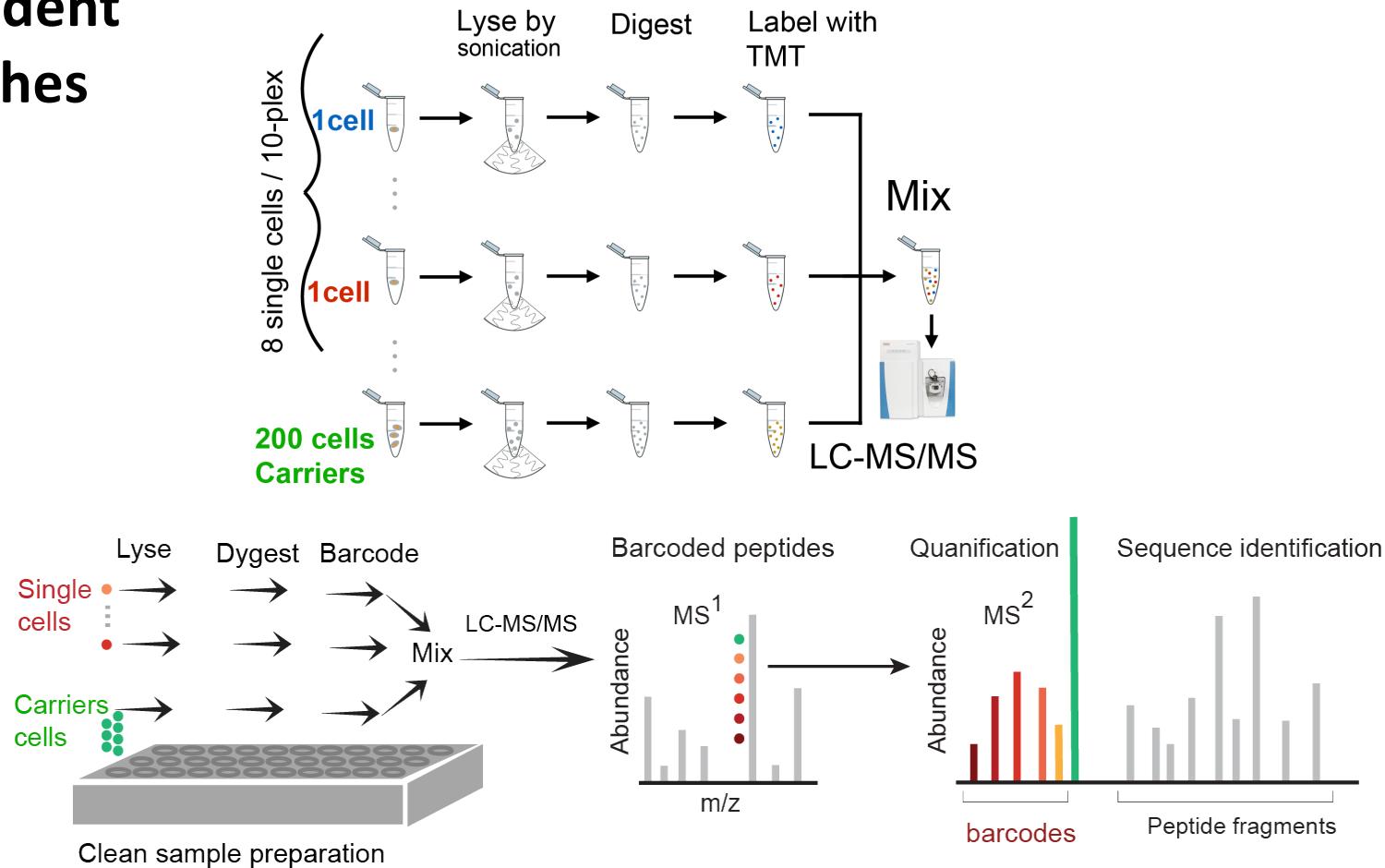
# Antibody-independent approaches

## SCoPE-MS (Single Cell ProtEomics by Mass Spectrometry)



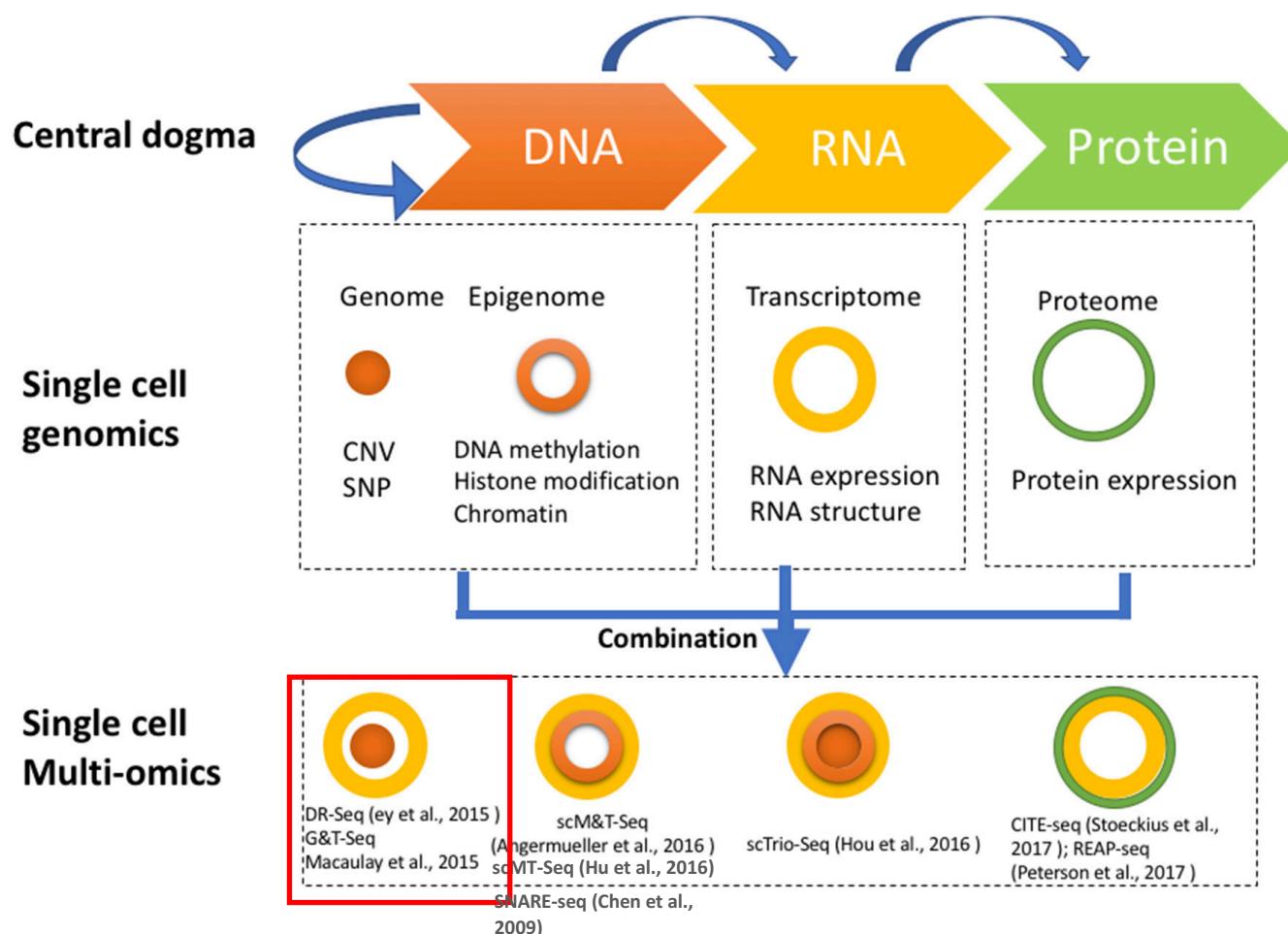
# Antibody-independent approaches

## SCoPE-MS (Single Cell ProtEomics by Mass Spectrometry)



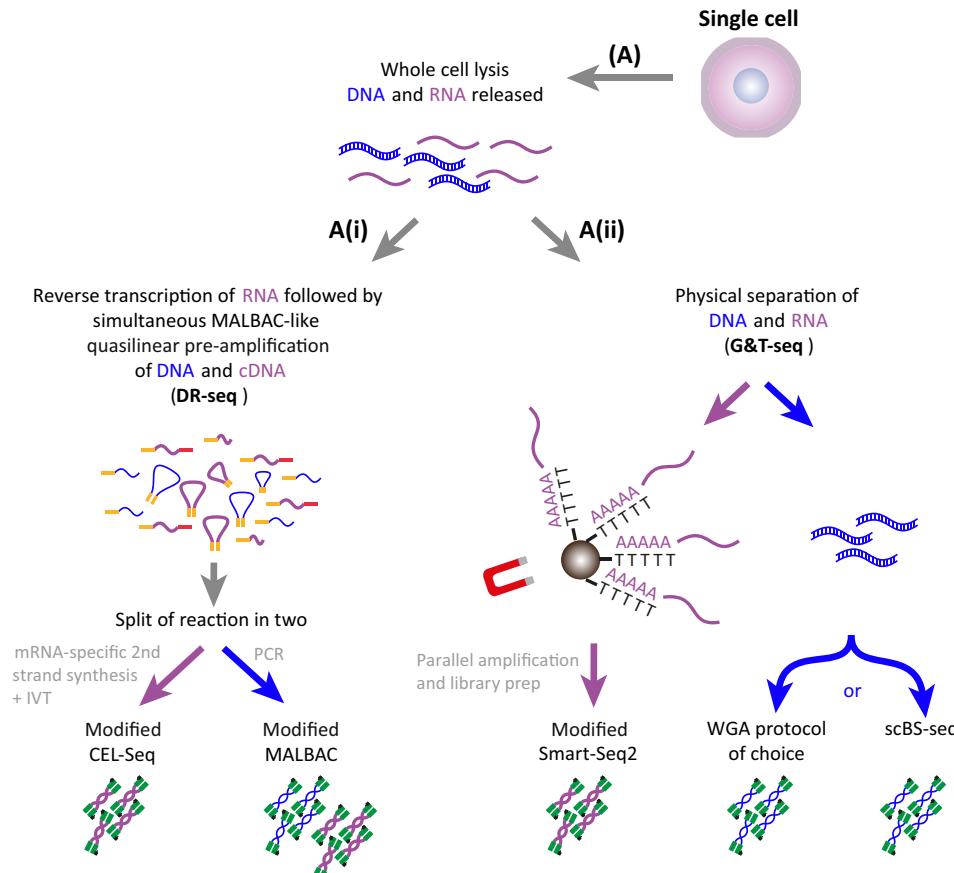
**Single cell multi–omics**

# Strategies for multi-omics profiling of single cells



# **Single cell DNA- and RNA-Sequencing**

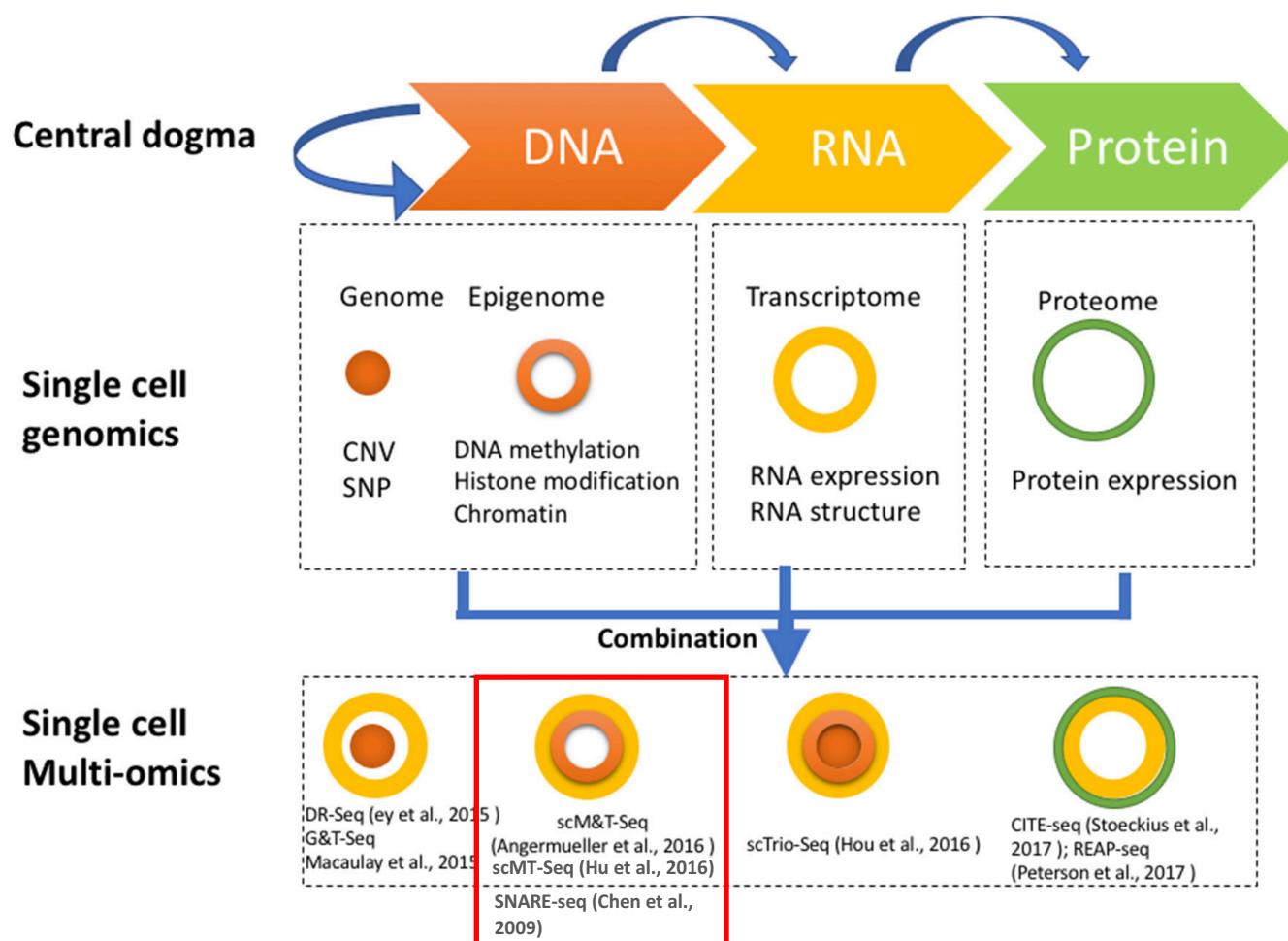
# Single cell DNA- and RNA-Sequencing



# Single cell DNA- and RNA-Sequencing

(C)	Loss of nucleic acids	Nature of RNA-seq	Nature of gDNA-seq	Shown amenable to bisulphite-sequencing
DR-seq	Minimal risk of loss	3'end tag transcript seq	MALBAC-like amplified gDNA, contaminated with co-amplified cDNA	no
G&T-seq (like)	Potential loss of mRNA and DNA molecules	Full-length transcript seq	In line with chosen WGA	yes

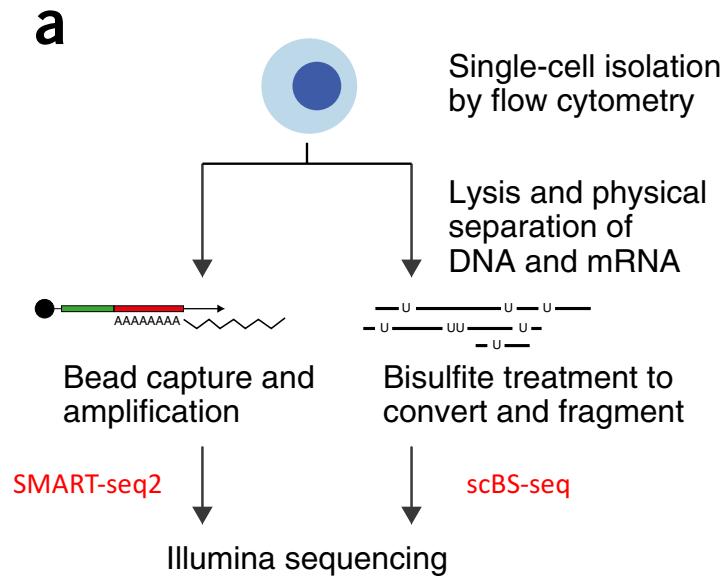
# Strategies for multi-omics profiling of single cells



# **Single cell RNA- and methylation- Sequencing**

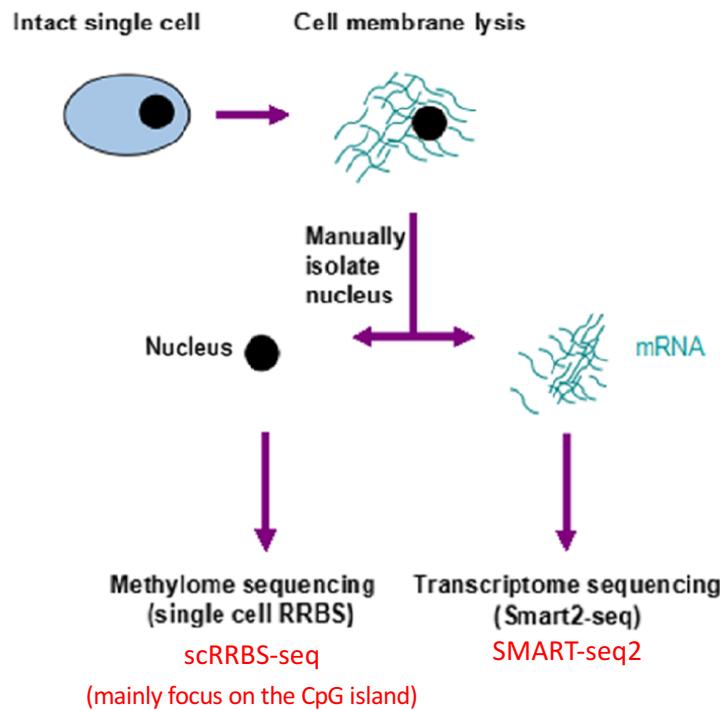
# scM&T-seq

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)



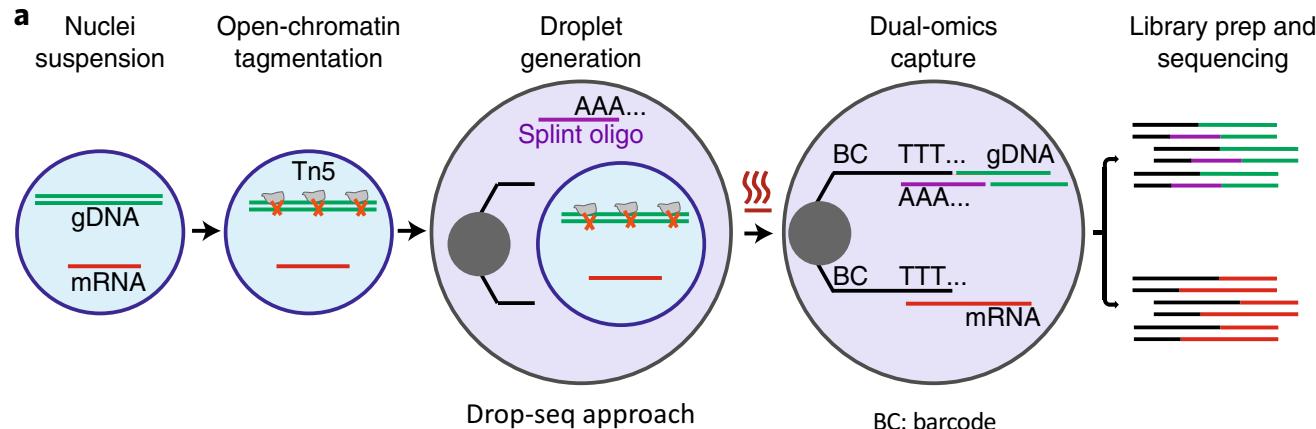
# **Single cell RNA- and ATAC-Sequencing**

# SNARE-seq

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

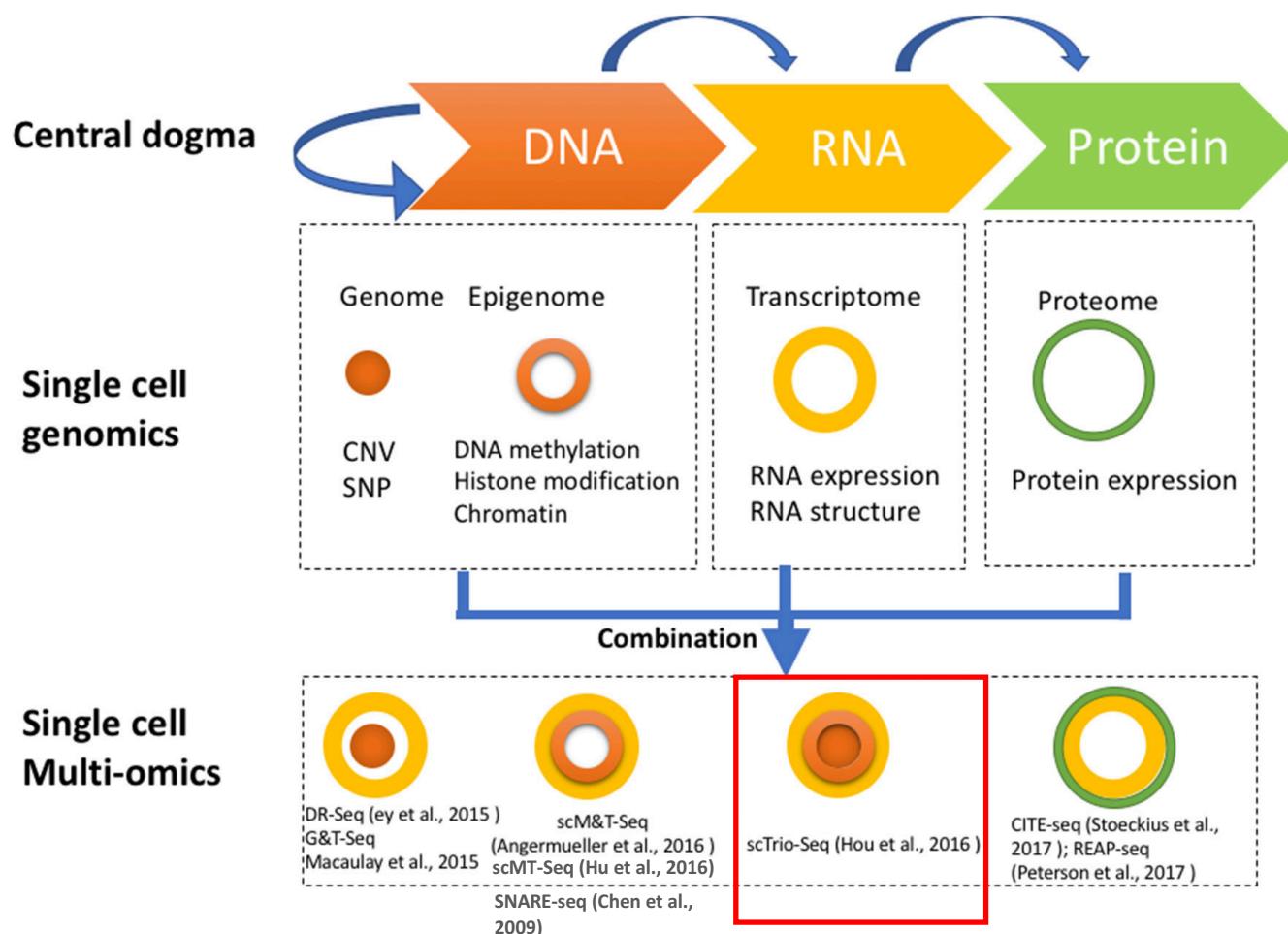
## High-throughput sequencing of the transcriptome and chromatin accessibility in the same cell

Song Chen<sup>ID</sup>, Blue B. Lake<sup>ID</sup> and Kun Zhang<sup>ID\*</sup>



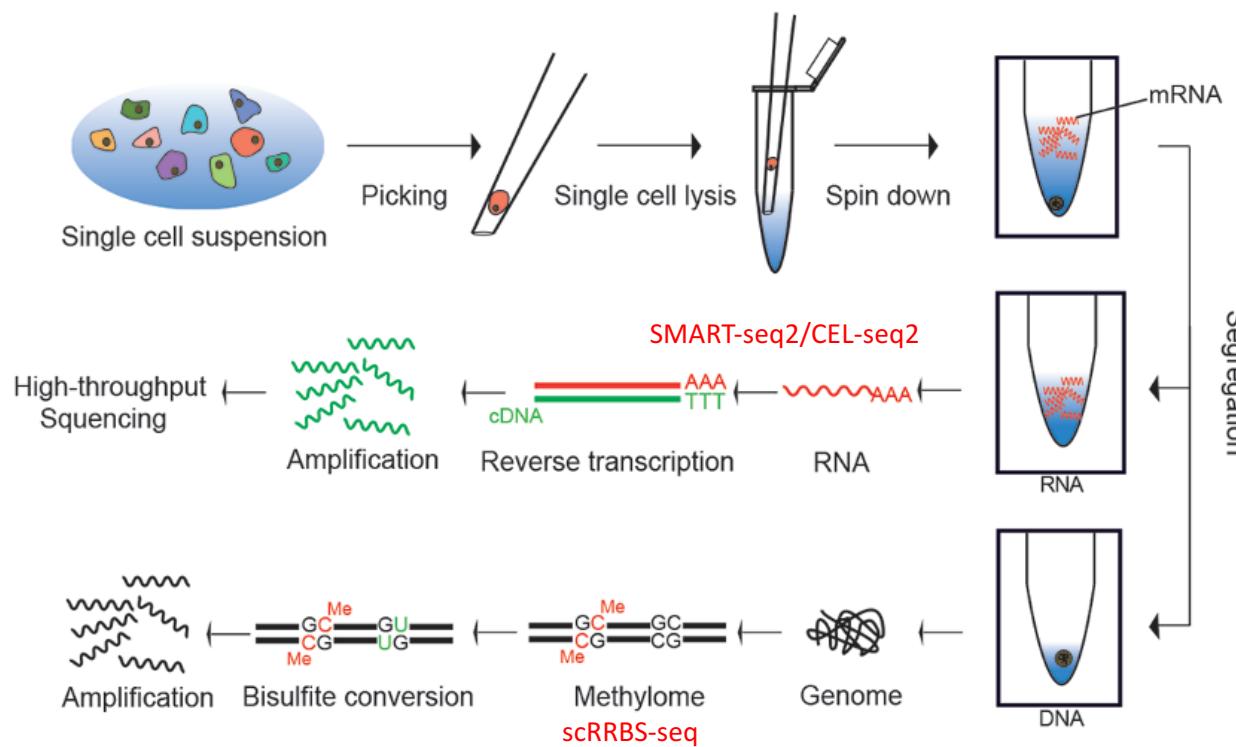
- a **splint oligonucleotide** with sequence complementary to the adaptor sequence inserted by transposition (5' end) and the poly(A) bases (3' end) allowing capture by oligo(dT)-bearing barcoded beads

# Strategies for multi-omics profiling of single cells



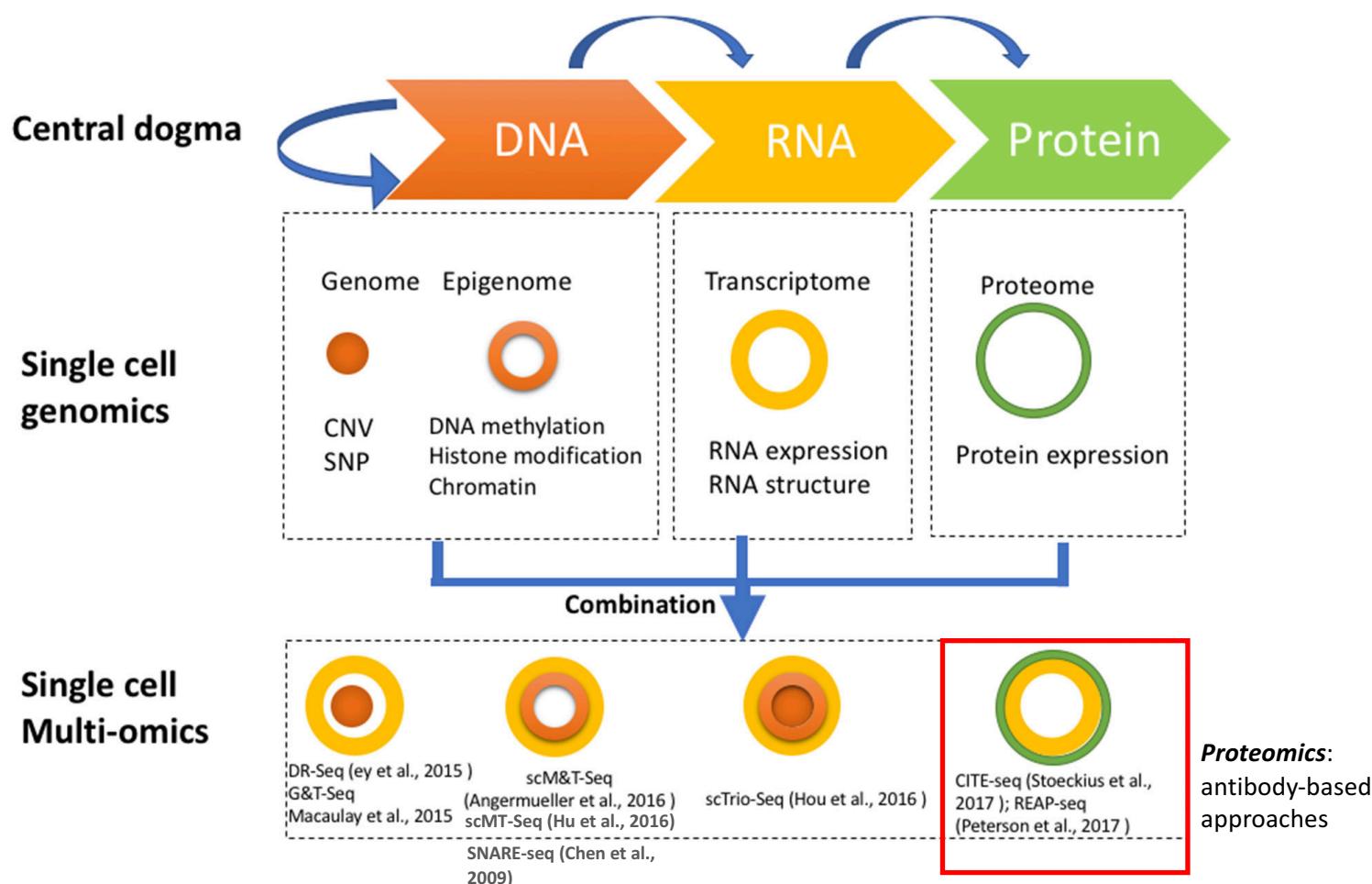
# scTrio-seq

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

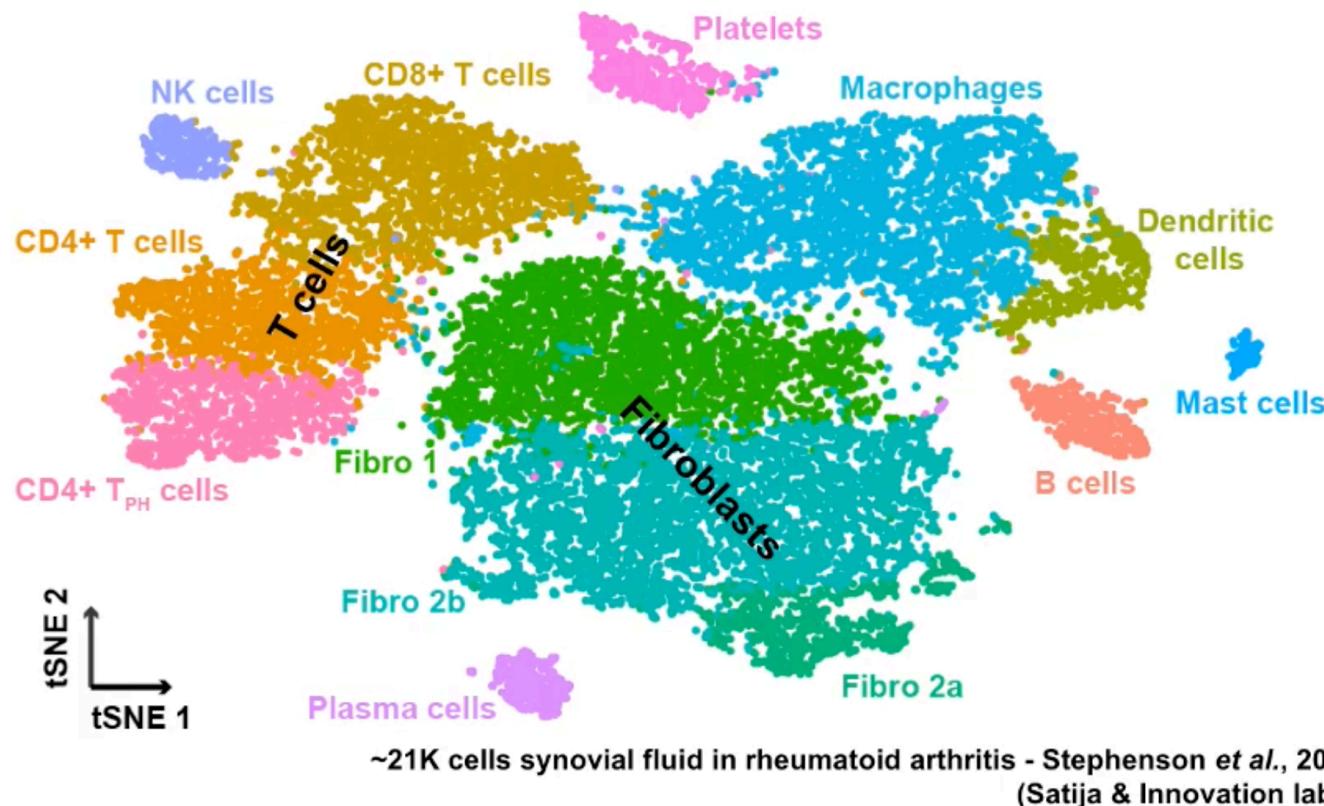


A flow chart illustrating the scTrio-seq technique. After a single cell was lysed with mild lysis buffer, the lysis product was centrifuged. The **supernatant** was transferred to a new tube for **transcriptome sequencing** analyses, while the pellet (containing the nucleus) was **bisulfite-converted**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

# Strategies for multi-omics profiling of single cells

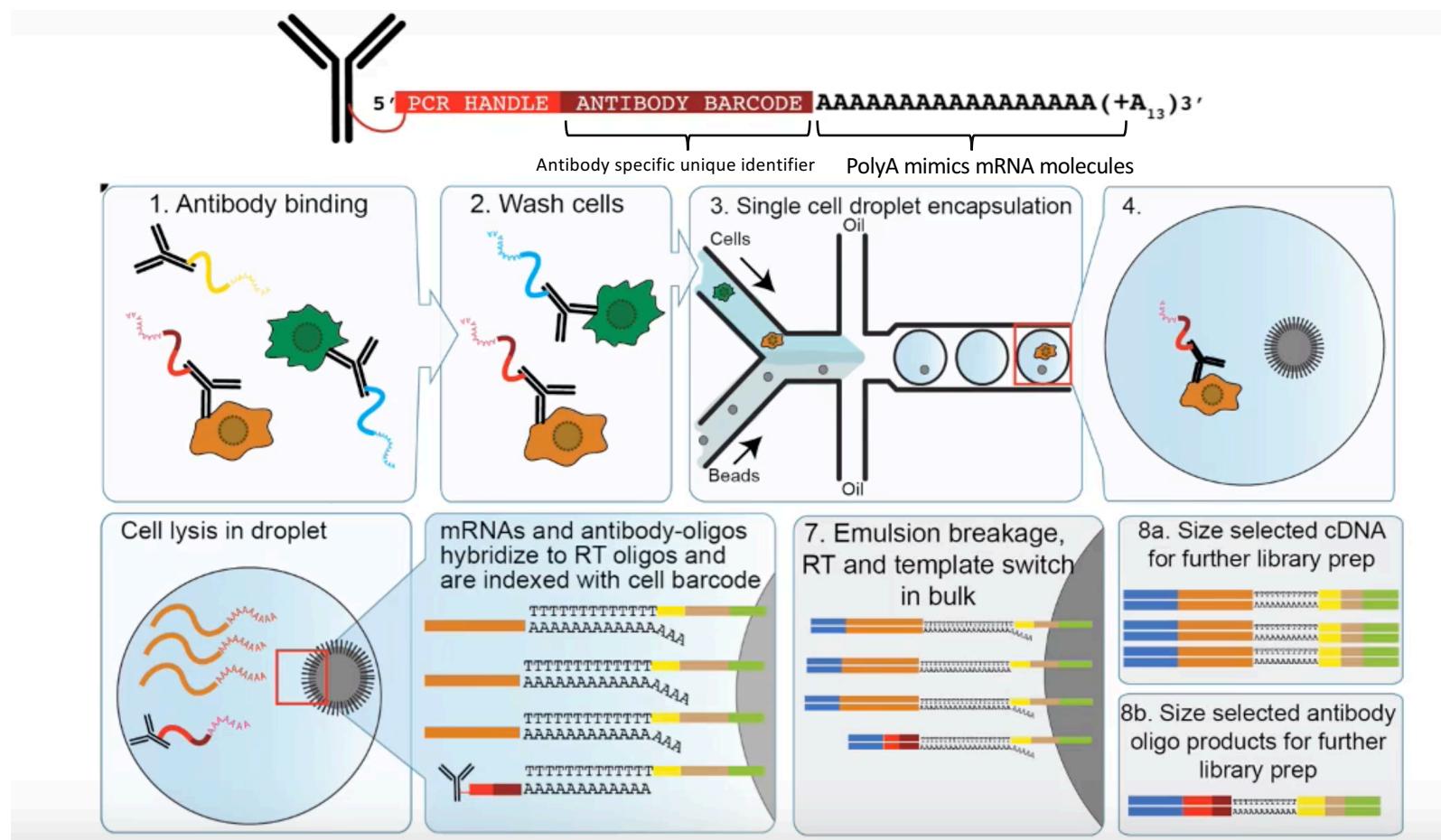


# Distinguishing cells/states that are transcriptomically similar is difficult in scRNA-seq



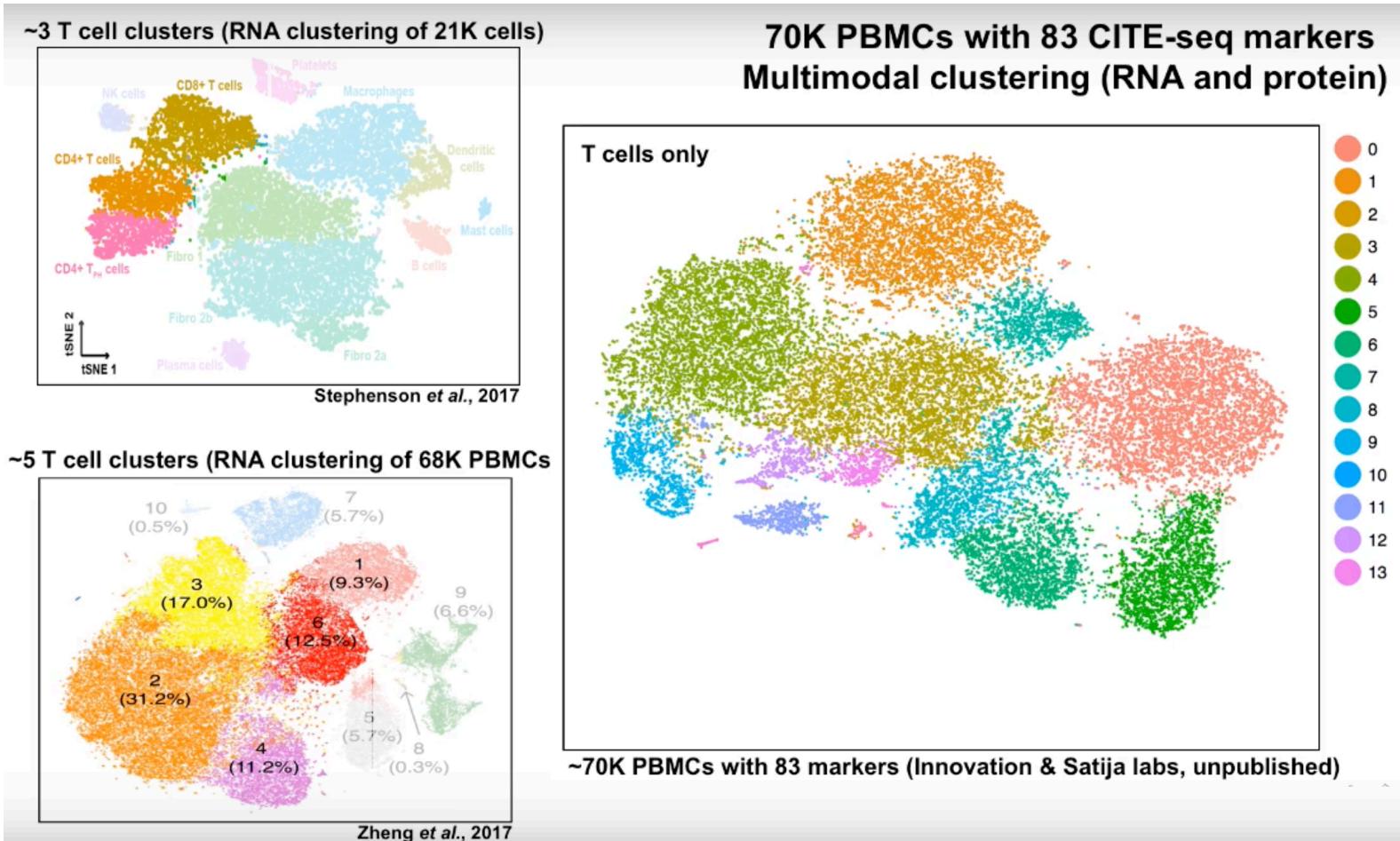
- It's often that many subtypes of cells have robust surface proteins/antigens  
=> recognized by antibodies

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

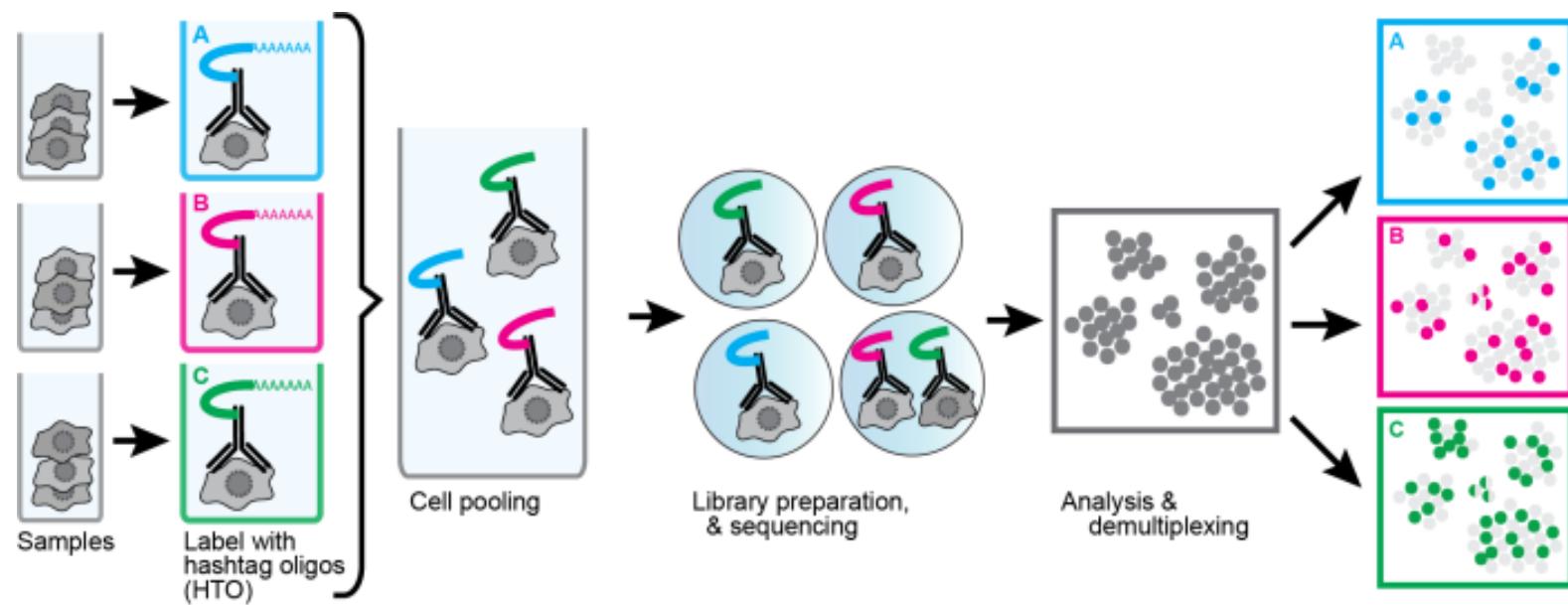


# Multimodal clustering resolves more T cell clusters

13 T cell clusters with distinct protein and RNA markers

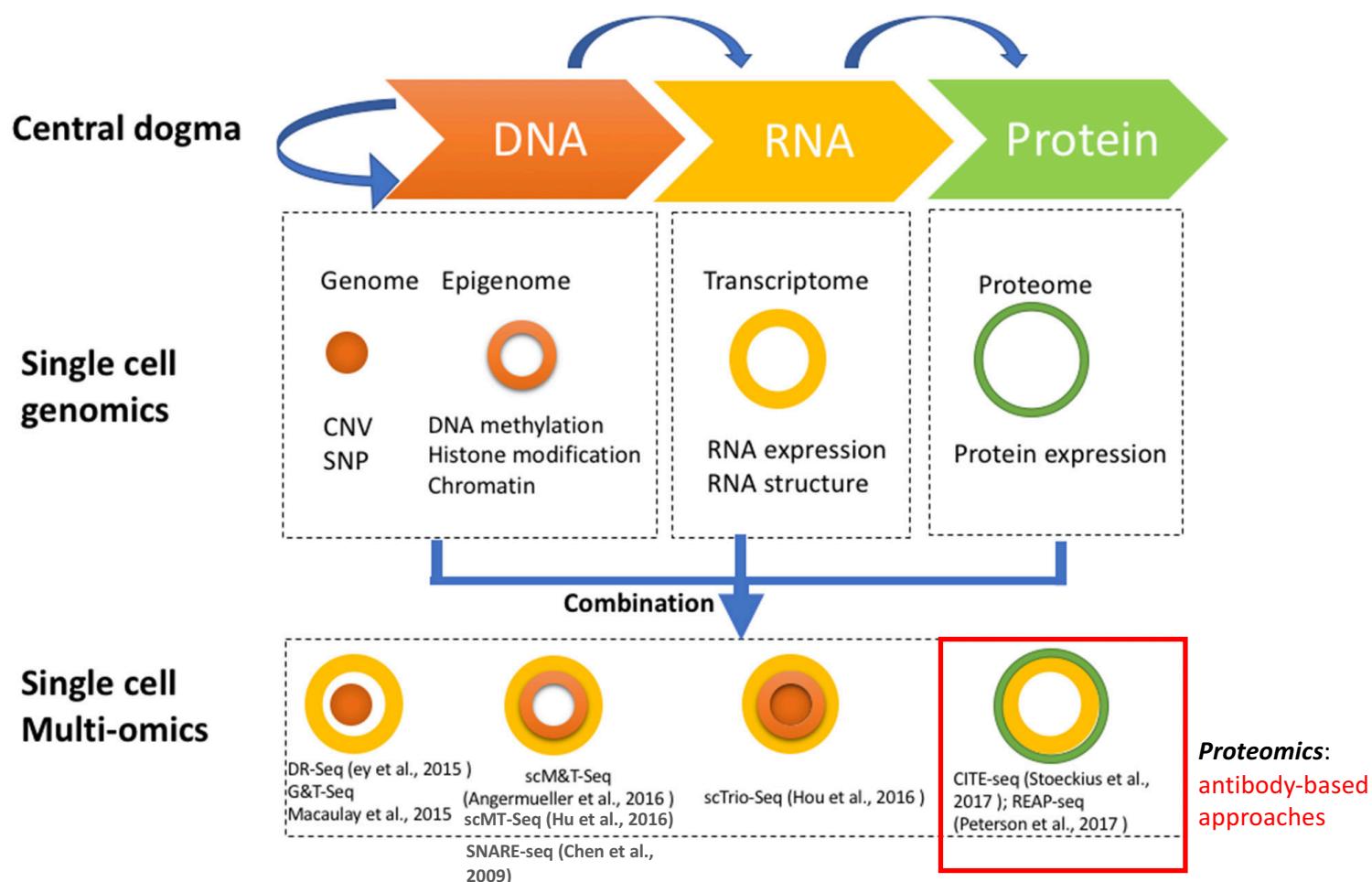


# Cell Hashing: apply CITE-seq to multiplex multiple samples



Available from 10X Genomics & BD Rhapsody

# Strategies for multi-omics profiling of single cells



# Single cell whole transcriptome & proteome analysis

RESEARCH

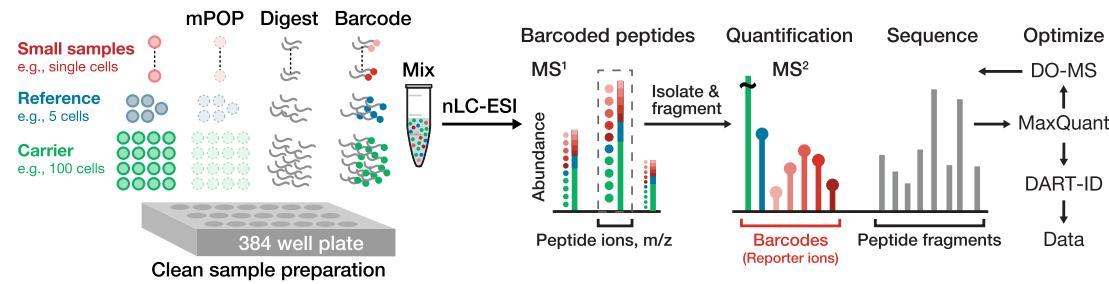
Open Access

## Single-cell proteomic and transcriptomic analysis of macrophage heterogeneity using SCoPE2



Harrison Specht<sup>1\*</sup>, Edward Emmott<sup>1,2</sup>, Aleksandra A. Petelski<sup>1</sup>, R. Gray Huffman<sup>1</sup>, David H. Perlman<sup>1,3</sup>, Marco Serra<sup>4</sup>, Peter Kharchenko<sup>4</sup>, Antonius Koller<sup>1</sup> and Nikolai Slavov<sup>1\*</sup>

## Single-Cell ProtEomics by Mass Spectrometry (SCoPE2)



Antibody-independent  
single cell proteomics

scRNAseq

Integrated  
analysis

10X GENOMICS

Genome Biology (2021) 22:50

# Conclusion

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