

Single cell multi-omics

Miao-Ping Chien

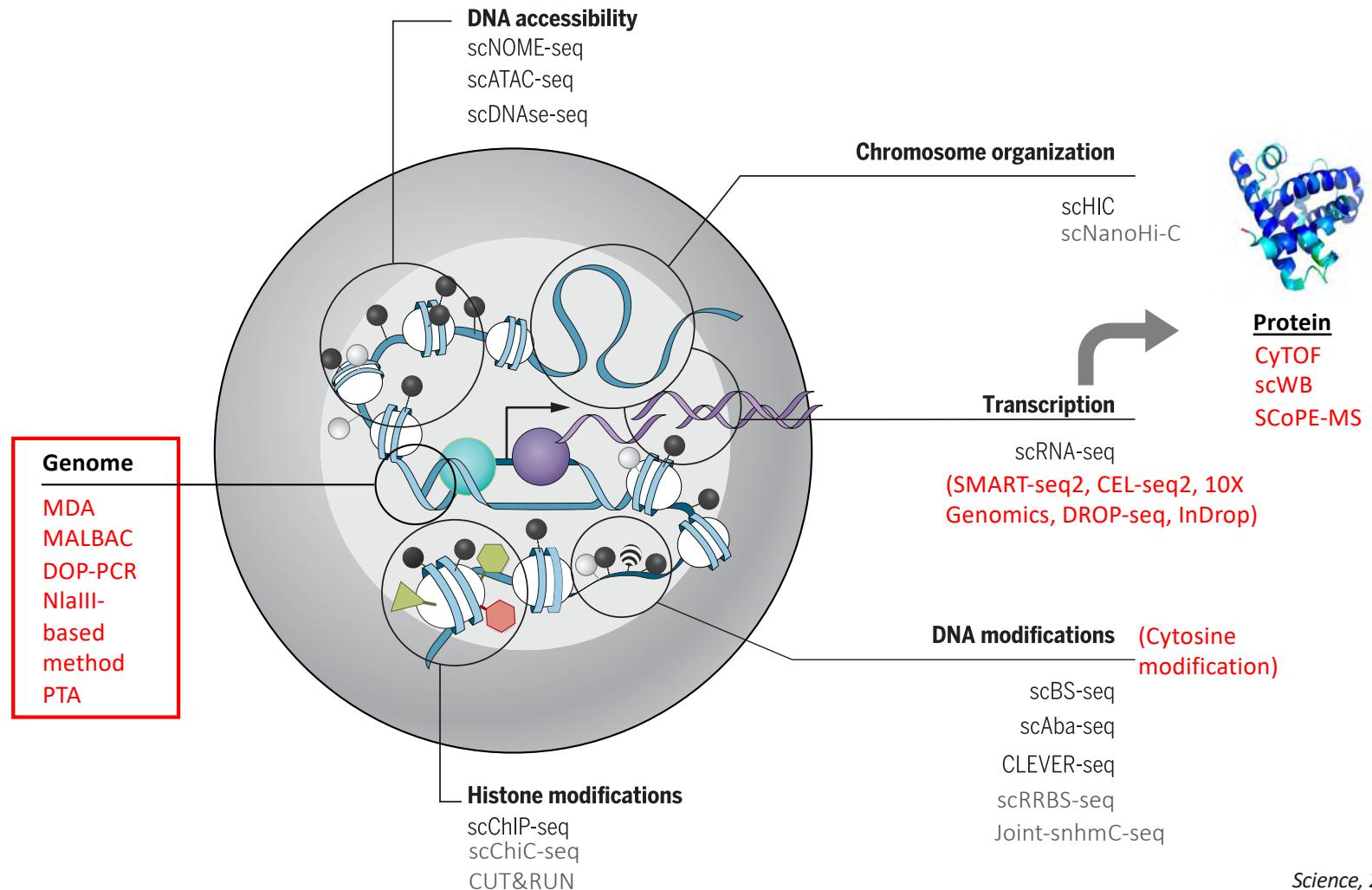
Erasmus MC, Associate professor

2023 Single Cell Analysis Workshop, 2023/10/24

Outline

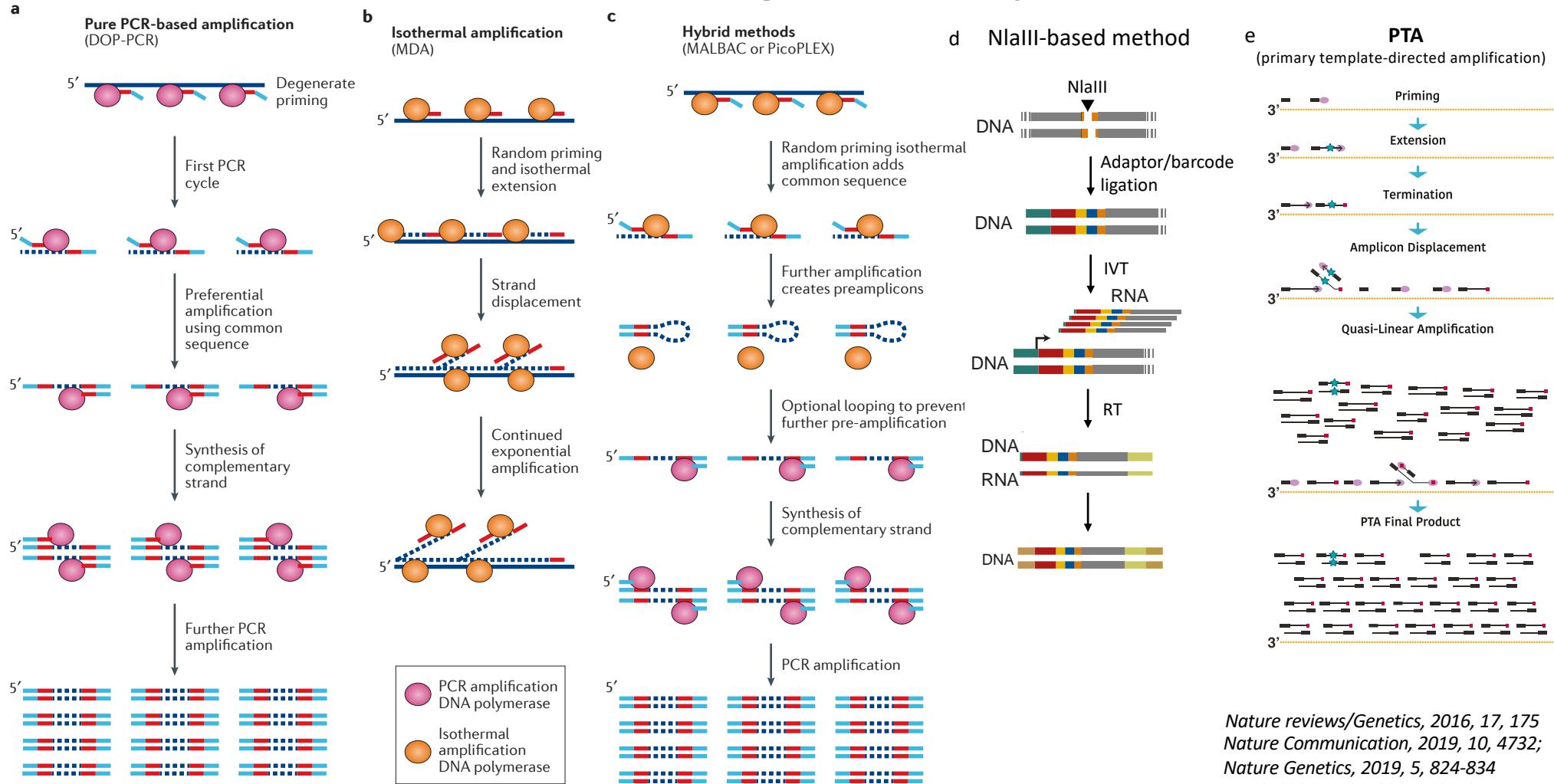
- (Quick) overview of different single cell –omics methods
- Different single cell multi–omics methods

Overview of single cell -omics



Single cell genomic sequencing

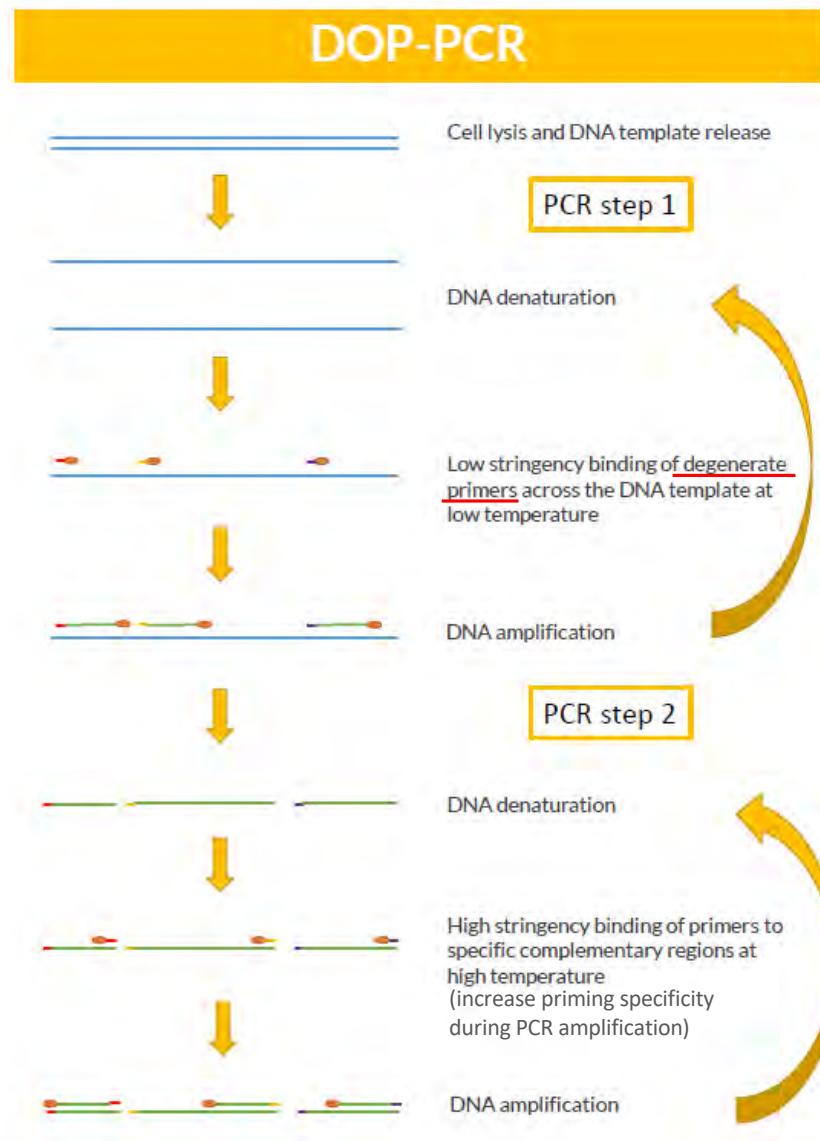
Overview of the main whole-genome amplification methods



Nature reviews/Genetics, 2016, 17, 175
Nature Communication, 2019, 10, 4732;
Nature Genetics, 2019, 5, 824-834
PNAS, 2021, 118, e2024176118

Degenerate Oligonucleotide-Primed PCR (DOP-PCR)

- biased amplification
- error prone

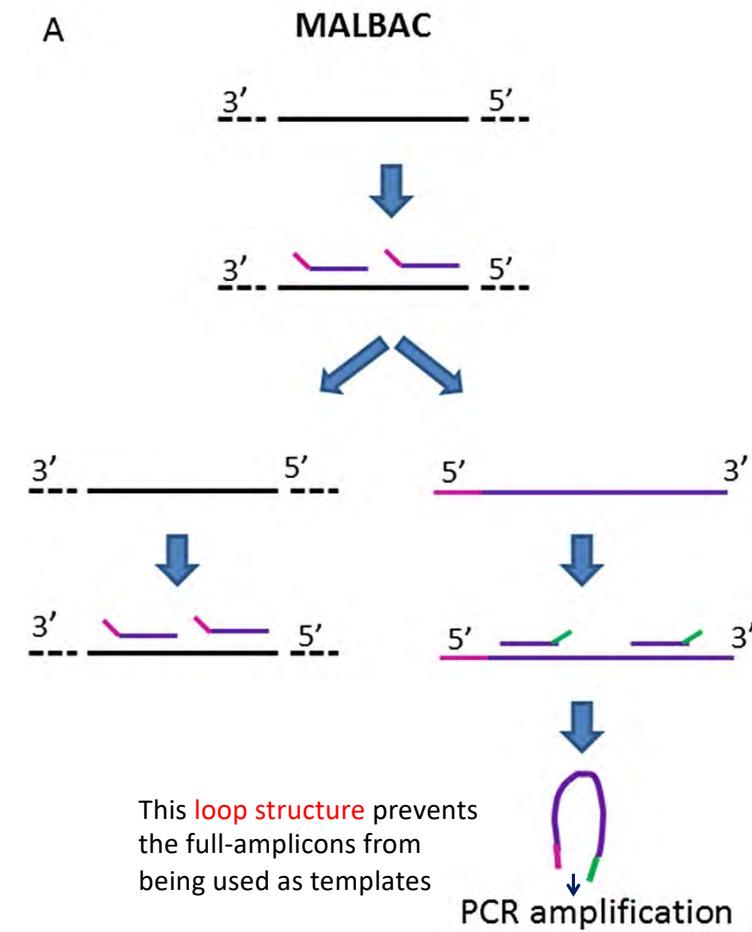


MALBAC vs MDA

Hybrid method

MALBAC: multiple annealing and looping based amplification cycles

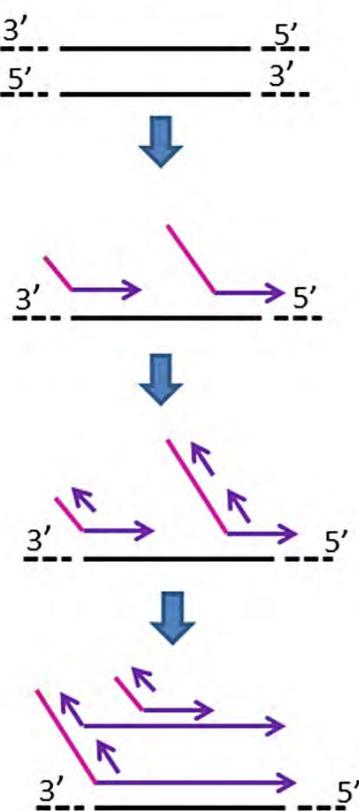
A



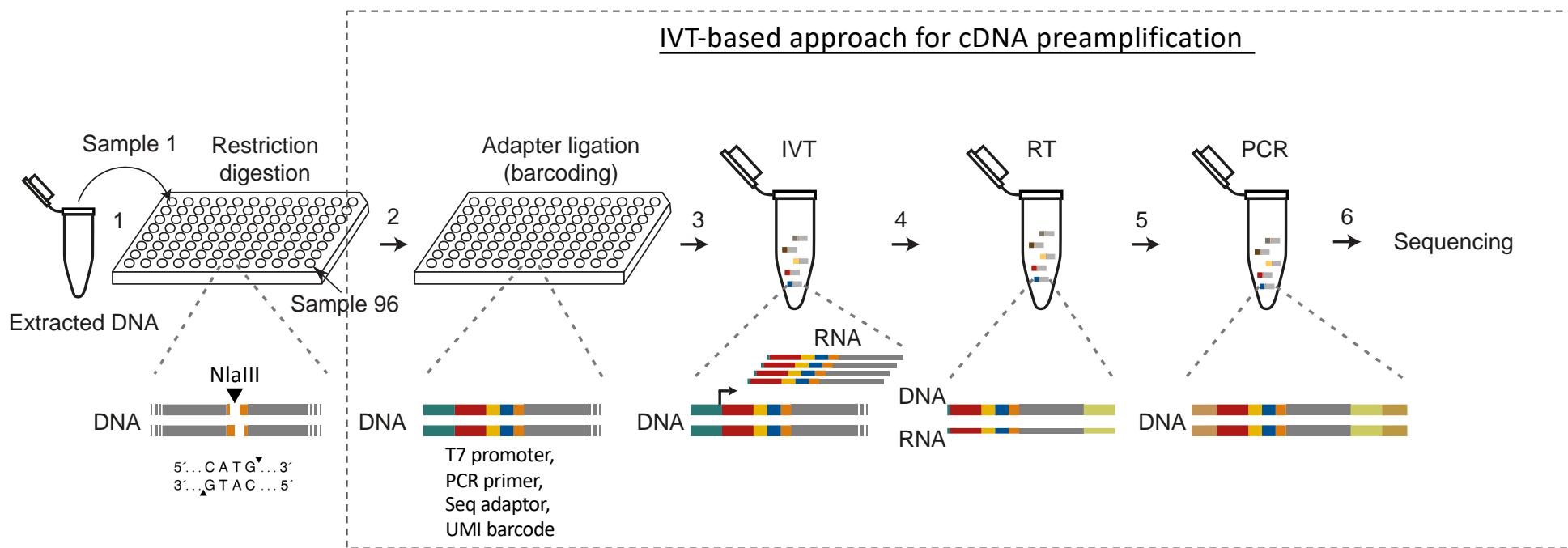
Isothermal amplification

MDA: multiple displacement amplification

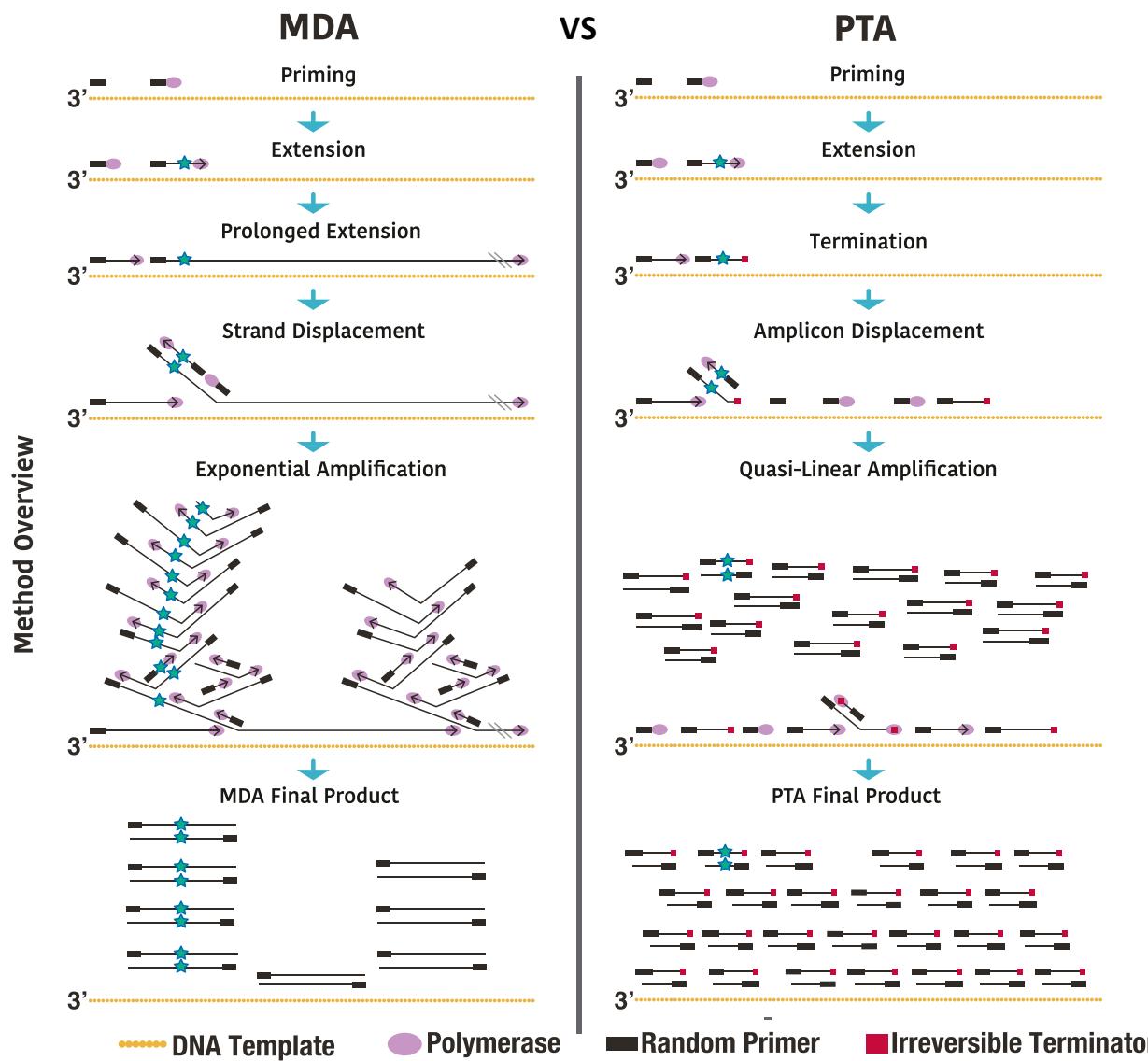
B



NlaIII-based scDNAseq method



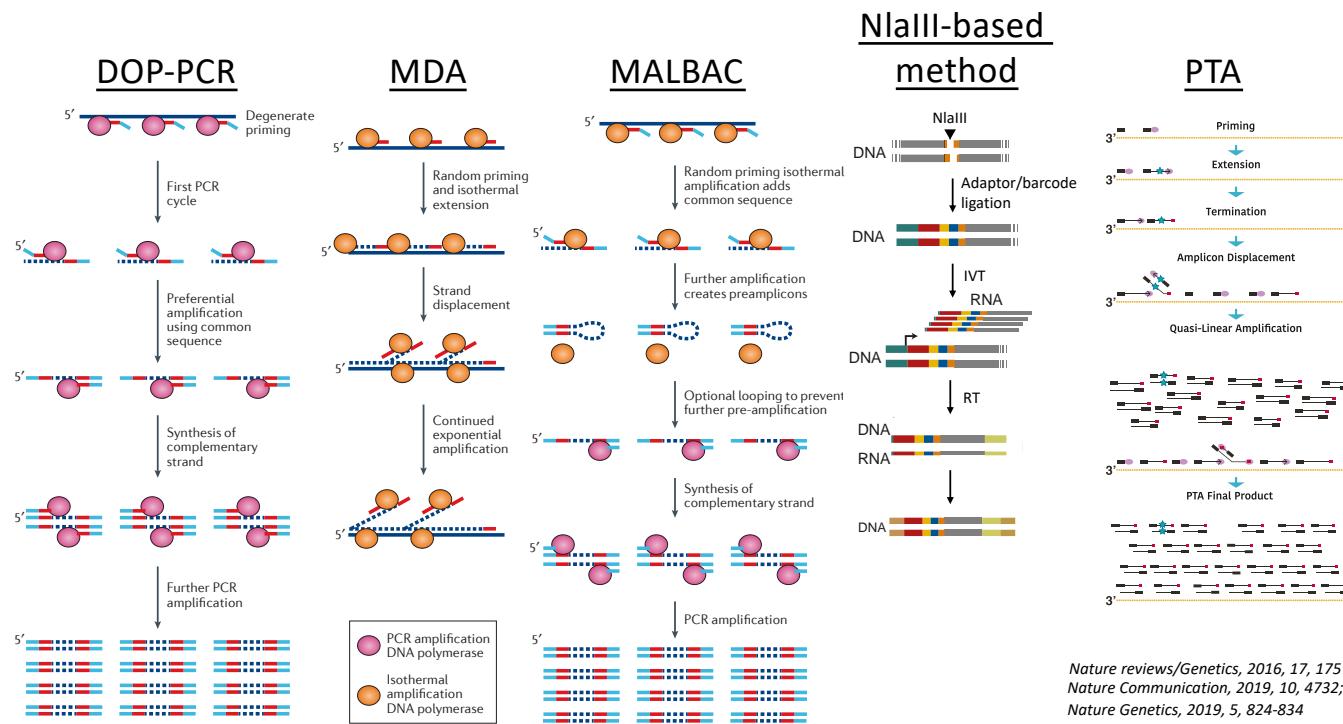
Nature Communication, 2019, 10, 4732;
Nature Genetics, 2019, 5, 824-834



PTA (primary template-directed amplification):

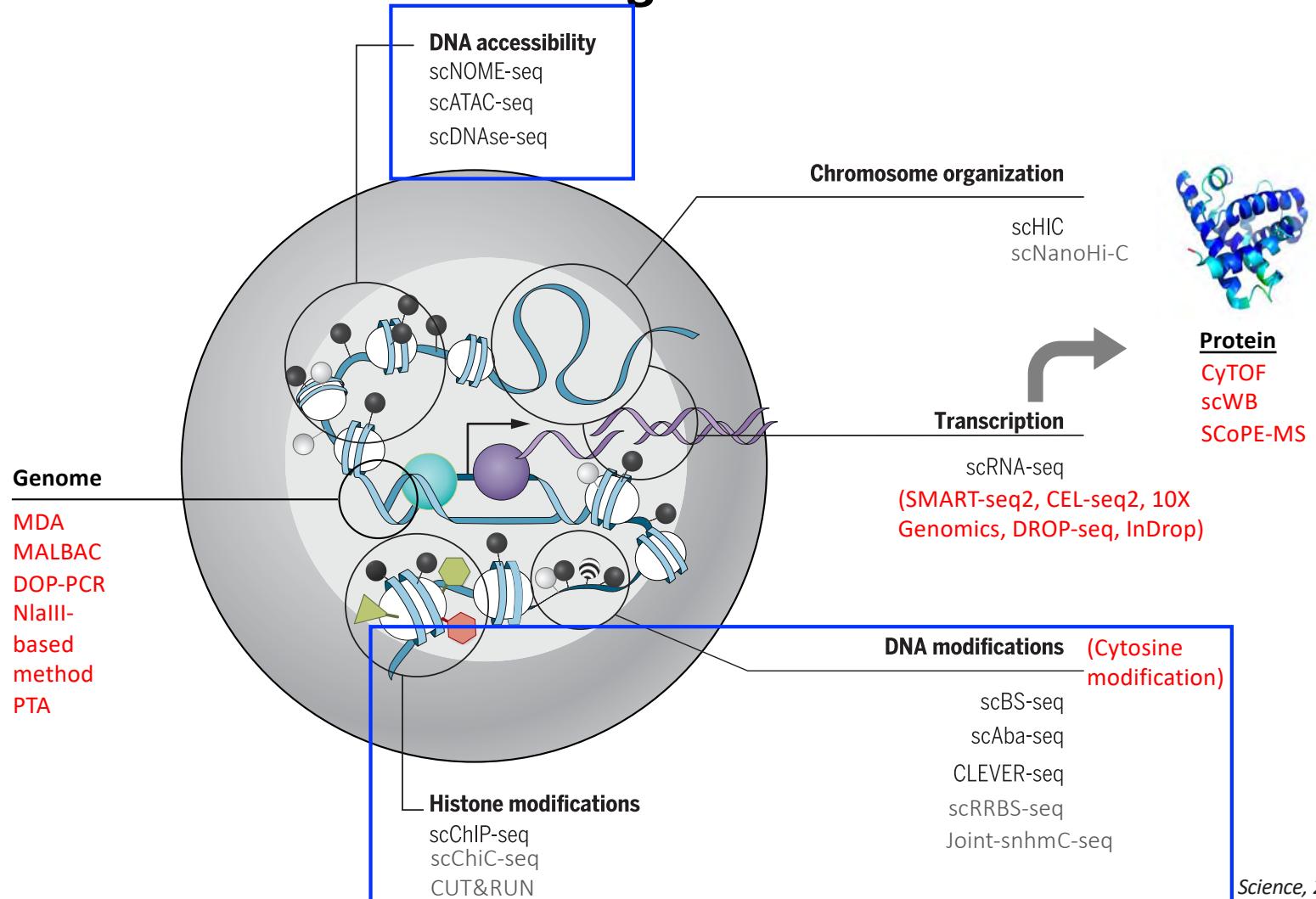
- incorporation of **exonuclease-resistant terminators** in the reaction result in smaller double-stranded amplification products
- This approach undergoes **limited subsequent amplification**, resulting in a quasilinear process with more amplification **originating from the primary template**.
- With this, **errors have limited propagation** from daughter amplicons during subsequent amplification compared to MDA.
- In addition, PTA has improved and reproducible genome **coverage breadth** and **uniformity**.

Overview of the main whole-genome amplification methods



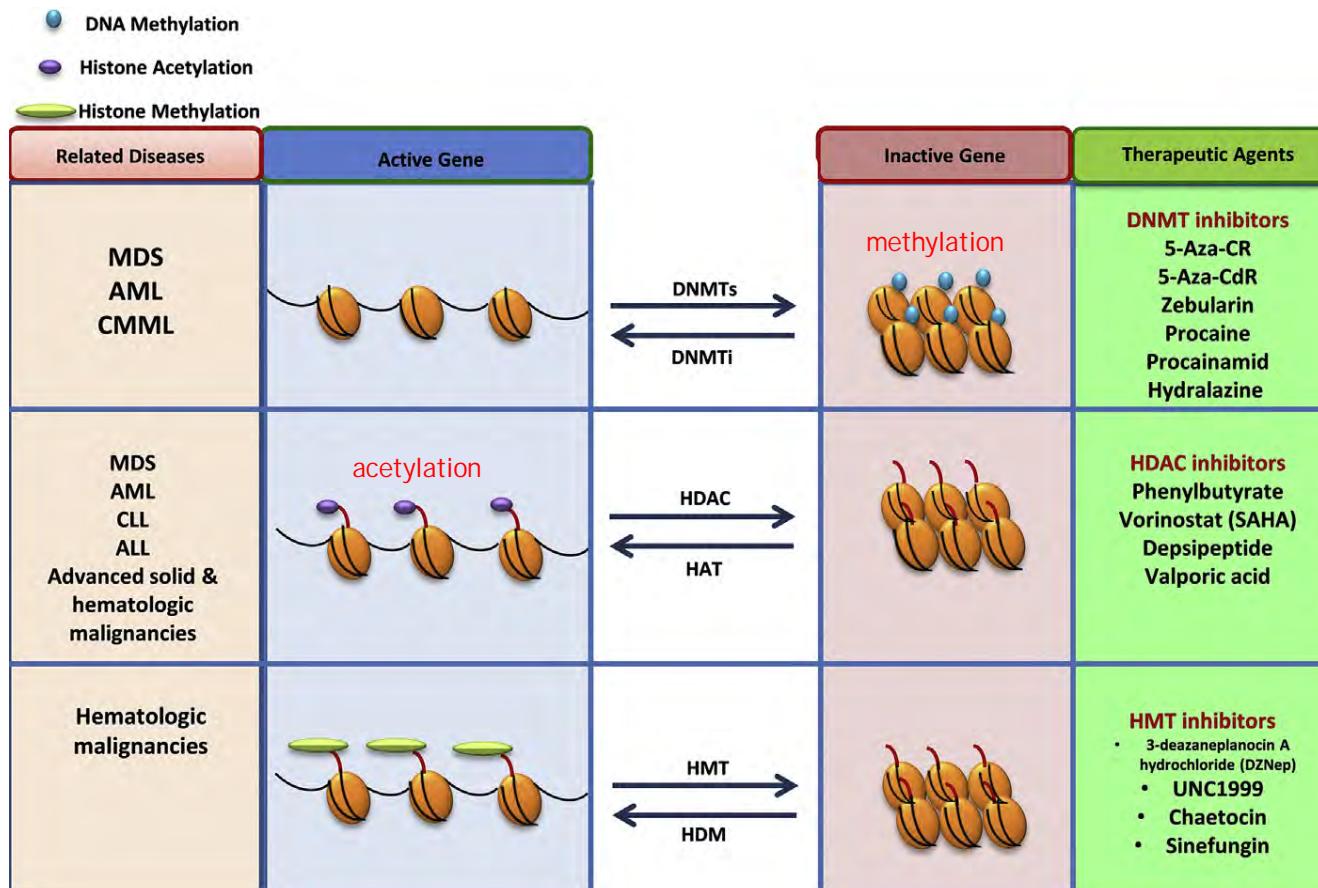
- Highest to lowest genome recovery rate?
PTA (>90%) > MDA (~80%) > MALBAC (~50%) > DOP-PCR (~6%) / NlaIII-based method (~5-10%)
- Highest to lowest duplication ratio (duplicate the product more than the original template)?
DOP-PCR >> MDA > MALBAC > NlaIII-based method, PTA
- Homogeneous amplification (uniformity)?
PTA > MALBAC, NlaIII-based method > MDA > DOP-PCR

Overview of single cell -omics



Single cell epigenomic sequencing

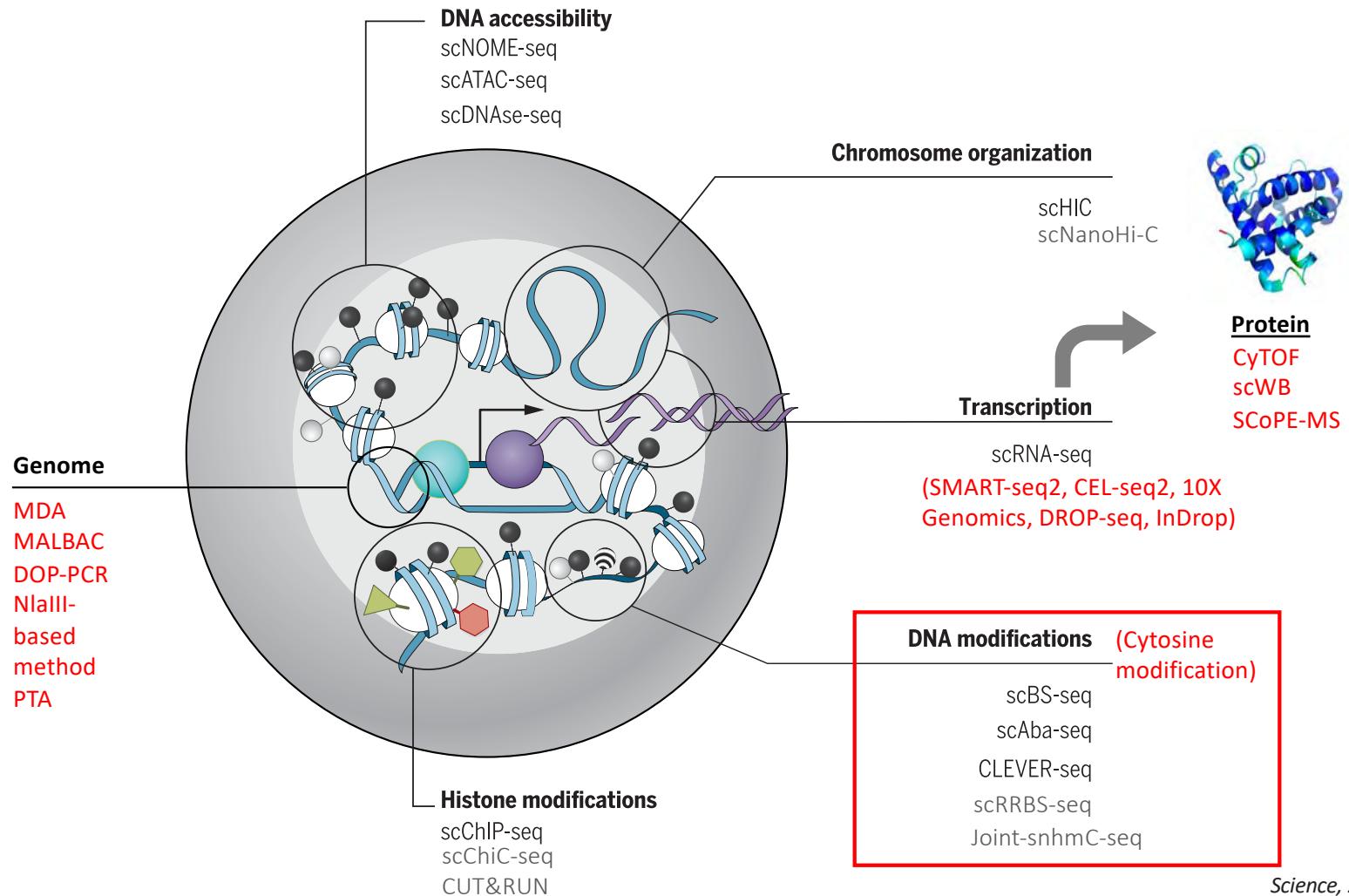
Why epigenomics?



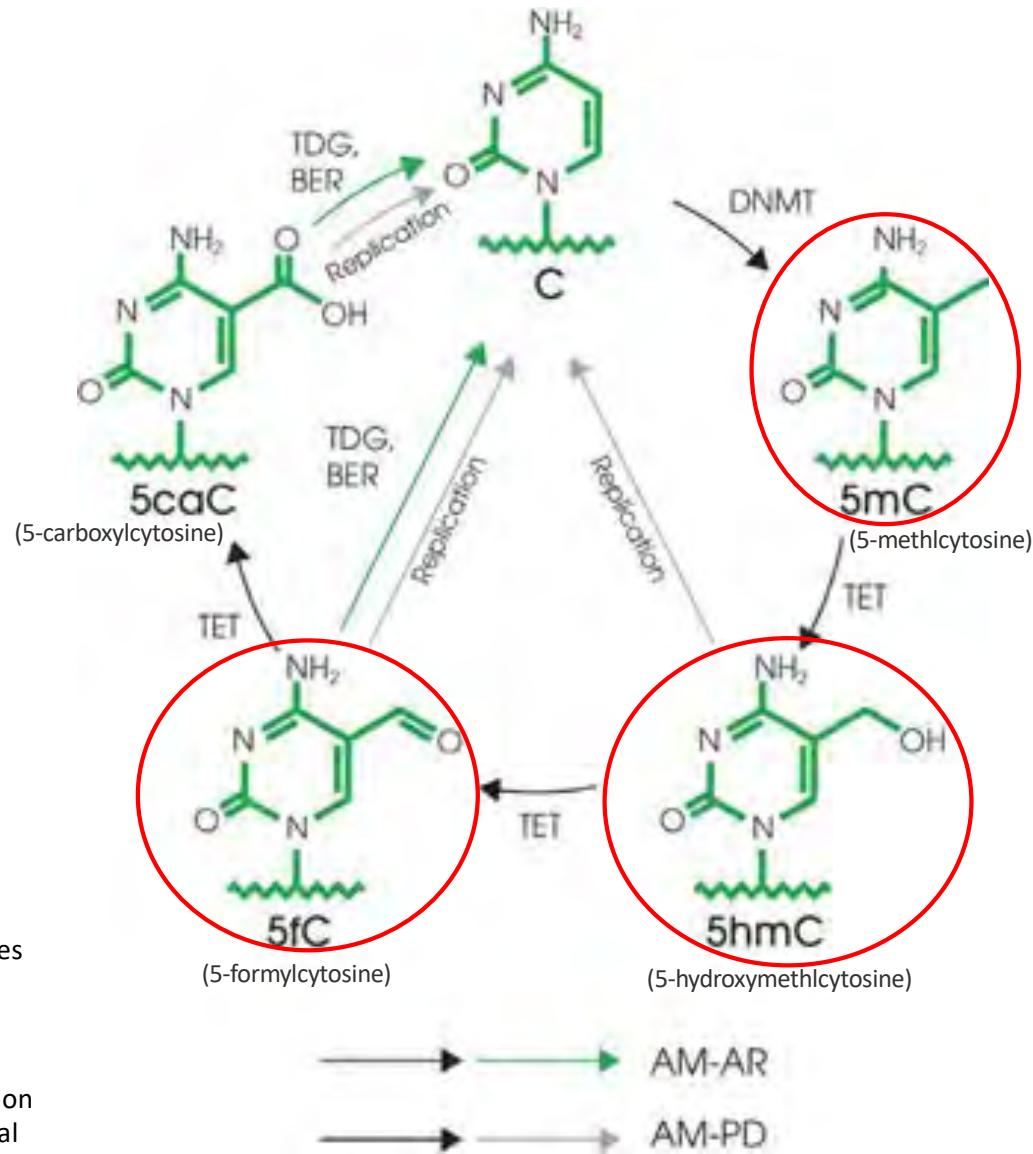
Histone acetylation: opening of the chromatin mass & the onset of transcription

DNA Methylation: condenses chromatin & accompanies transcriptional inhibition

Overview of single cell -omics



The cycle of DNA (de)methylation



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

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

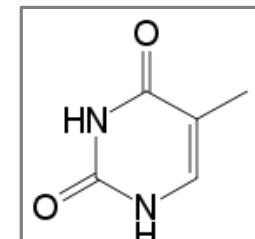
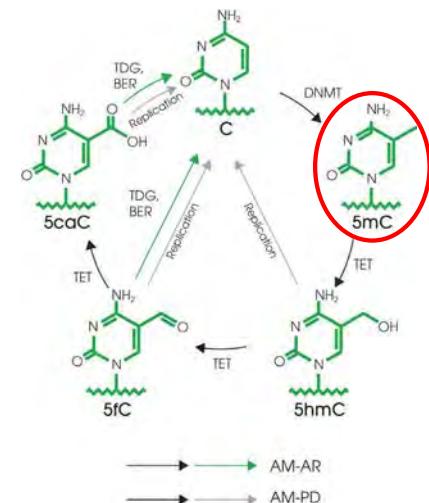
Step 3

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

Fragmented Genomic DNA Samples → Cytosine → $\text{NaHSO}_3, \text{pH } 5.0$ → 5-sulfocytosine → $+ \text{H}_2\text{O}, - \text{NH}_3$ → 5-hydroxycytosine → OH → $+ \text{NaHSO}_3$ → Uracil

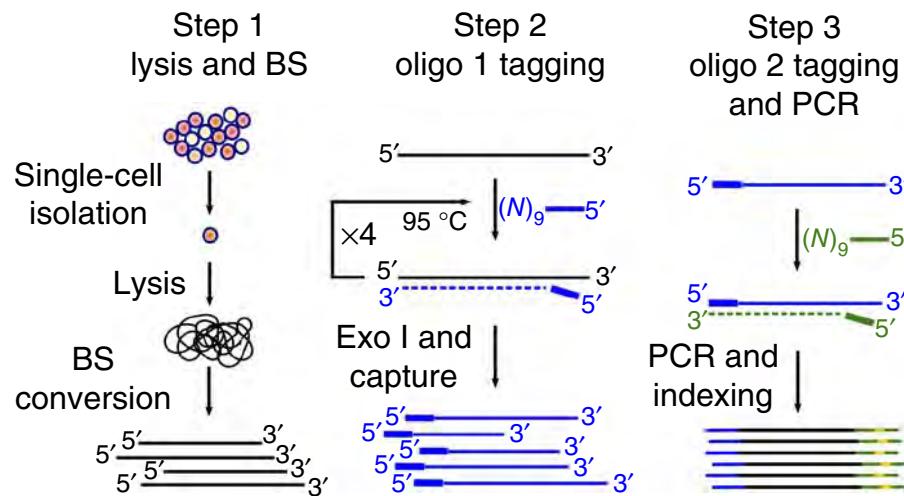
5-Methylcytosine (5-mC) → $\text{NaHSO}_3, \text{pH } 5.0$ (X) → Not converted

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



Single cell bisulfite sequencing (scBS-seq)

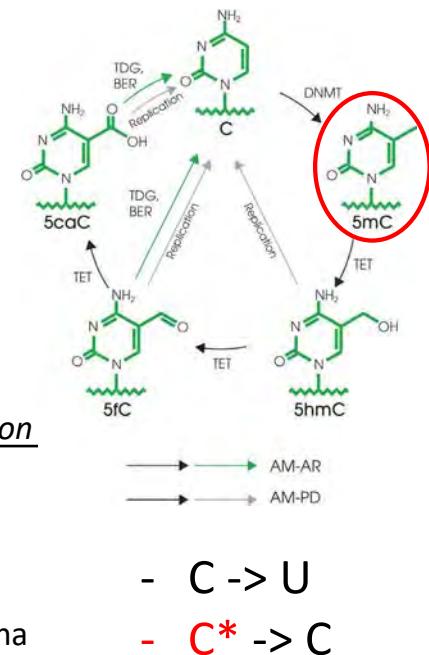
a



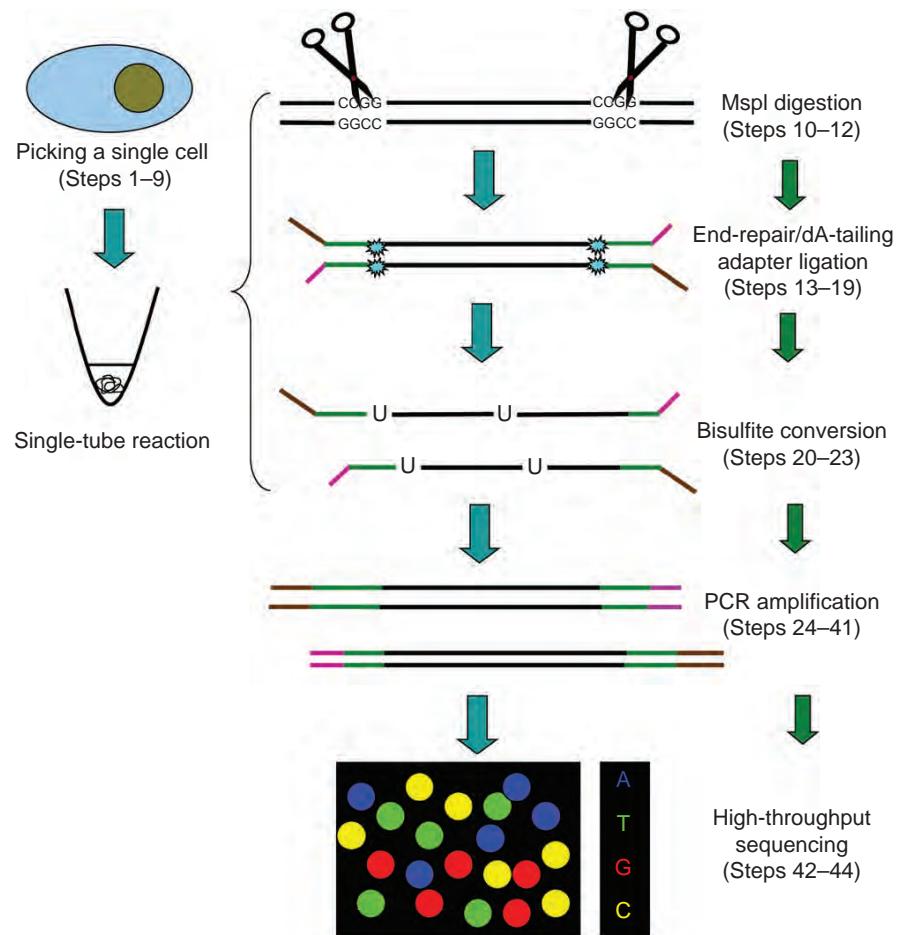
Step 1: bisulfite treatment -> DNA fragmentation & conversion of unmethylated cytosines to thymine

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

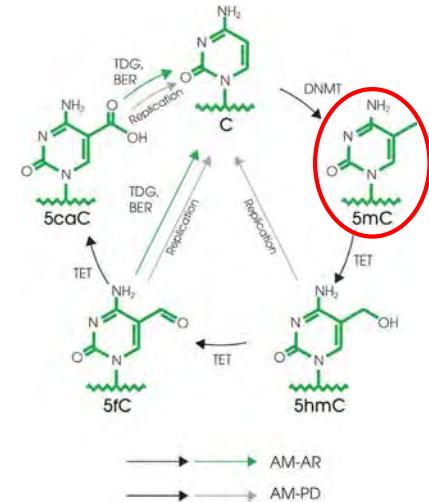
Step 3: After capturing the tagged strands, a second adaptor is similarly integrated, and PCR amplification is performed with indexed primers



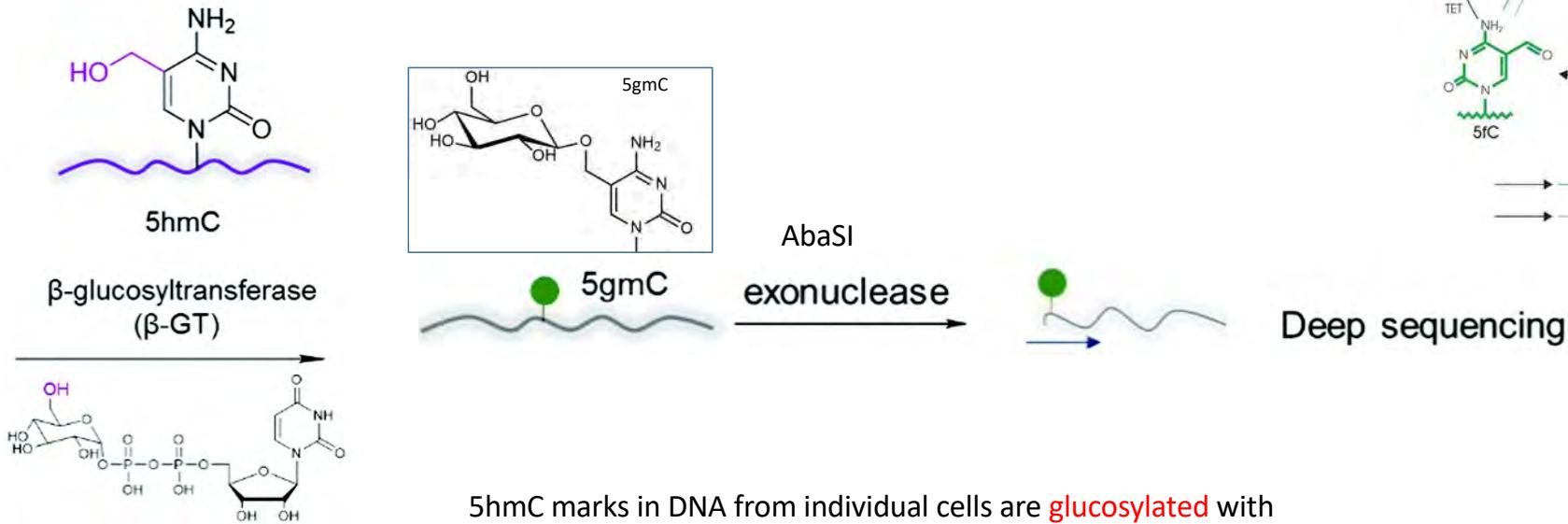
scRRBS-seq: single-cell reduced-representation bisulfite sequencing



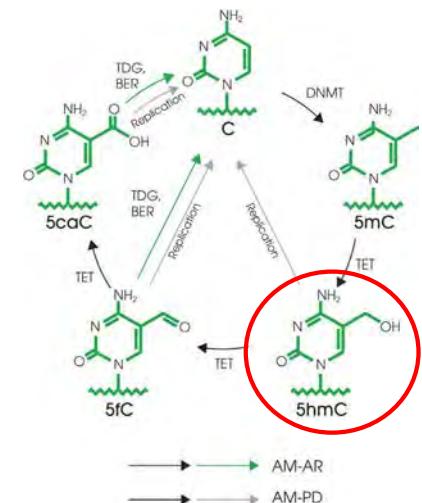
Mainly focus on the **CpG island** (related to gene expression regulation -> methylation of "C" in CpG island silences gene expression)

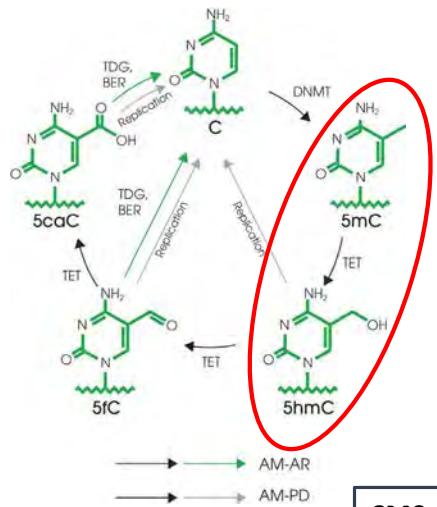


scAba-seq : single-cell 5hmC sequencing



5hmC marks in DNA from individual cells are **glucosylated** with **T4 phage- 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.

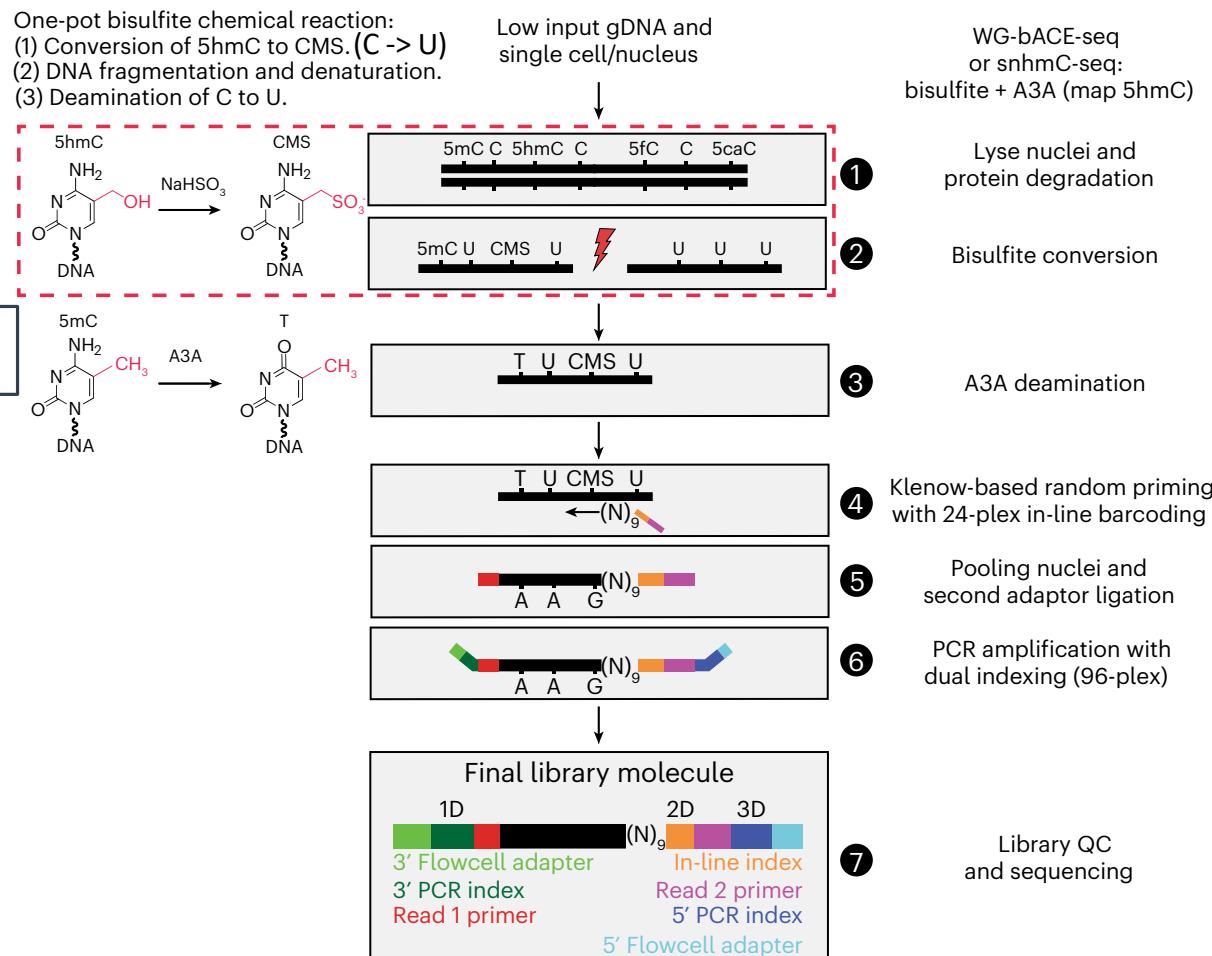




joint single-nucleus (hydroxy)methylcytosine sequencing (Joint-snhmC-seq)

a scalable and quantitative approach that simultaneously profiles 5hmC and true 5mC in single cells

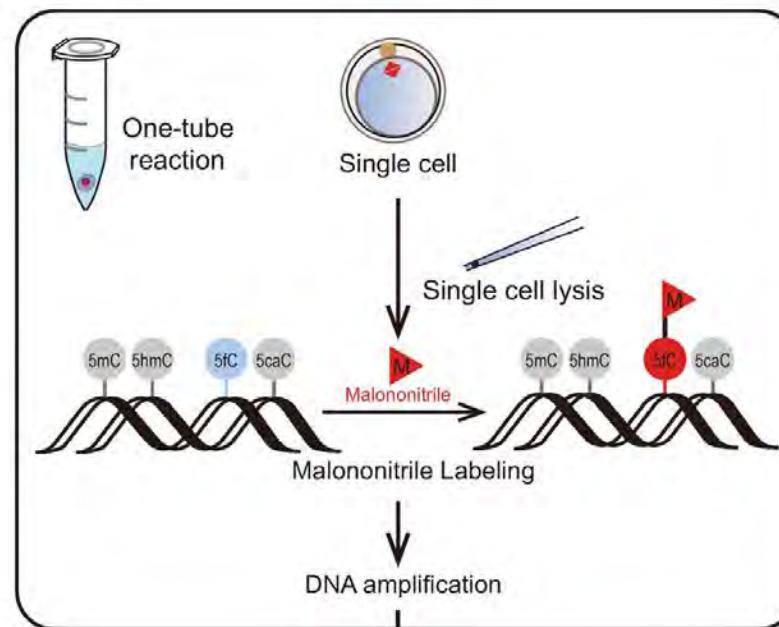
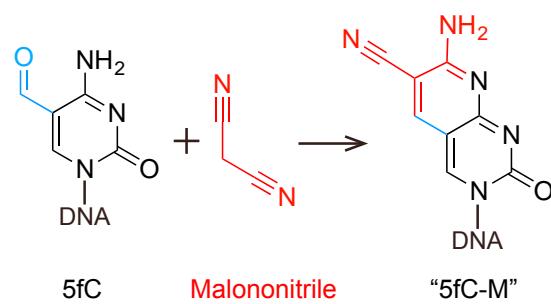
CMS: cytosine-5-methylenesulfonate
A3A: APOBEC3A deaminase



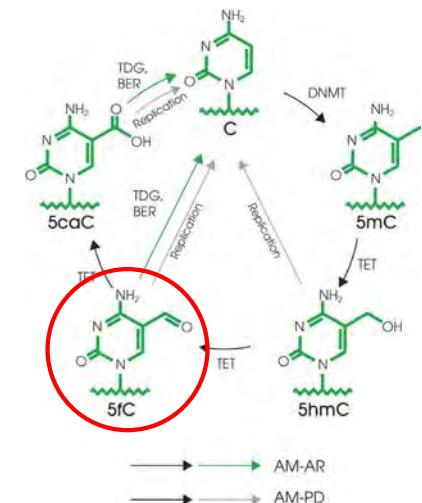
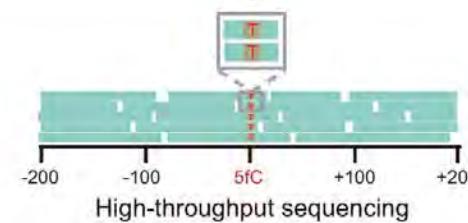
CLEVER-seq

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

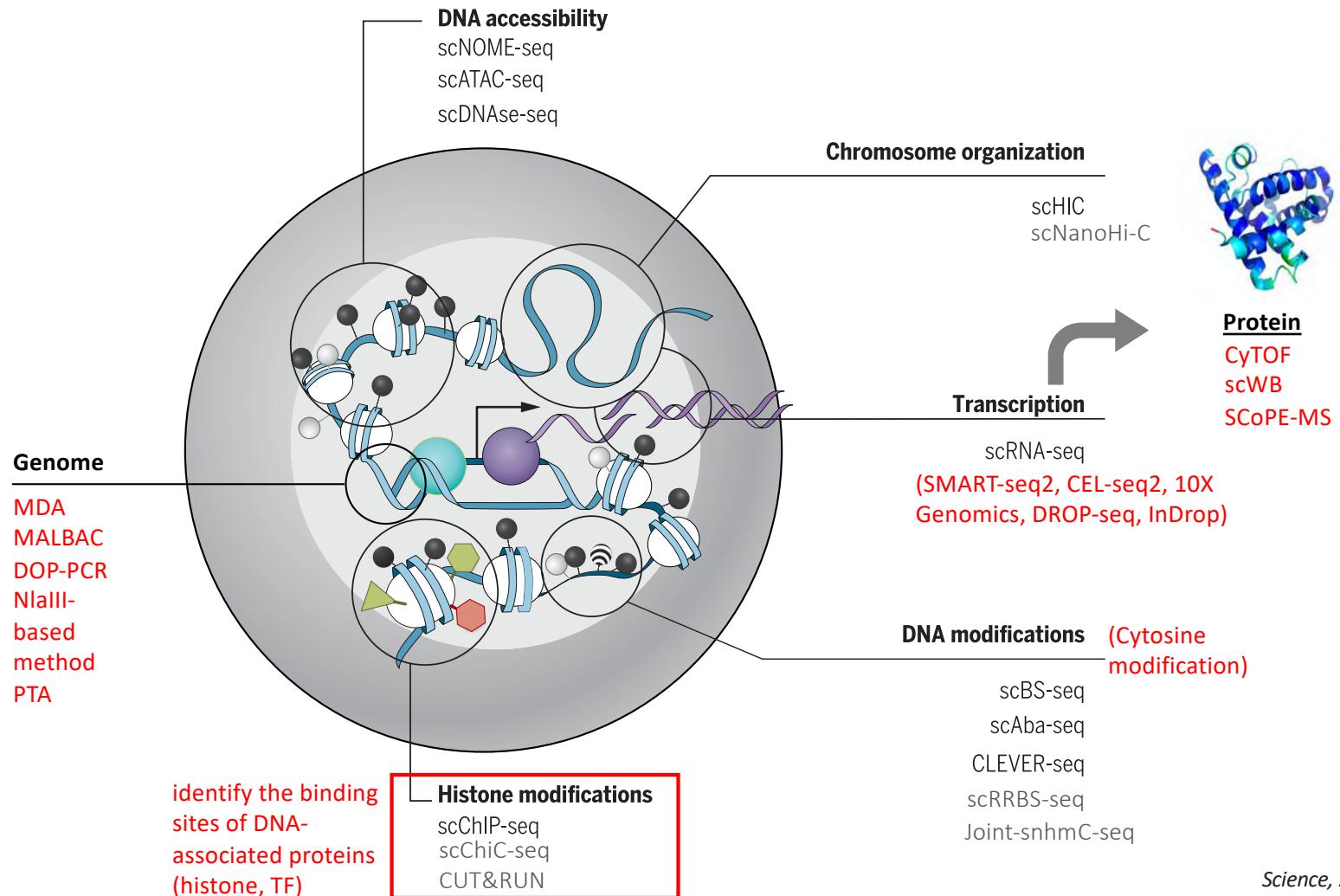
- A single-cell, single-base resolution whole-genome **5fC**-sequencing technology



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



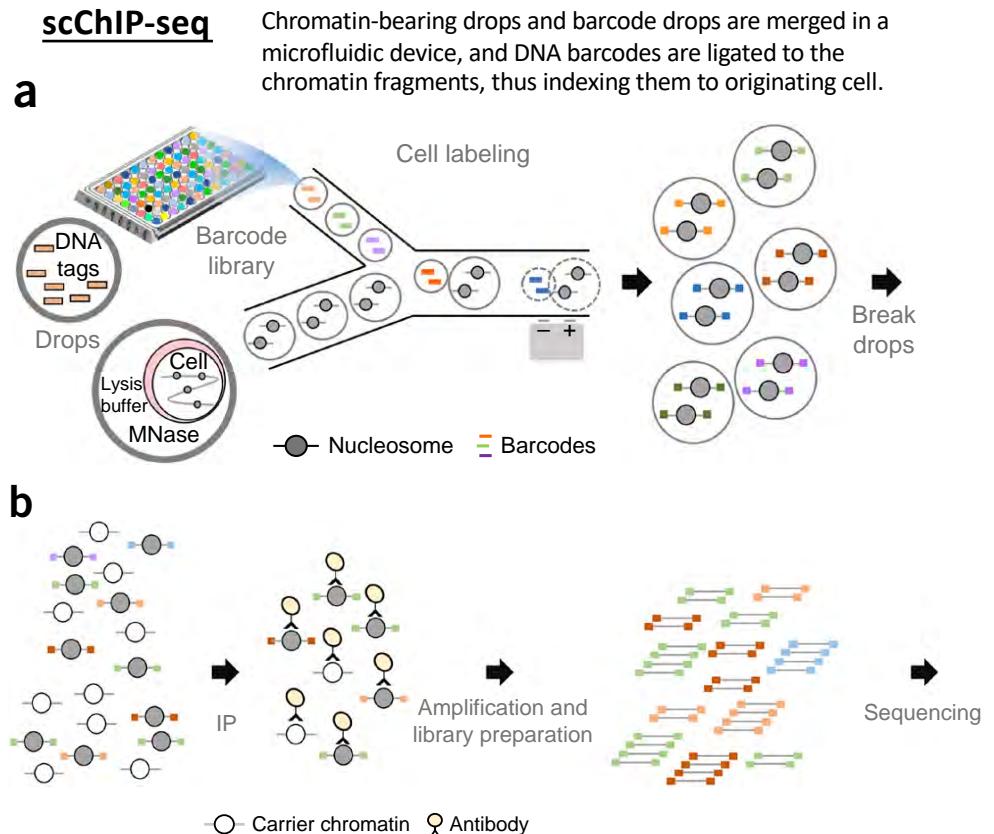
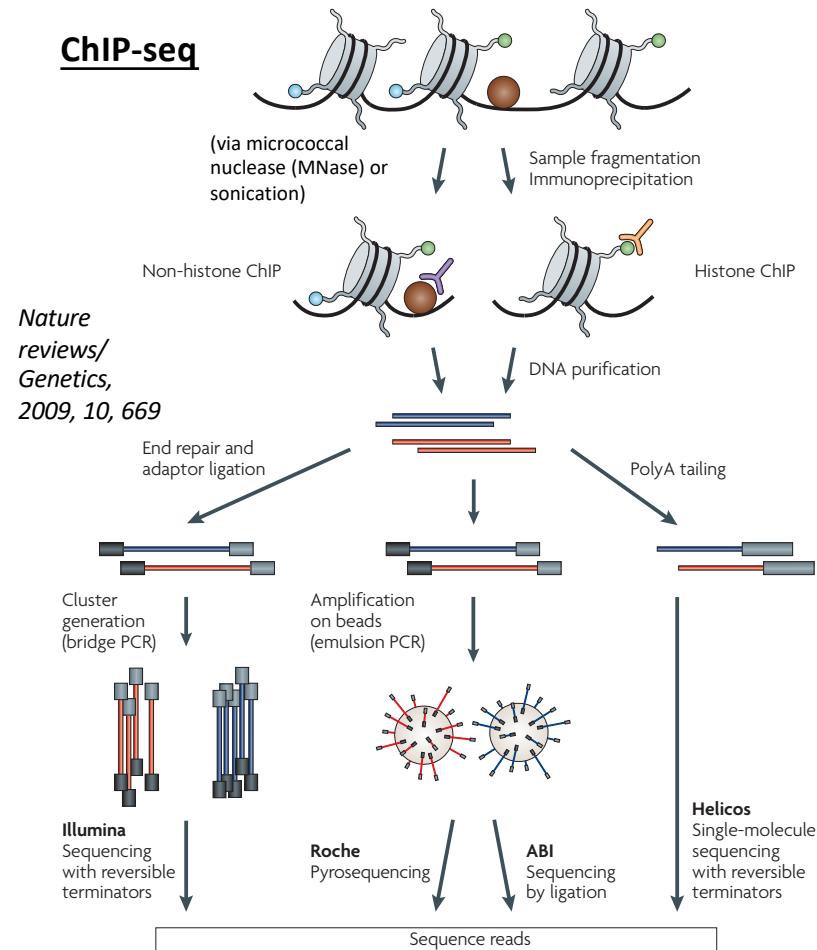
Overview of single cell -omics



Single cell sequencing for histone modifications

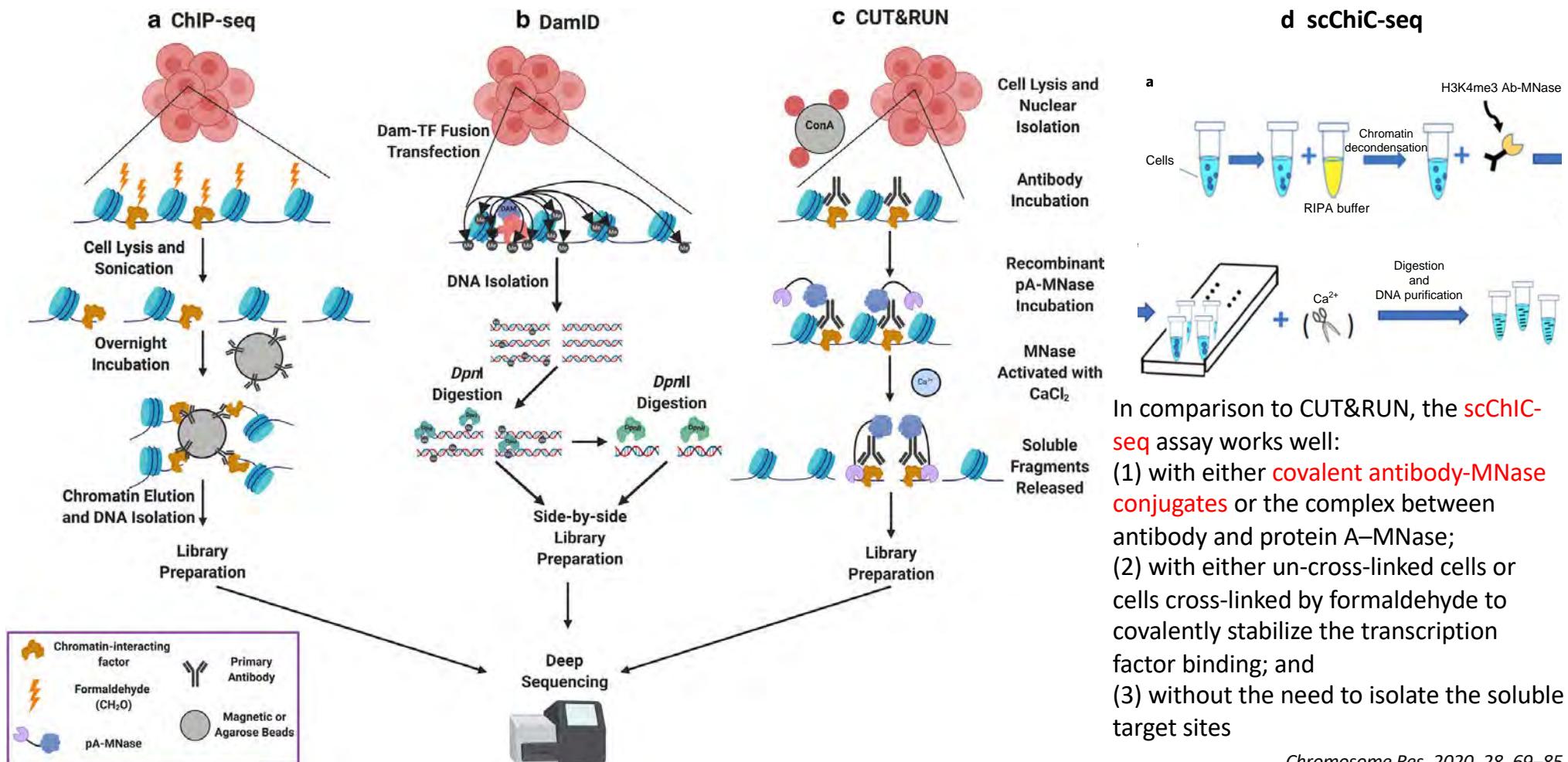
Single cell chromatin immunoprecipitation (ChIP) sequencing

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



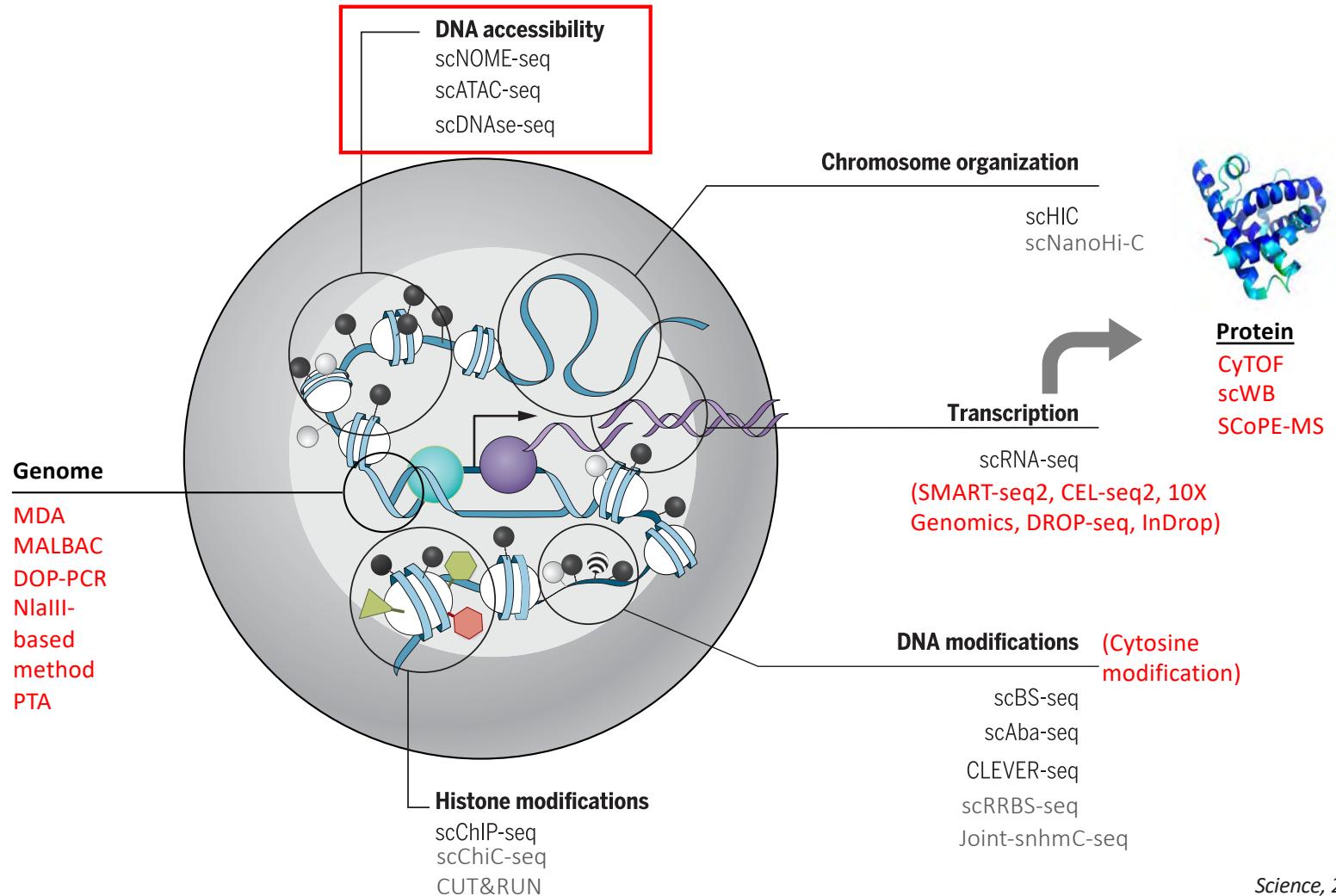
Nature Biotechnology, 2015, 33, 1165

Method comparison for histone modifications



*Chromosome Res, 2020, 28, 69–85
Nature Methods, 2019, 16, 323–325*

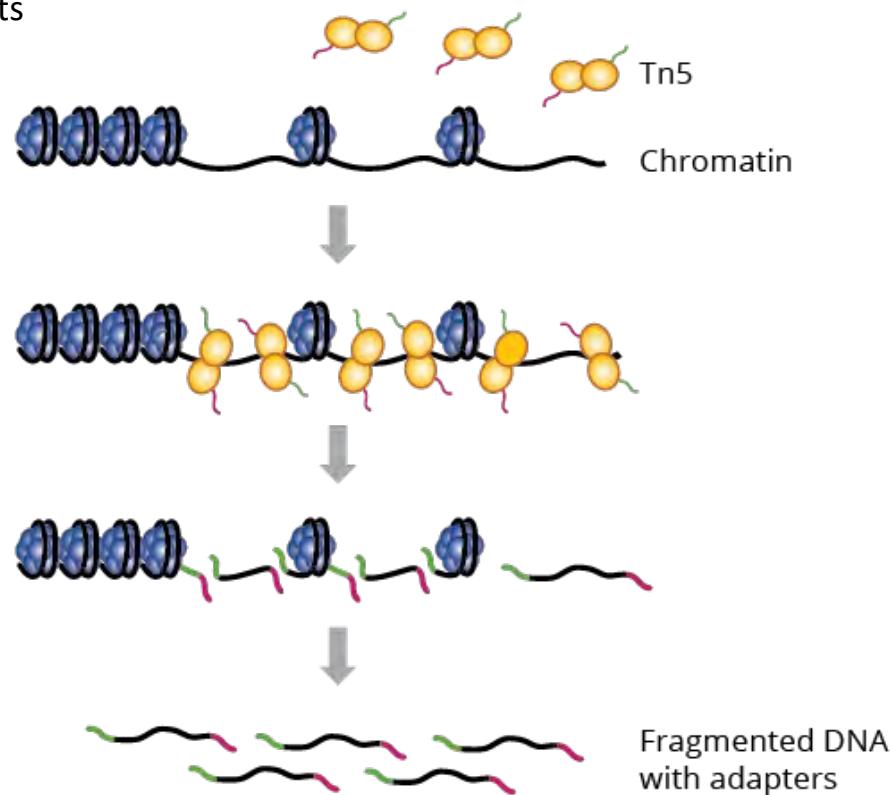
Overview of single cell -omics



Science, 2017, 358, 69–75

scATAC-seq: Assay for transposase-accessible chromatin

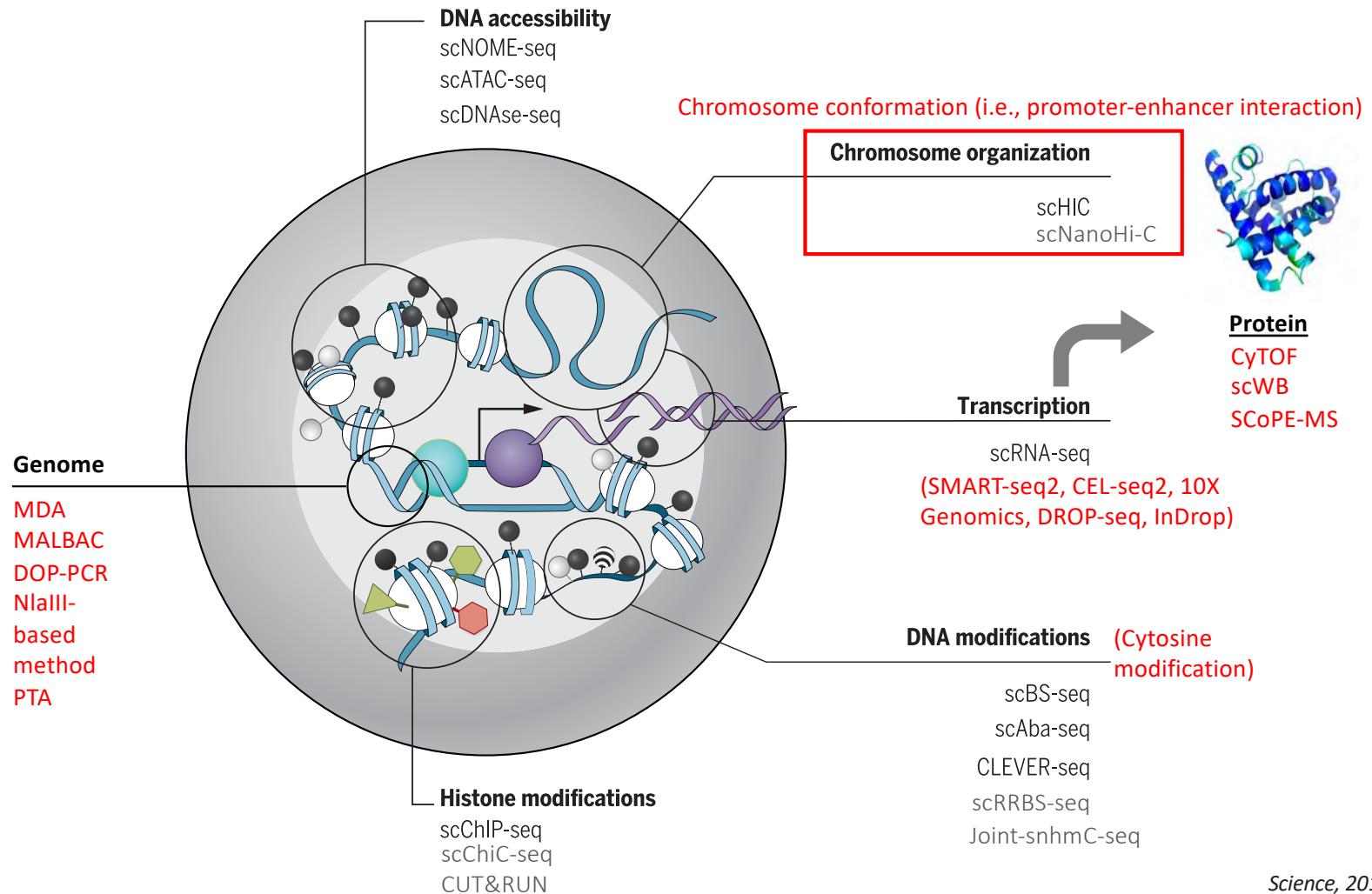
- scATACseq can be applied to
 - nucleosome mapping experiments
 - map transcription factor binding sites
 - map DNA methylation sites



Available at 10X Genomics

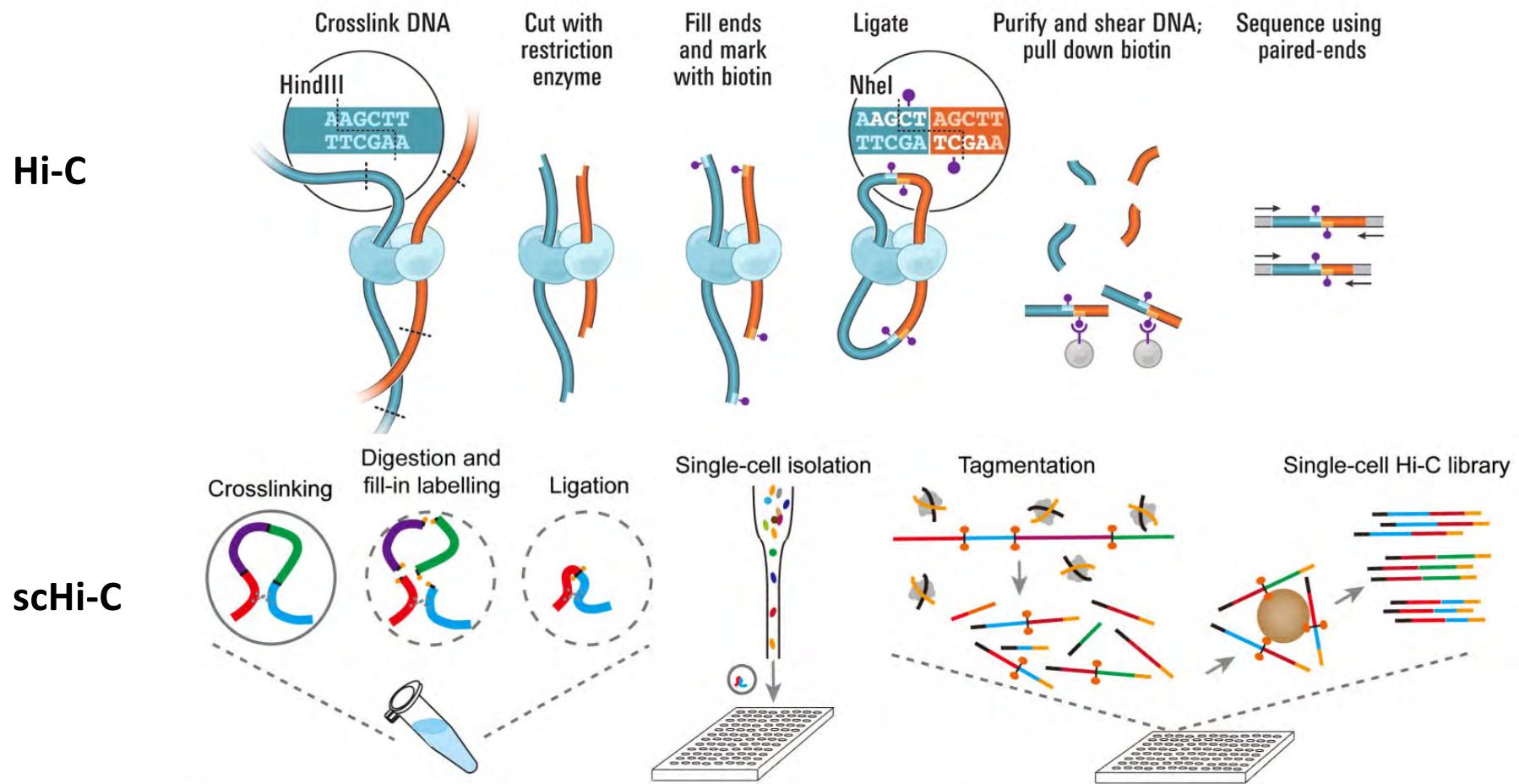
Buenrostro et al *Nat. Meth.* 2013

Overview of single cell -omics



Science, 2017, 358, 69–75

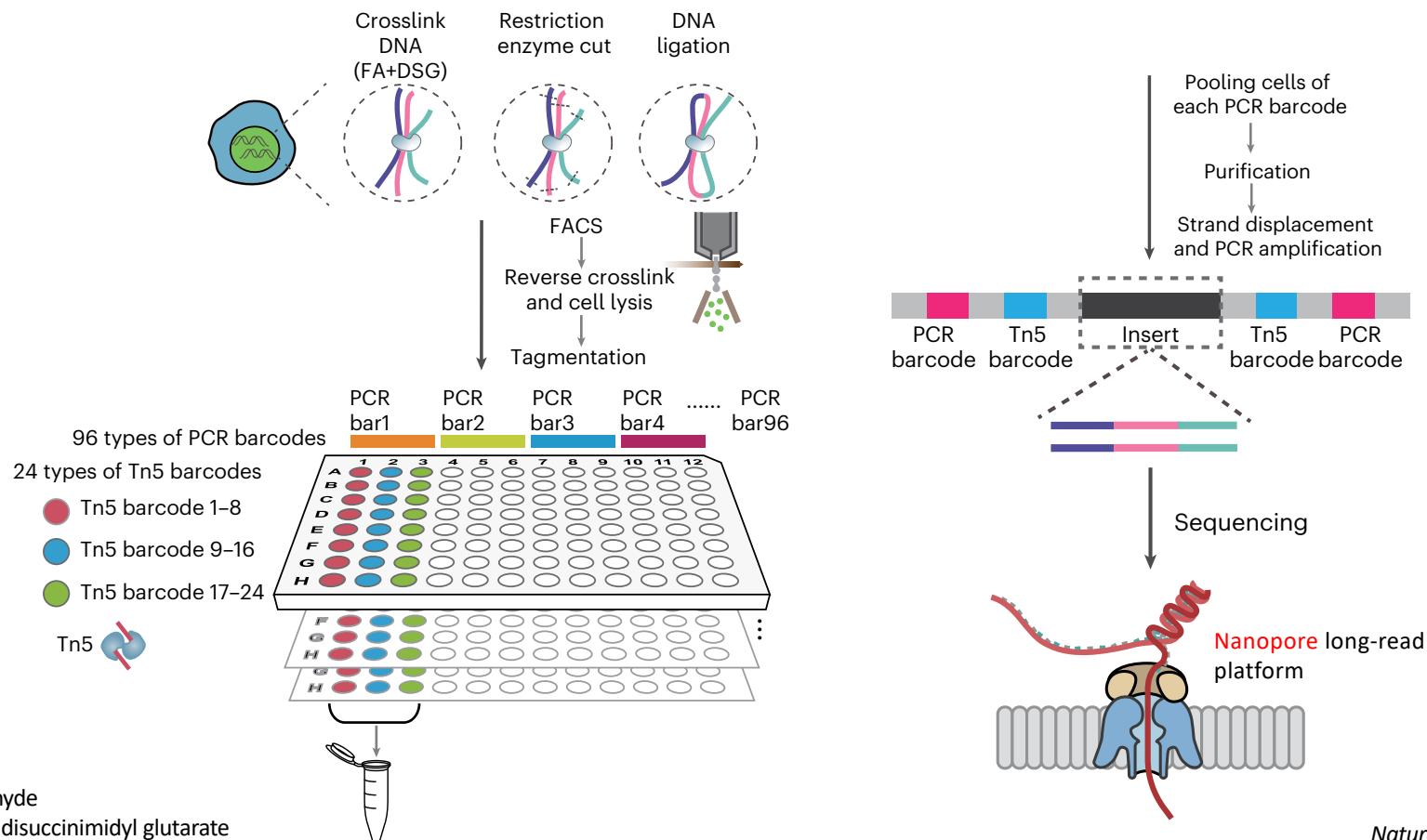
Single cell Hi-C (high-resolution chromosome conformation capture)



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

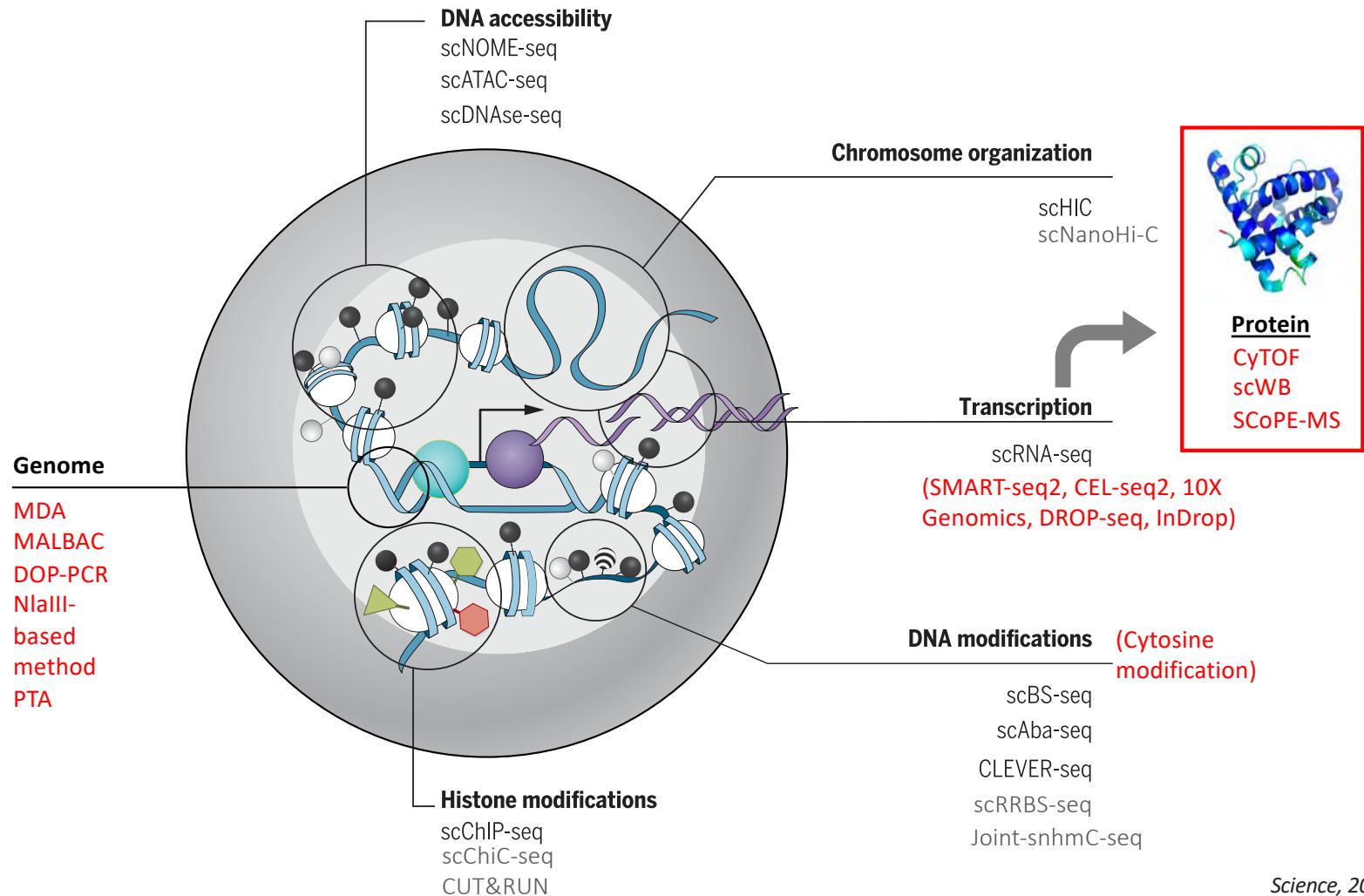
scNanoHi-C

a single-cell **long-read** concatemer sequencing method to reveal high-order chromatin structures & long-range chromosomal contacts within individual cells



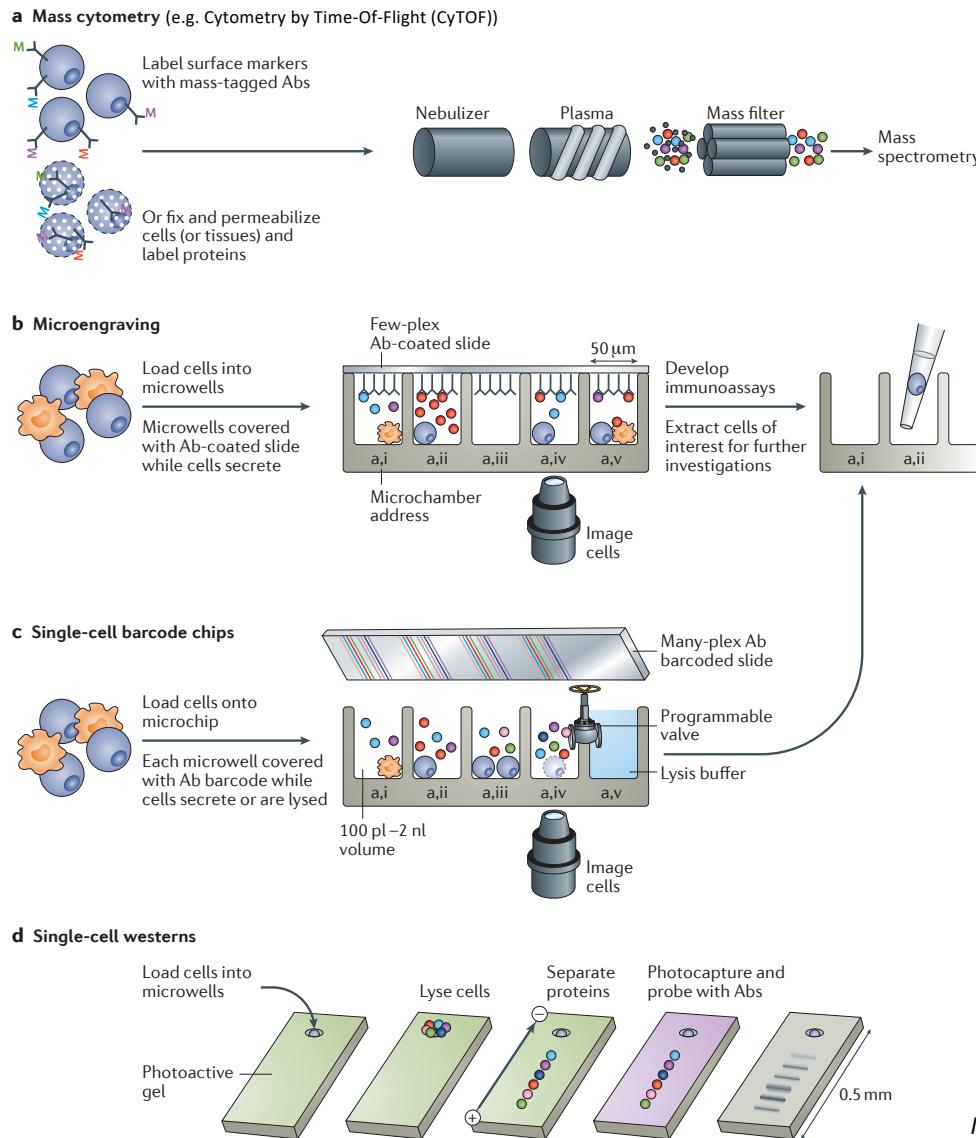
Nature Methods, 2023, 20, 1493-1505

Overview of single cell -omics



Single cell proteomics

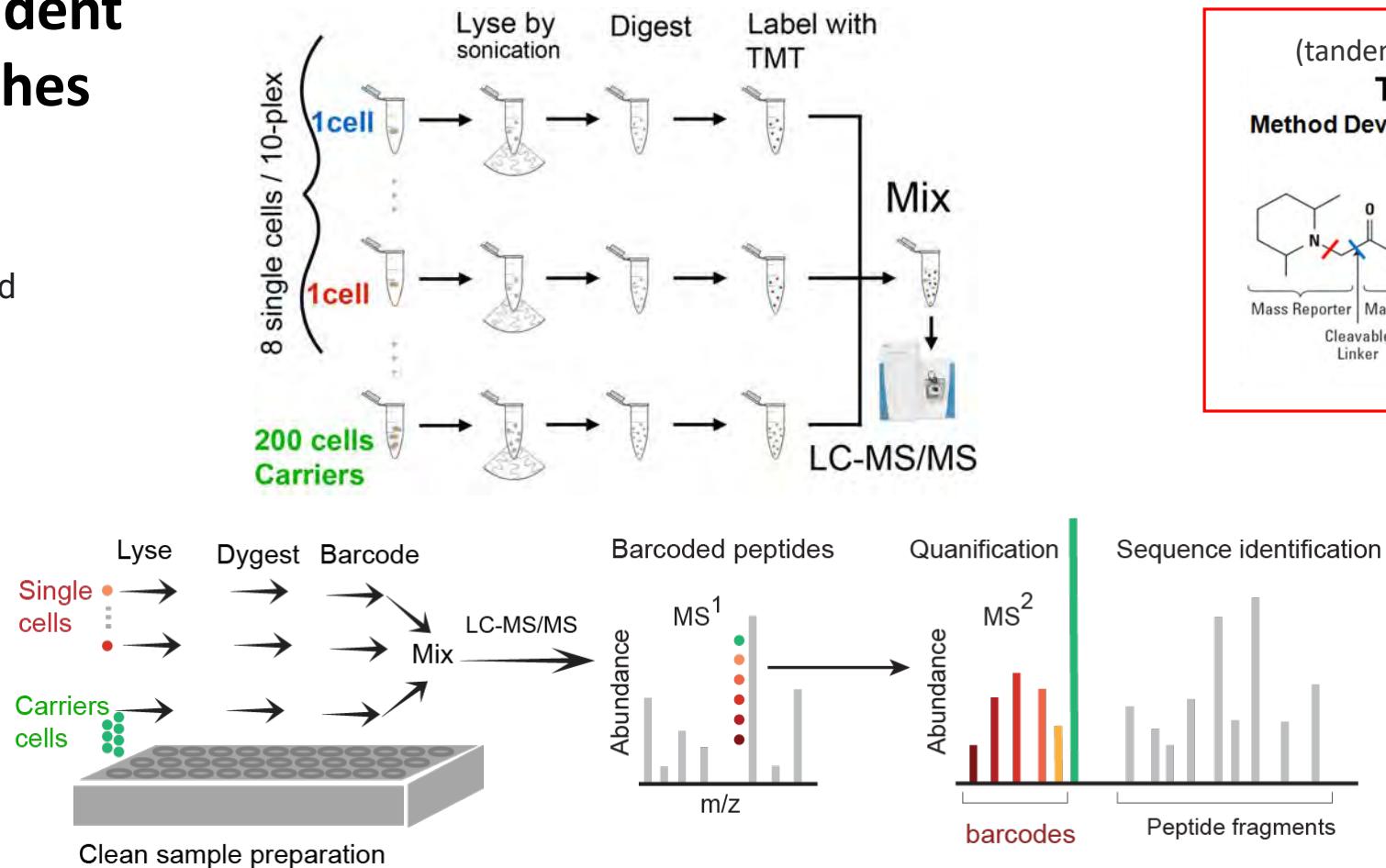
Emerging single-cell proteomics methods (antibody-dependent approaches)



Antibody-independent approaches

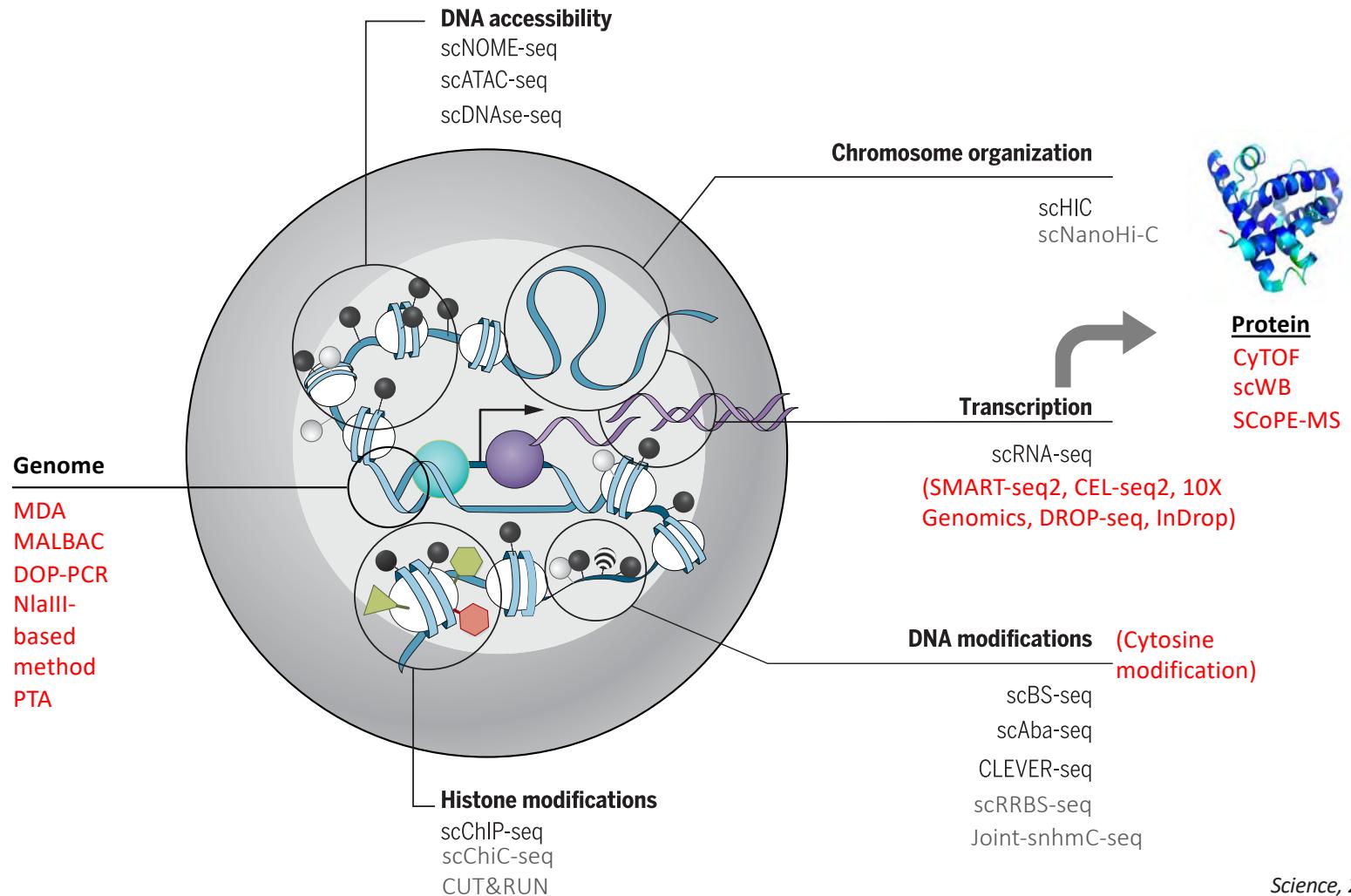
-> Mass spectrometry (Mass spec)-based method

SCoPE-MS (Single Cell ProtEomics by Mass Spectrometry)



Nikolai Slavov group
Genome Biology, 2018, 19, 161

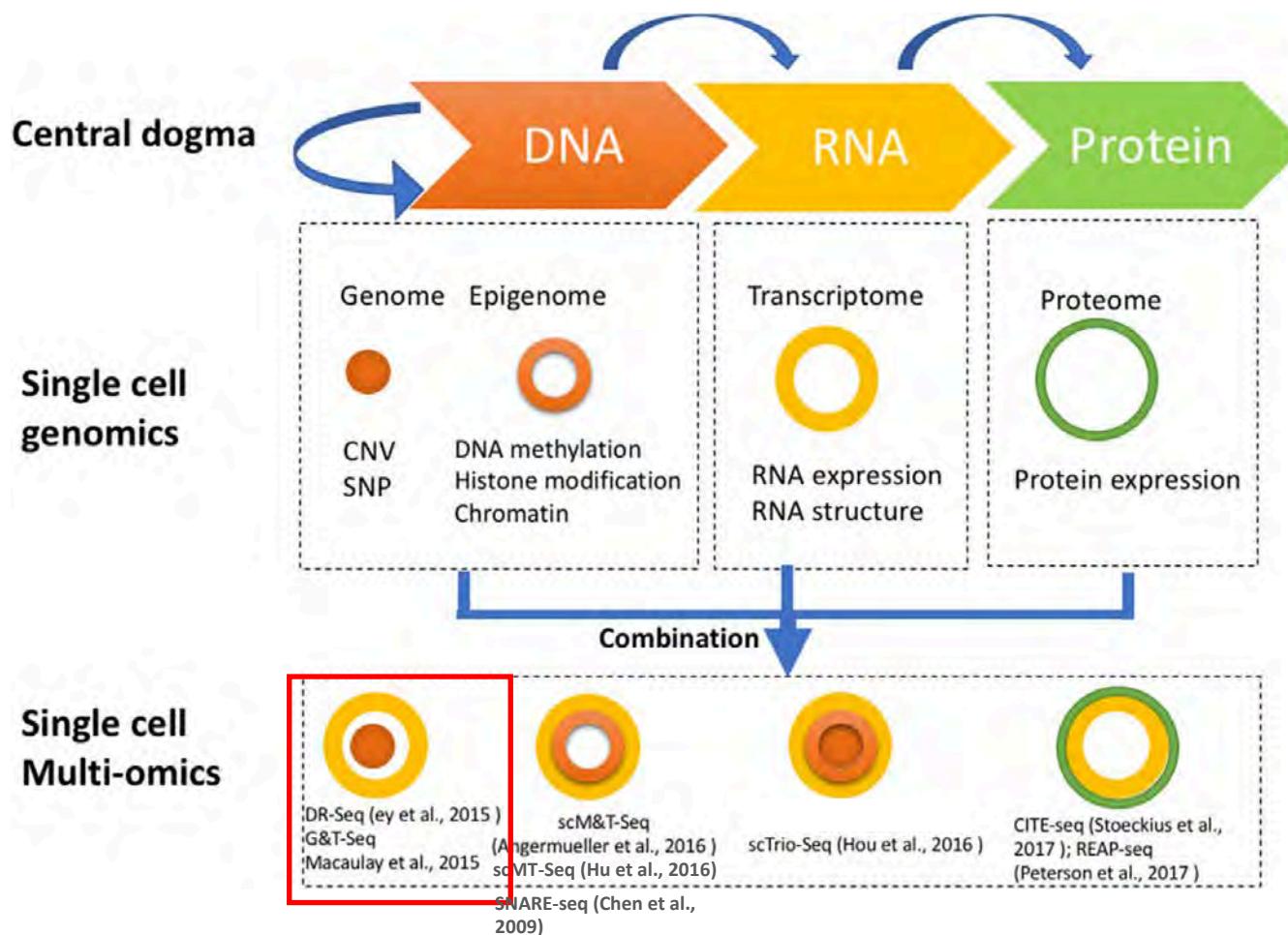
Overview of single cell -omics



Science, 2017, 358, 69–75

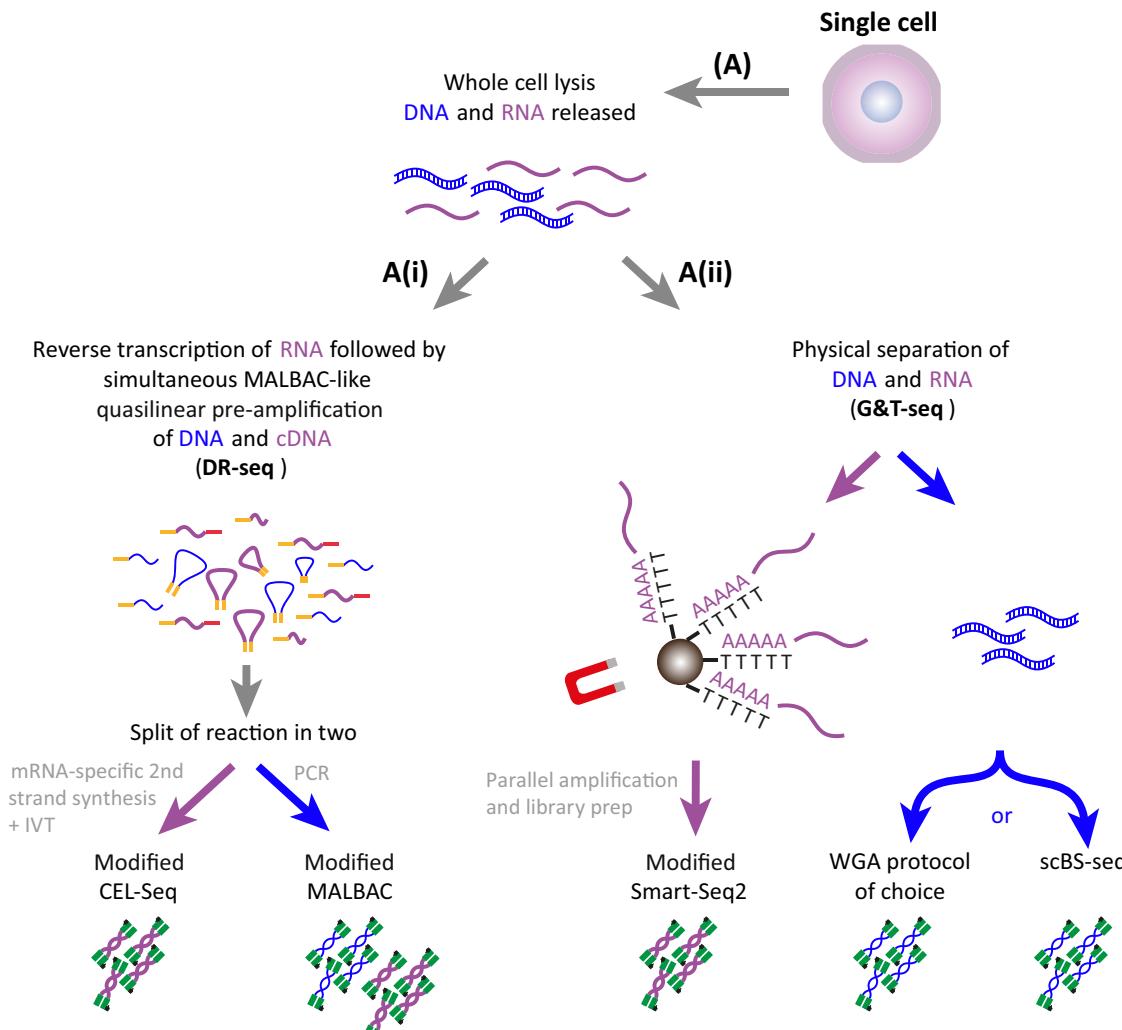
Single cell multi–omics

Strategies for multi-omics profiling of single cells

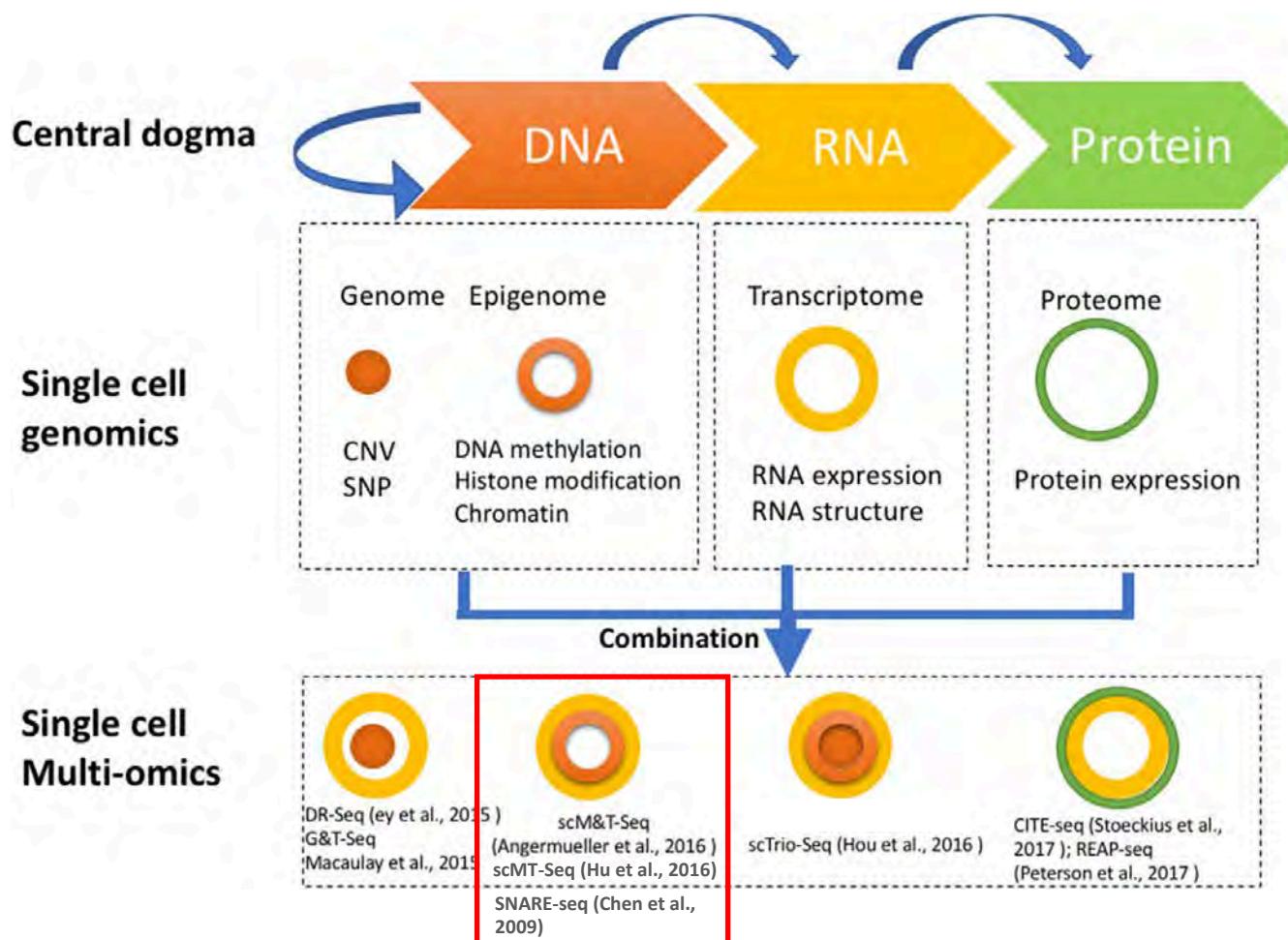


Single cell DNA- and RNA-Sequencing

Single cell DNA- and RNA-Sequencing



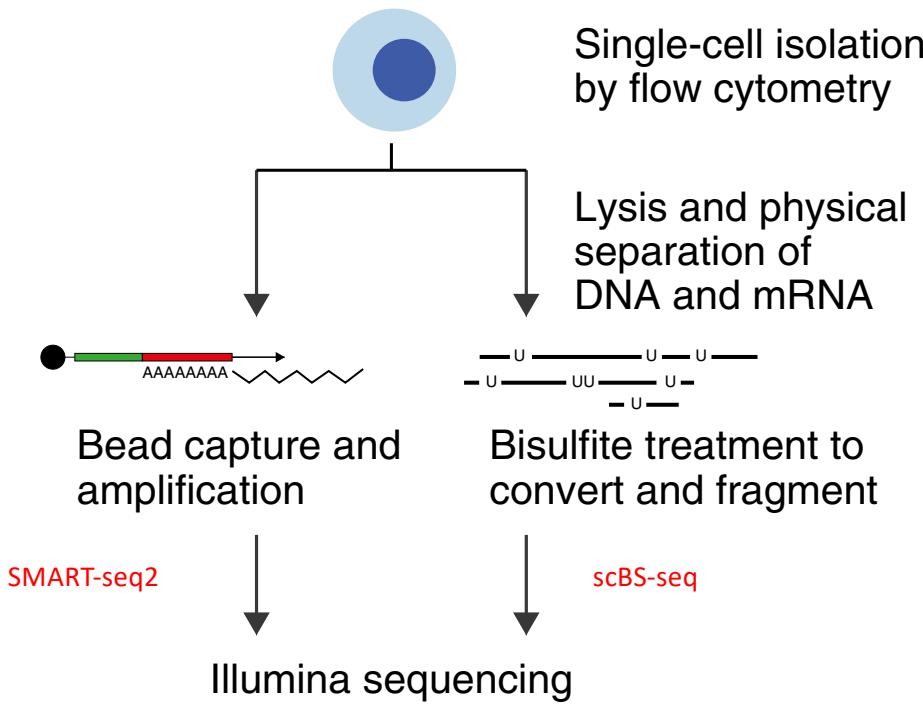
Strategies for multi-omics profiling of single cells



Single cell RNA- and methylation- Sequencing

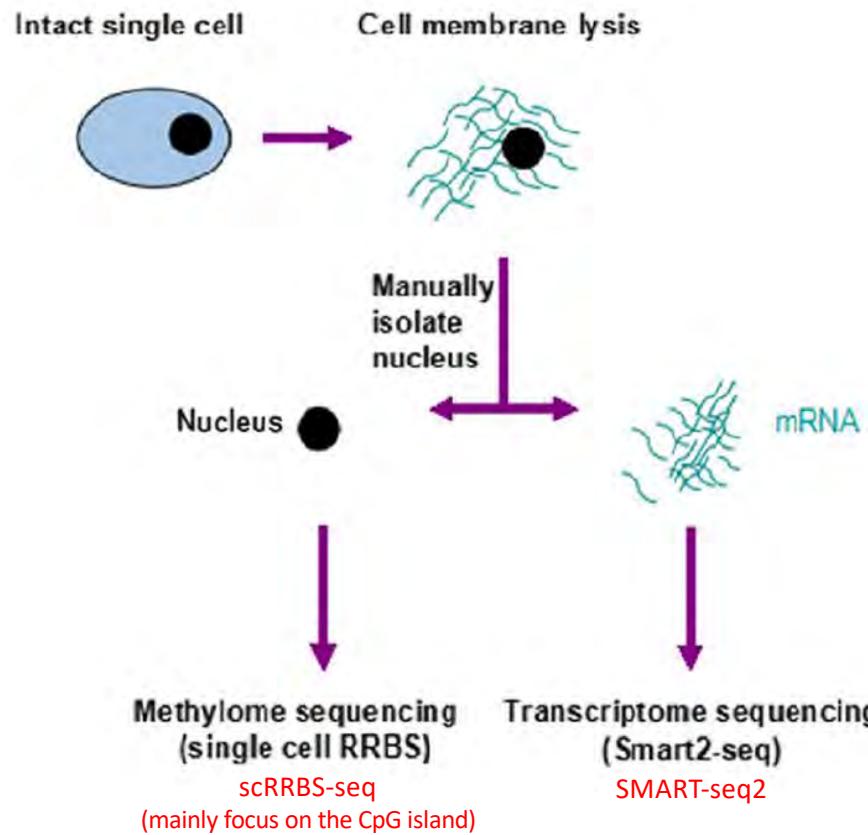
scM&T-seq

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)

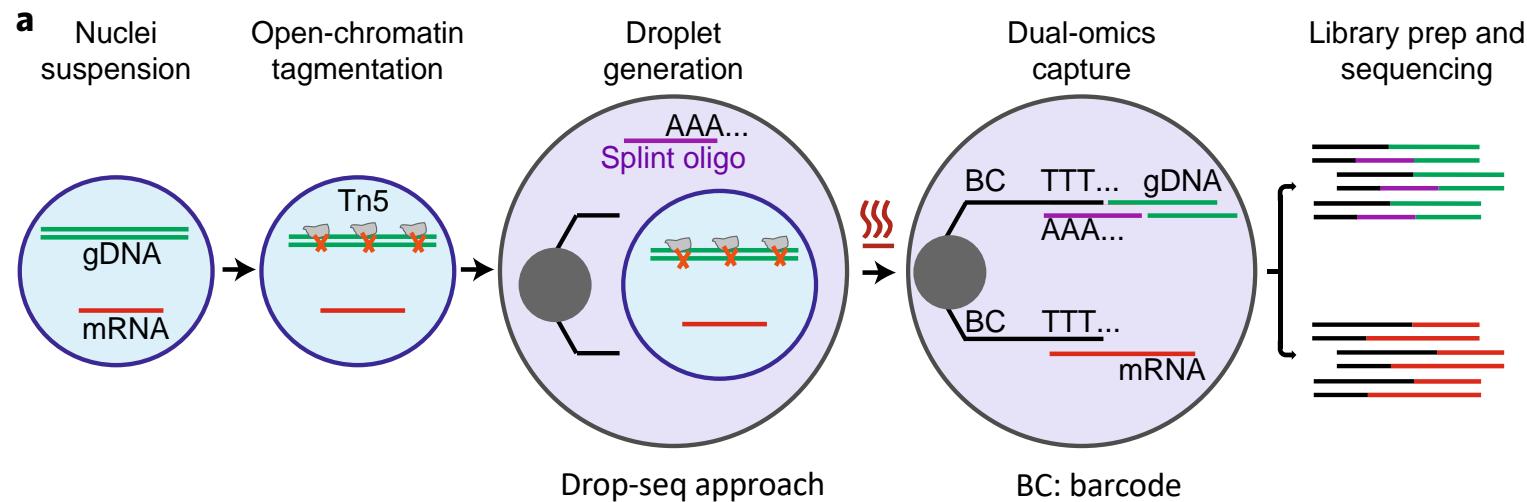
nature
biotechnology

LETTERS

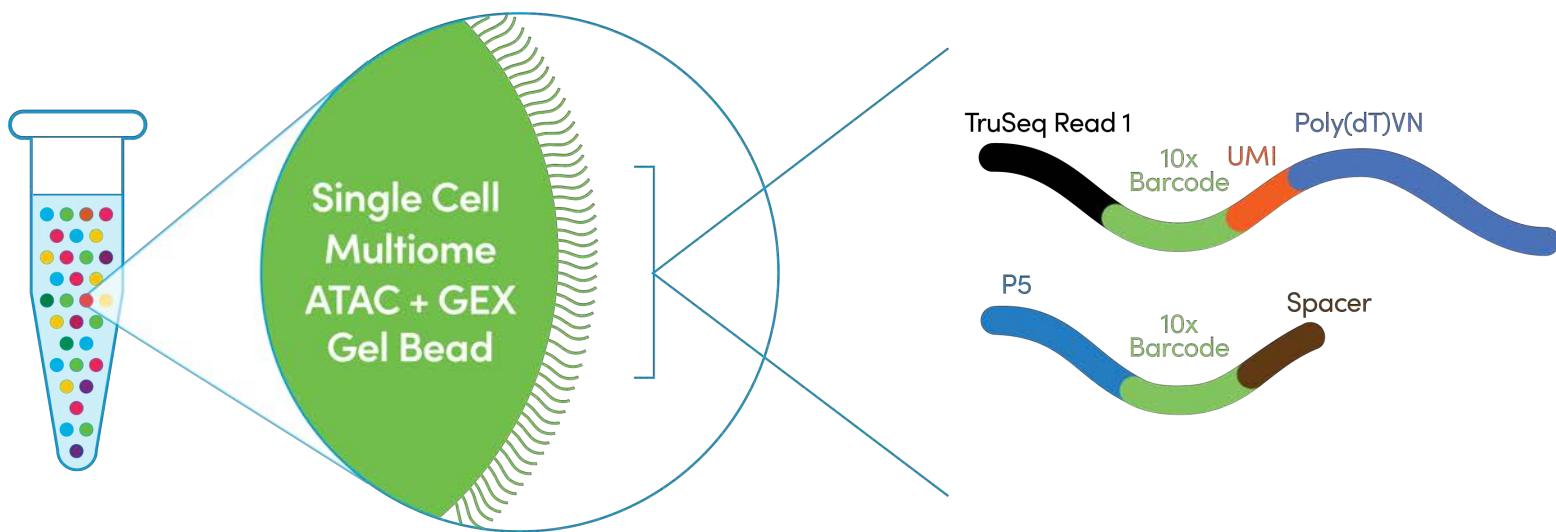
<https://doi.org/10.1038/s41587-019-0290-0>

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

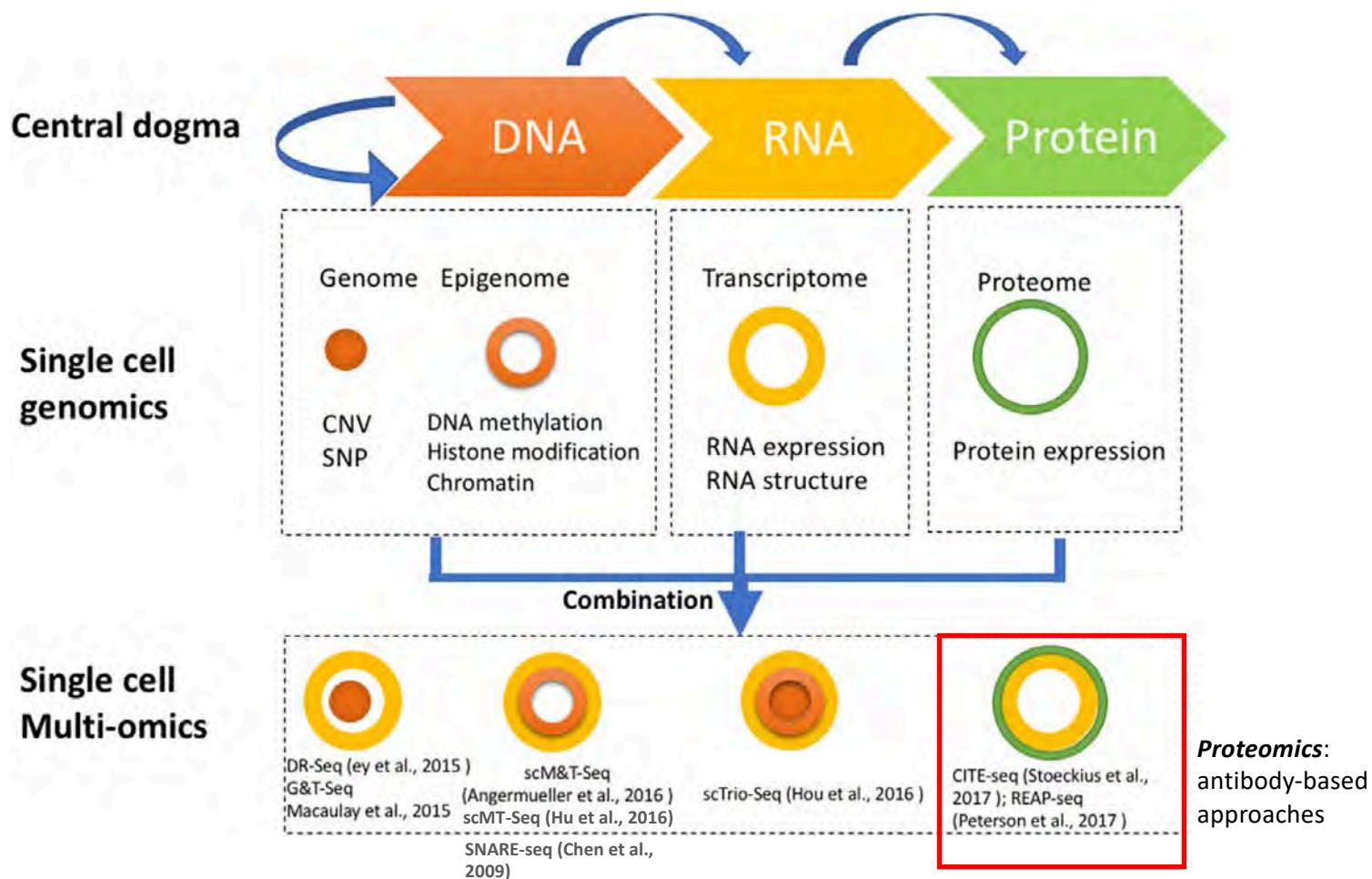
Song Chen^{ID}, Blue B. Lake^{ID} and Kun Zhang^{ID*}



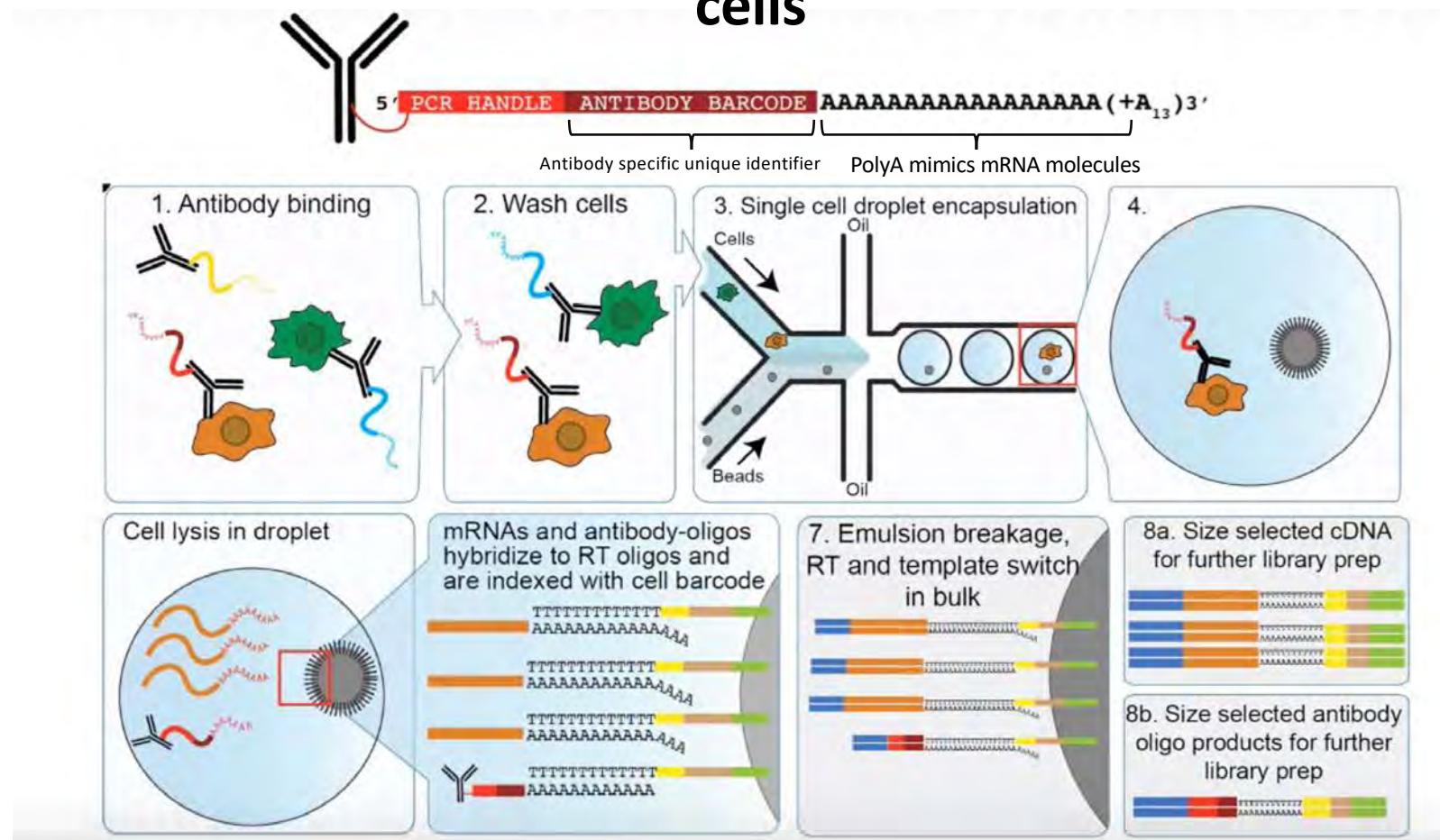
10X Single Cell Multiome (scATAC + scRNA)



Strategies for multi-omics profiling of single cells



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

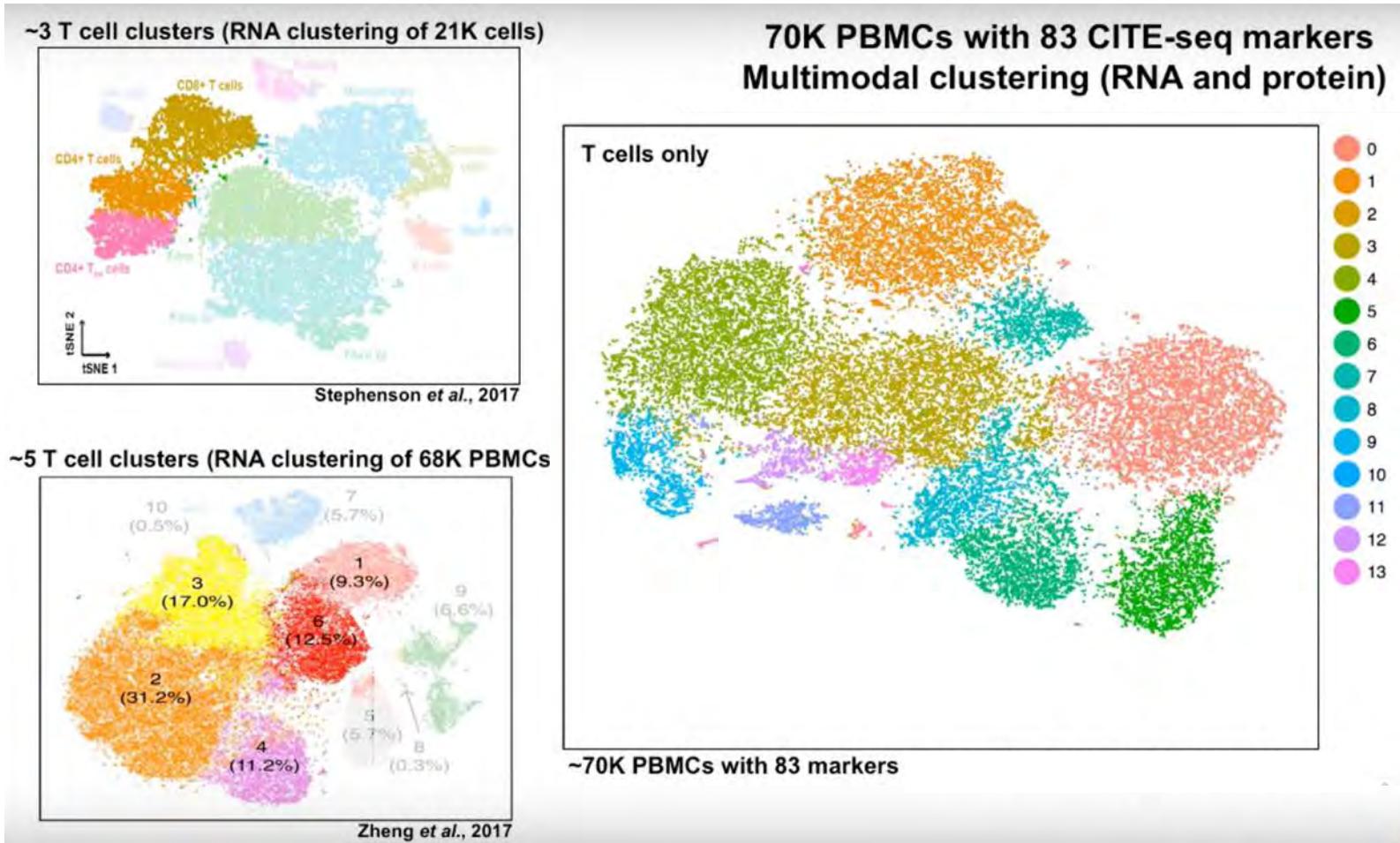


Marlon Stoeckius group

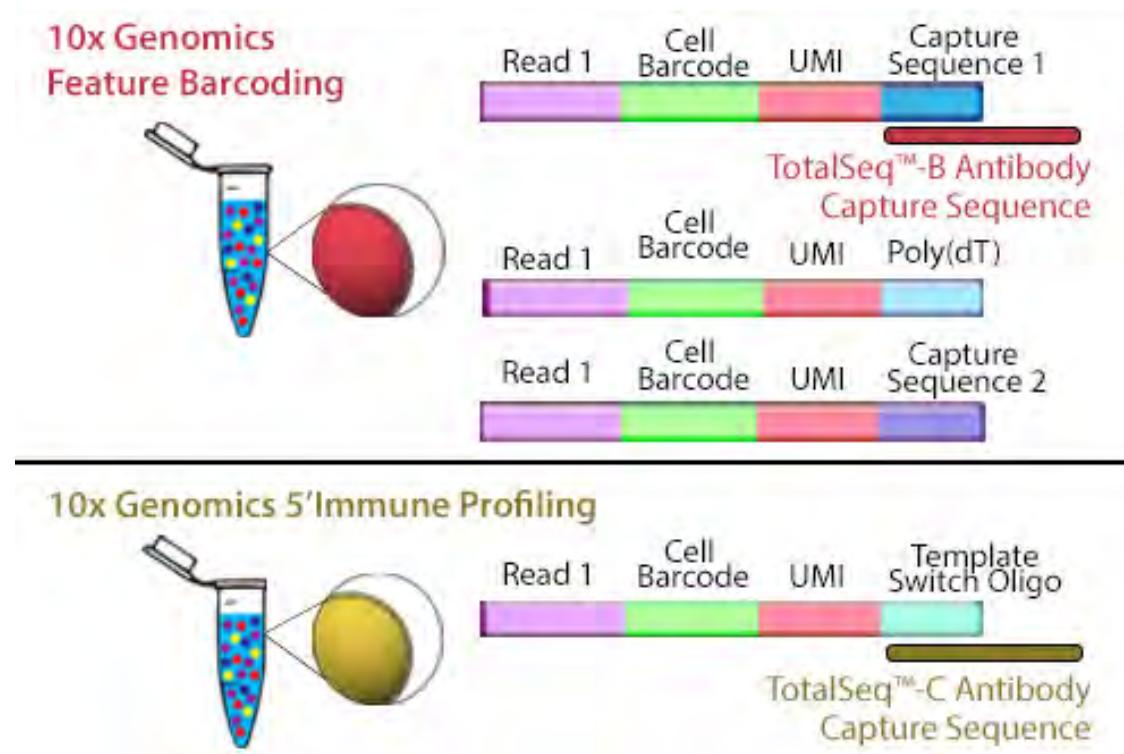
Nature Methods, 2017, 14, 865–868

Multimodal clustering resolves more T cell clusters

13 T cell clusters with distinct protein and RNA markers

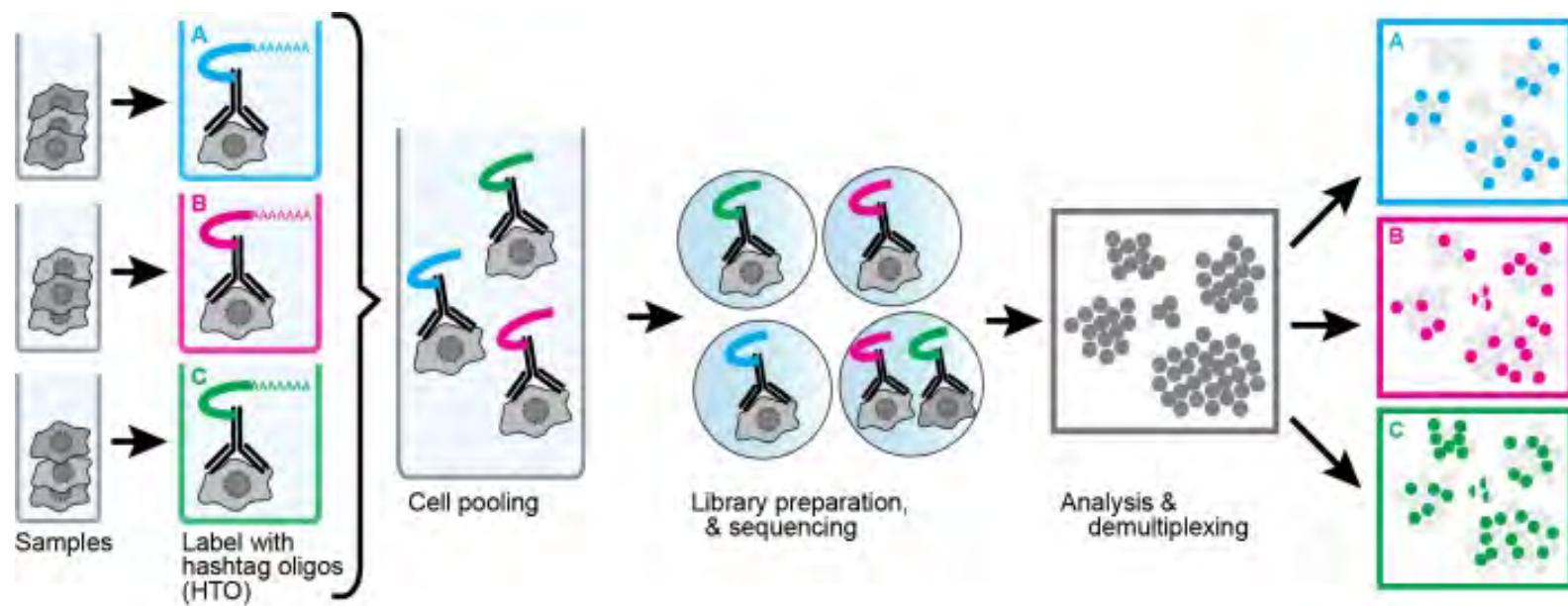


CITE-seq method is also available via 10X Genomics



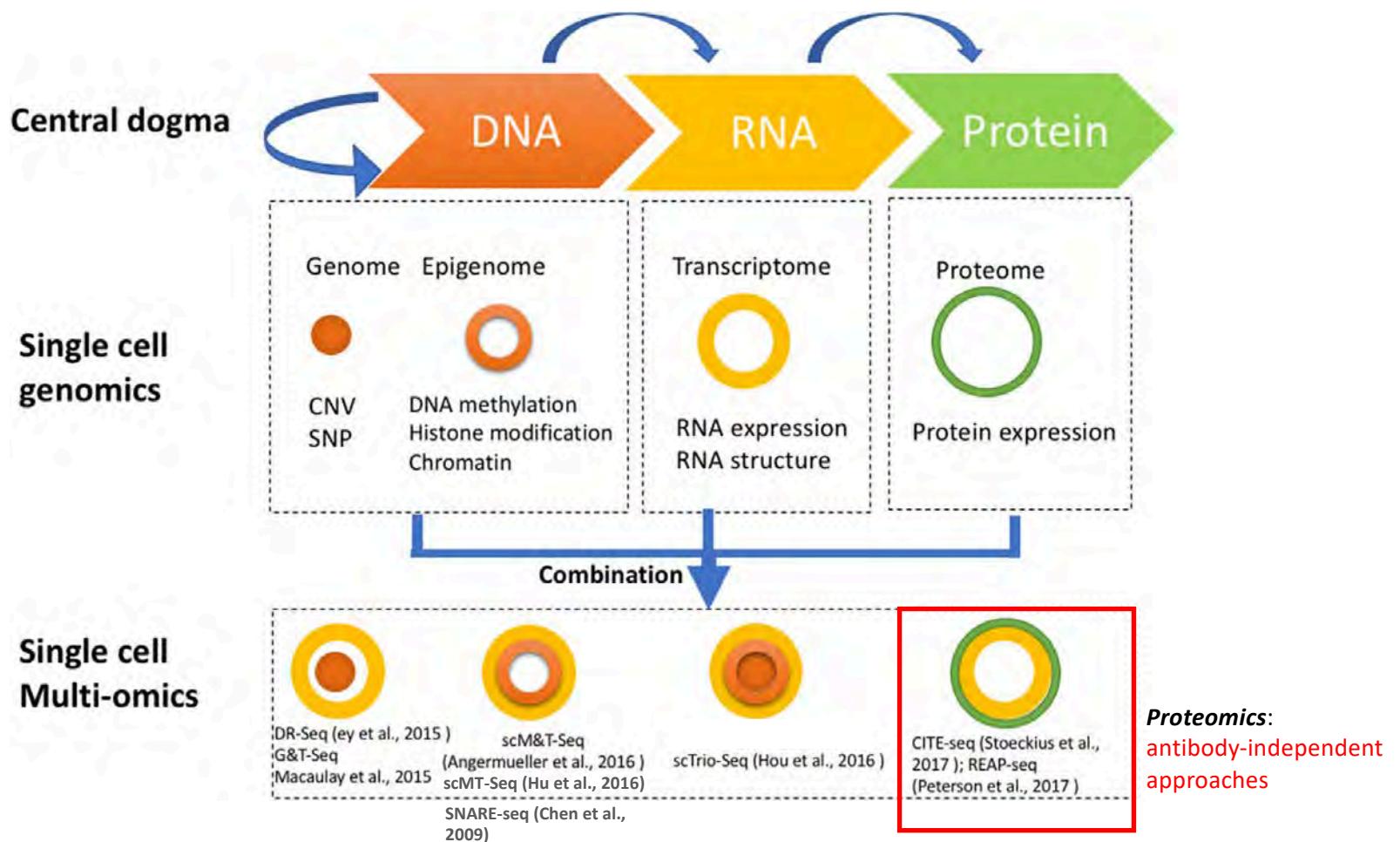
 BioLegend®
(oligo-conjugated antibodies)

Cell Hashing: apply CITE-seq to multiplex multiple samples



Available from 10X Genomics & BD Rhapsody (Ab-seq)

Strategies for multi-omics profiling of single cells



Single cell whole transcriptome & proteome analysis

RESEARCH

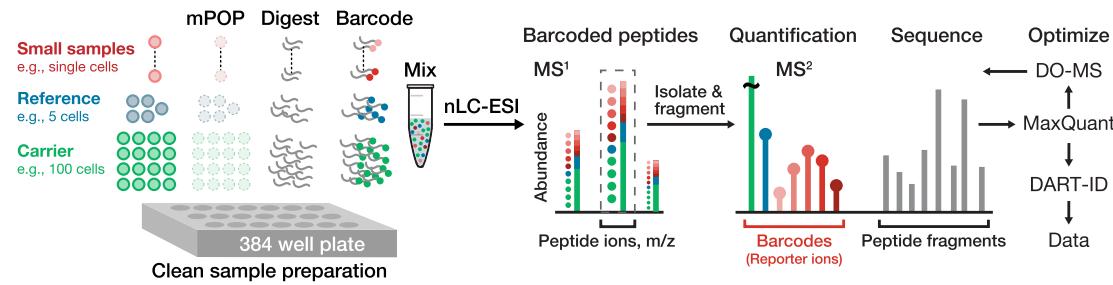
Open Access



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

Harrison Specht^{1*}, Edward Emmott^{1,2}, Aleksandra A. Petelski¹, R. Gray Huffman¹, David H. Perlman^{1,3}, Marco Serra⁴, Peter Kharchenko⁴, Antonius Koller¹ and Nikolai Slavov^{1*}

Single-Cell ProtEomics by Mass Spectrometry (SCoPE2)



Antibody-independent
single cell proteomics

Integrated
analysis

scRNAseq

Nikolai Slavov group
Genome Biology (2021) 22:50

10X GENOMICS

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

