

Single cell multi-omics

Miao-Ping Chien

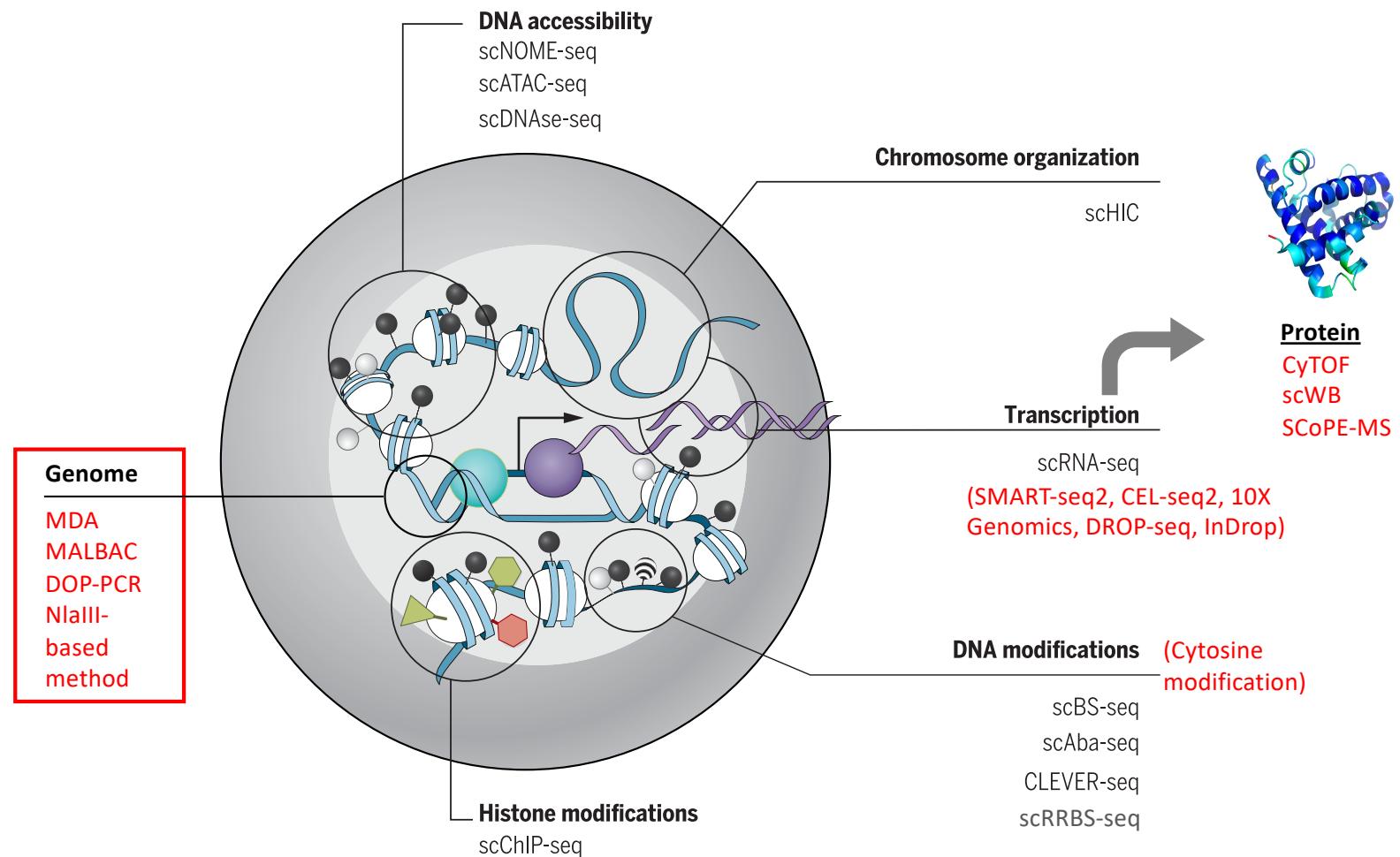
Erasmus MC, Assistant professor

2022 Single Cell Analysis Workshop, 2022/10/11

Outline

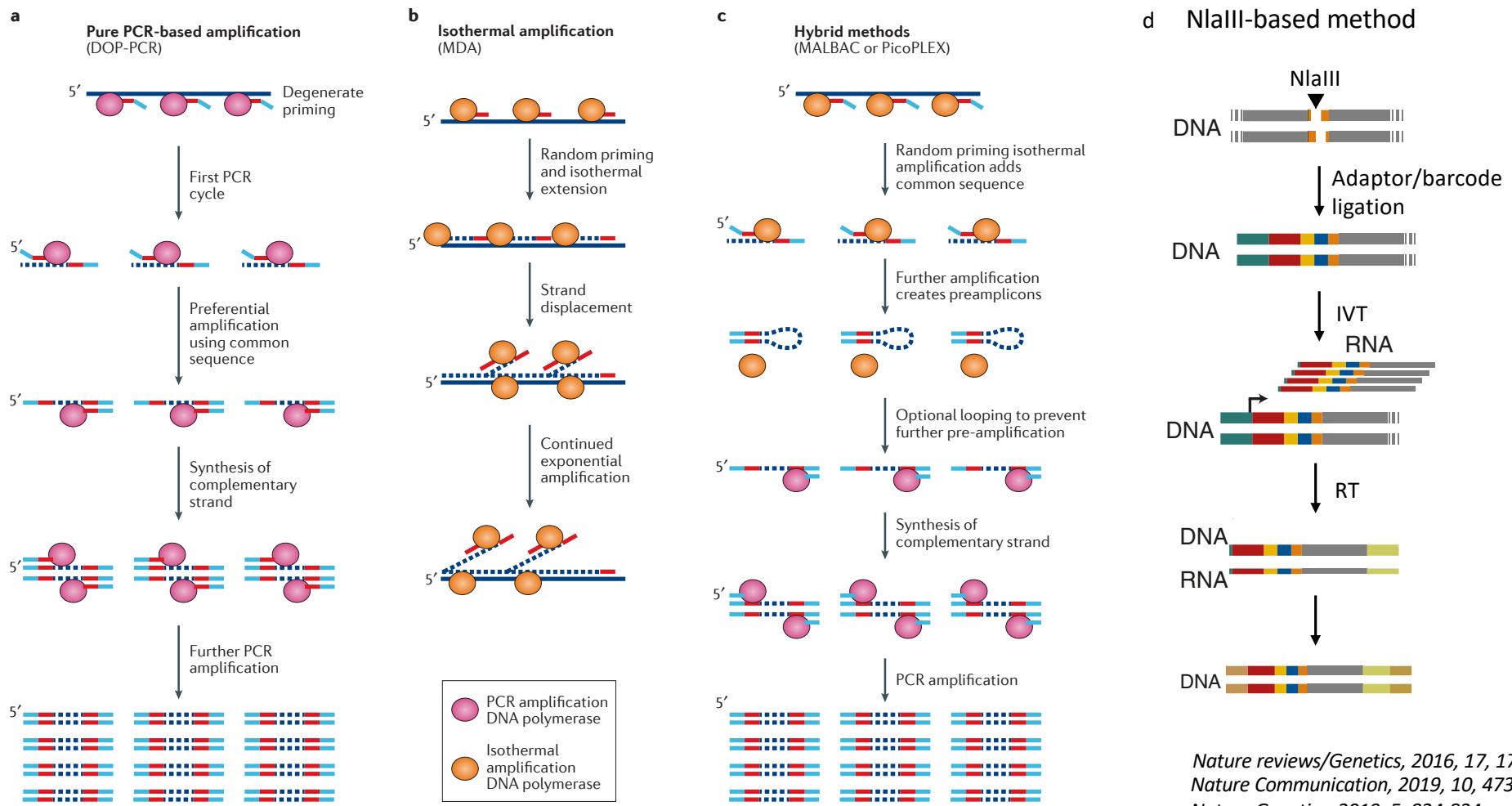
- (Quick) overview of different single cell –omics methods
- Different single cell multi–omics methods

Overview of single cell -omics



Single cell genomic sequencing

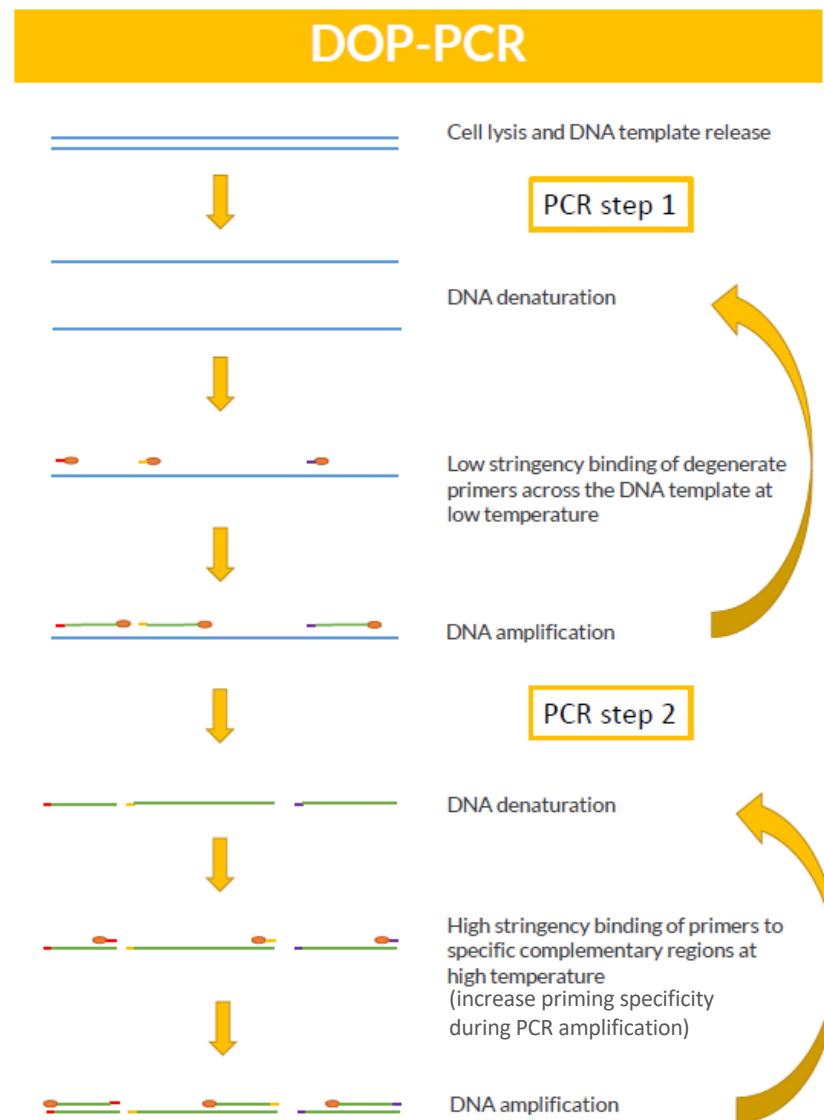
Overview of the main whole-genome amplification methods



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

Degenerate Oligonucleotide-Primed PCR (DOP-PCR)

- biased amplification
- error prone

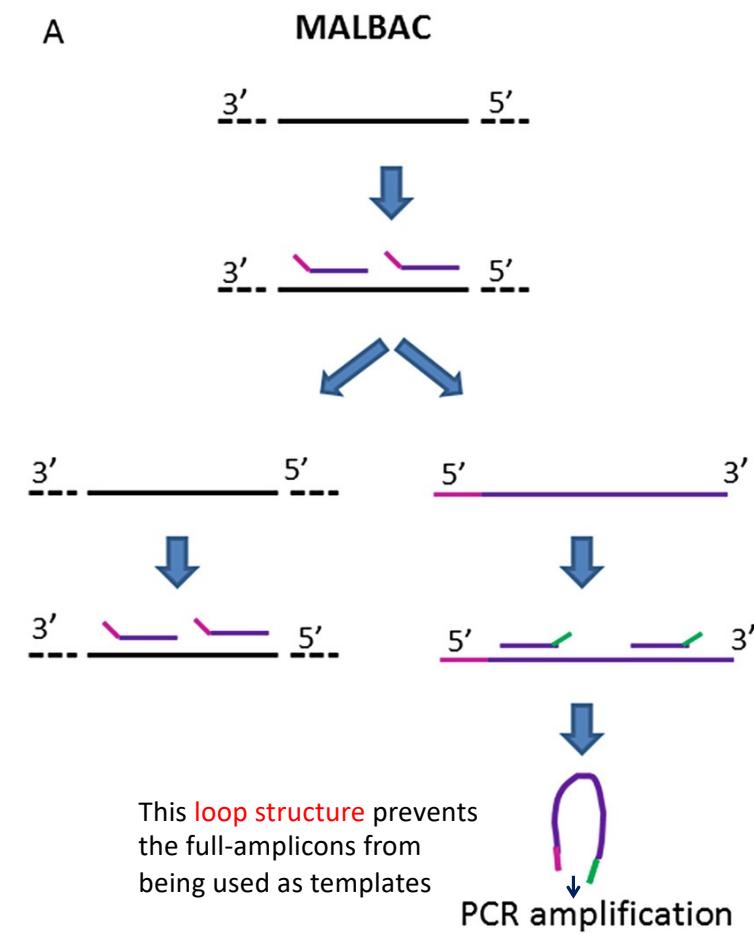


MALBAC vs MDA

Hybrid method

MALBAC: multiple annealing and looping based amplification cycles

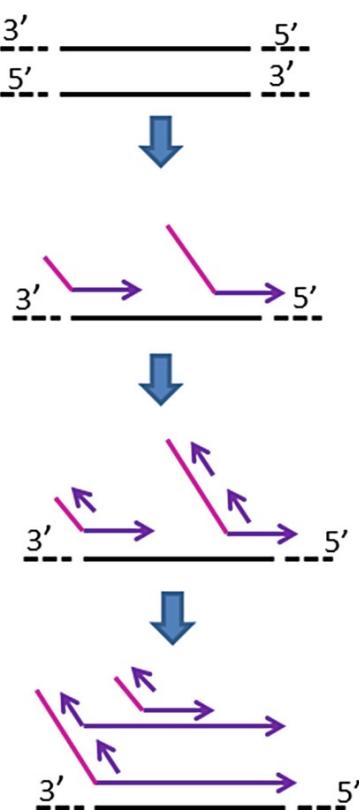
A



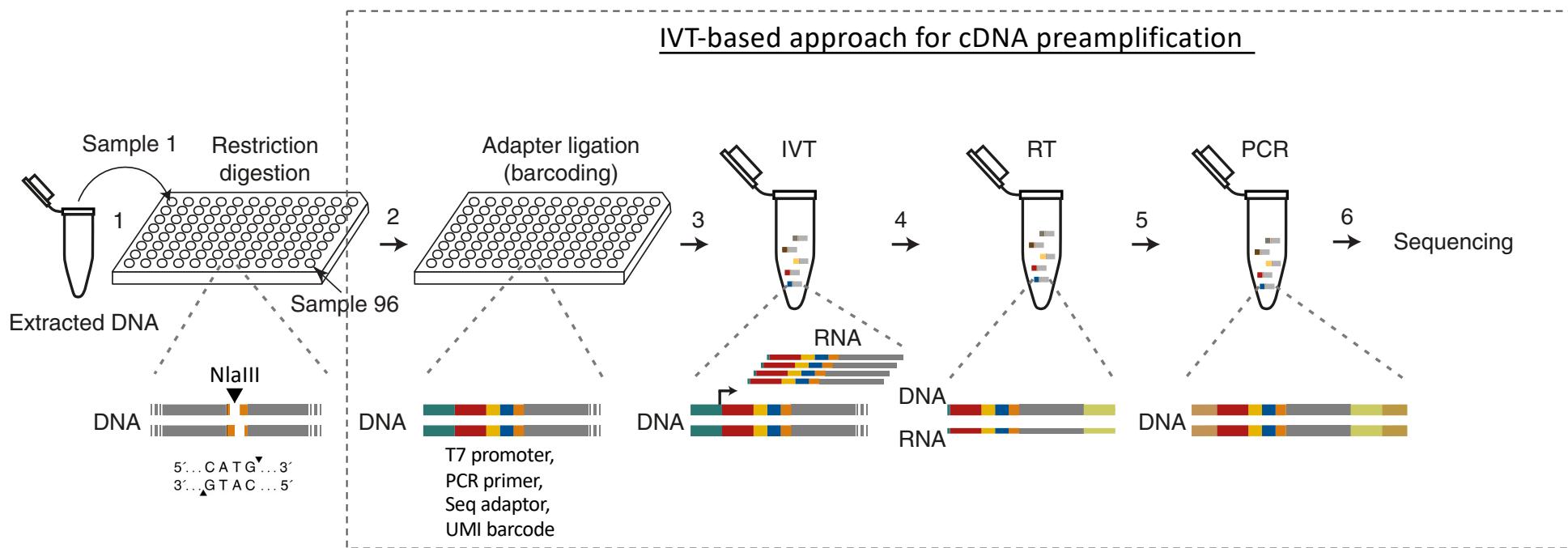
Isothermal amplification

MDA: multiple displacement amplification

B

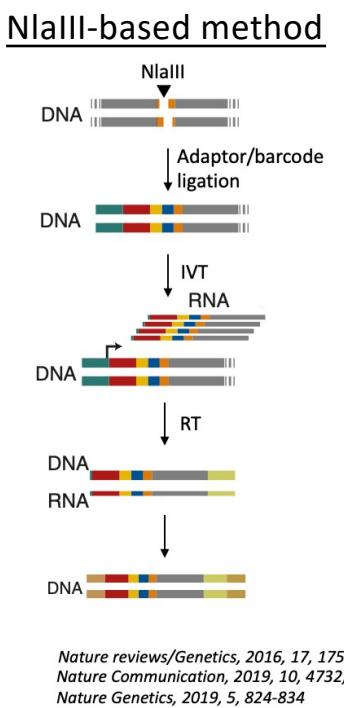
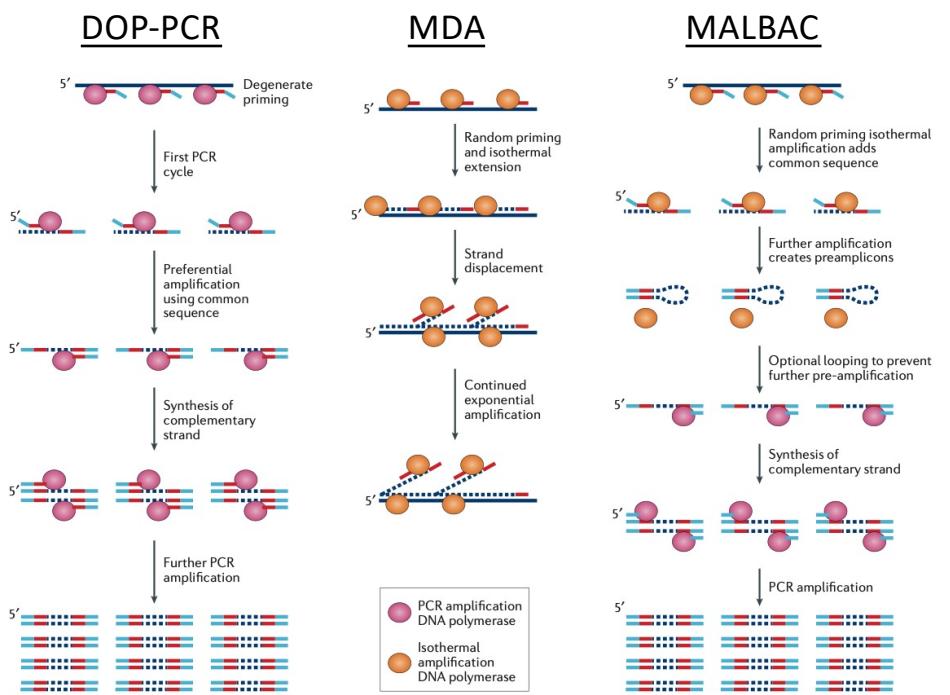


NlaIII-based scDNAseq method



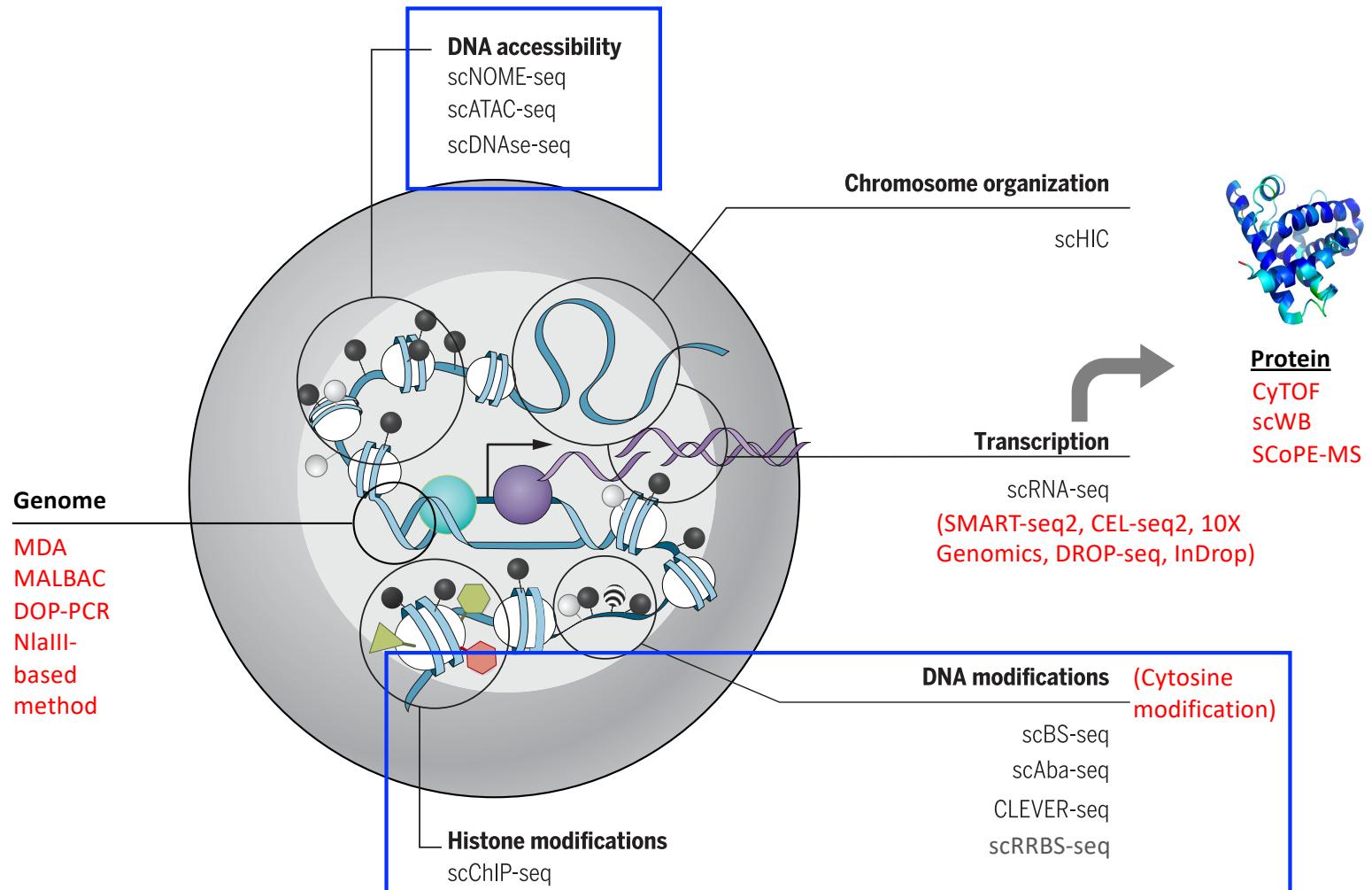
Nature Communication, 2019, 10, 4732;
Nature Genetics, 2019, 5, 824-834

Overview of the main whole-genome amplification methods



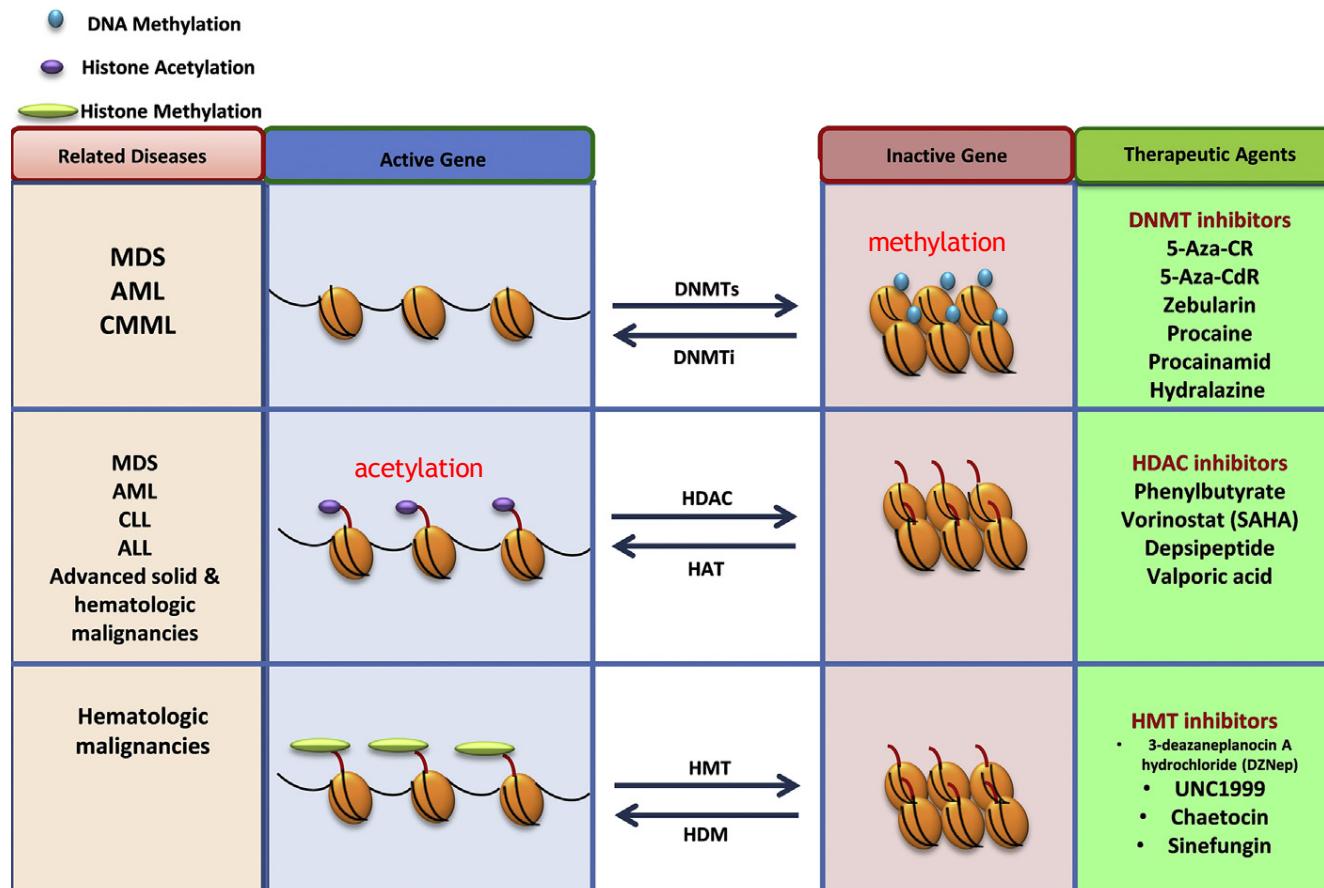
- Highest to lowest genome recovery rate?
MDA (~80%) > MALBAC (~50%) > DOP-PCR (~6%) / NlaIII-based method (~5-10%)
- Highest to lowest duplication ratio (duplicate the product more than the original template)?
DOP-PCR >> MDA > MALBAC, NlaIII-based method
- Homogeneous amplification?
MALBAC, NlaIII-based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

Overview of single cell -omics



Single cell epigenomic sequencing

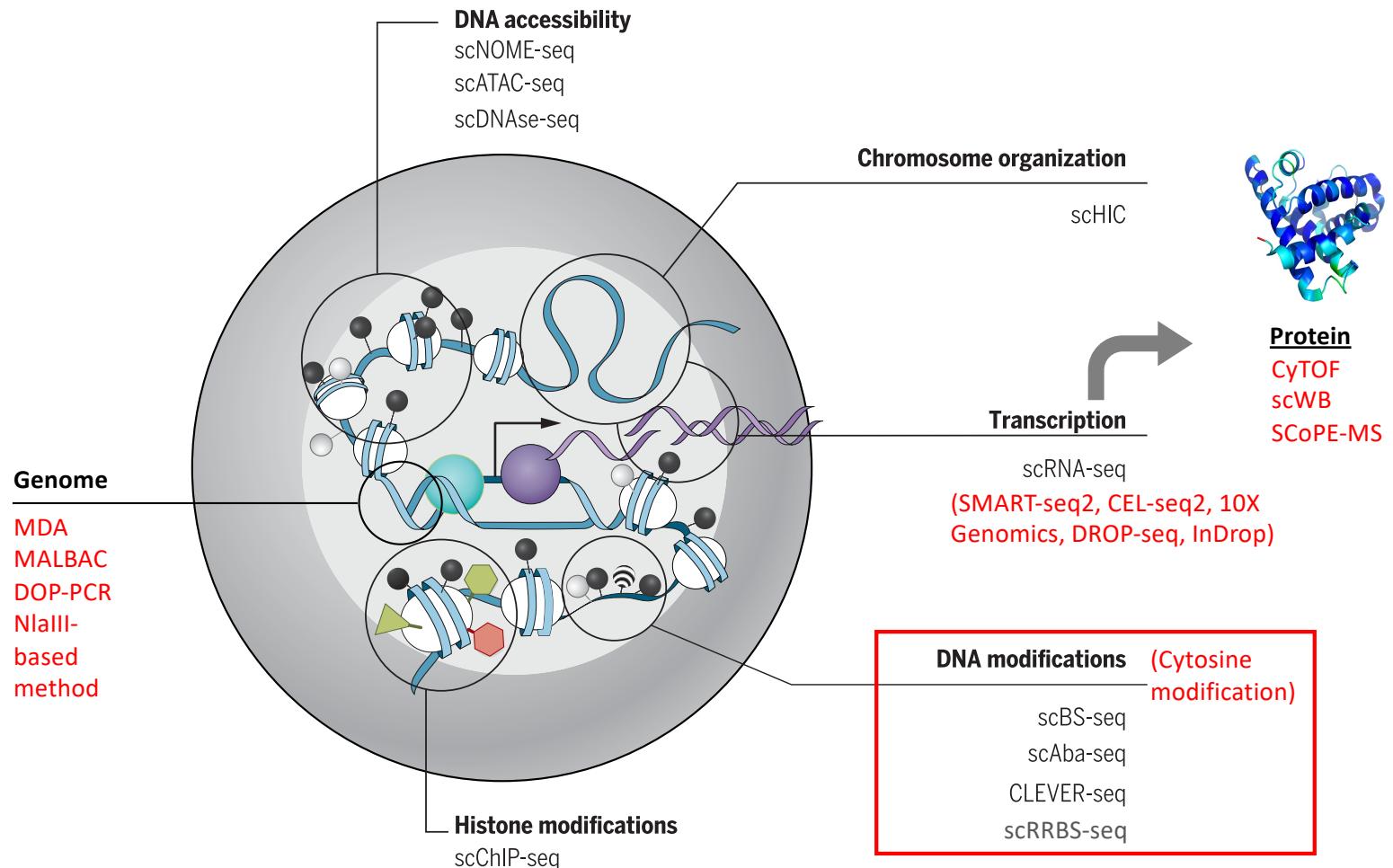
Why epigenomics?



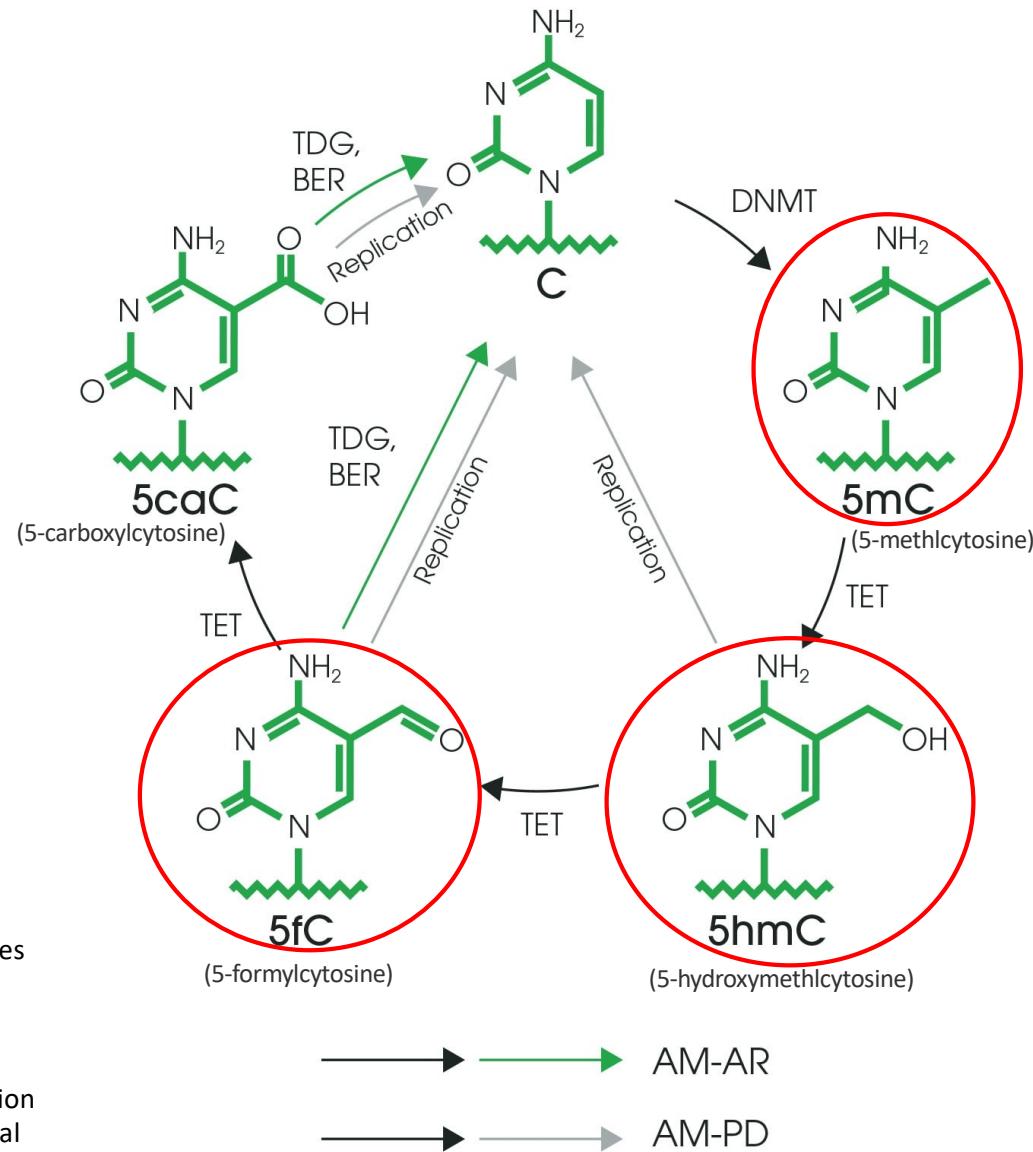
Histone acetylation: opening of the chromatin mass & the onset of transcription

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

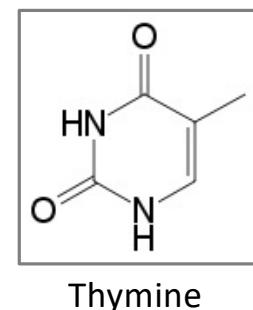
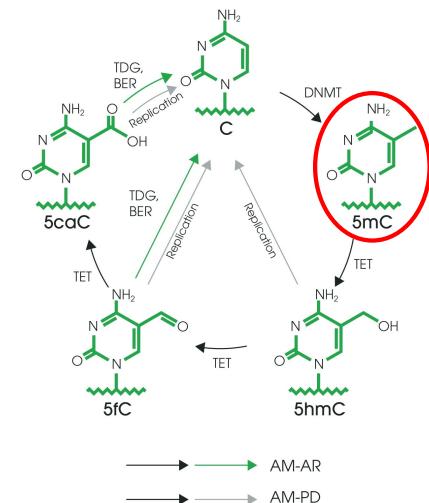
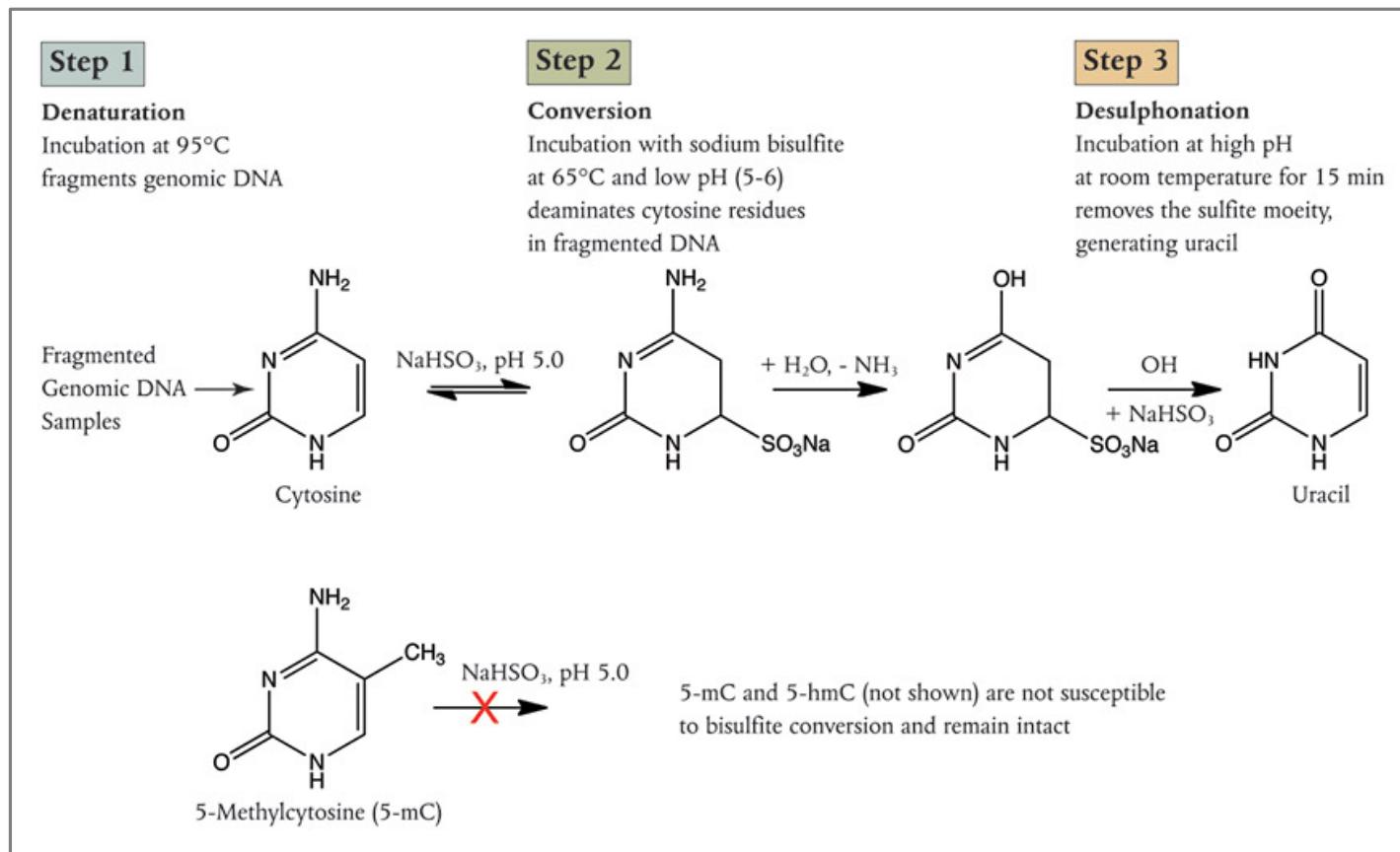
BER: base excision repair

AM-PD: Active modification—passive dilution

AM-AR: active modification—active removal

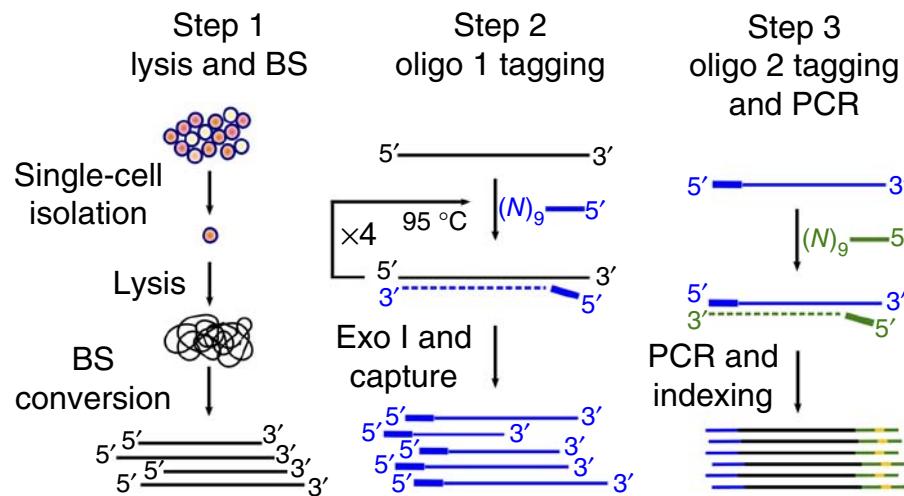
Bisulfite conversion

- C → U
- C* → C



Single cell bisulfite sequencing (scBS-seq)

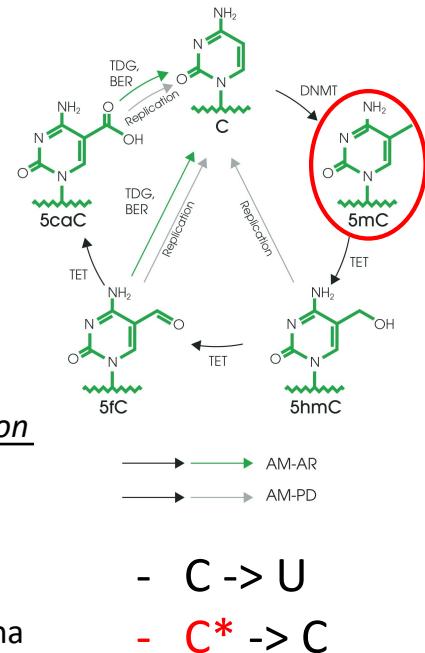
a



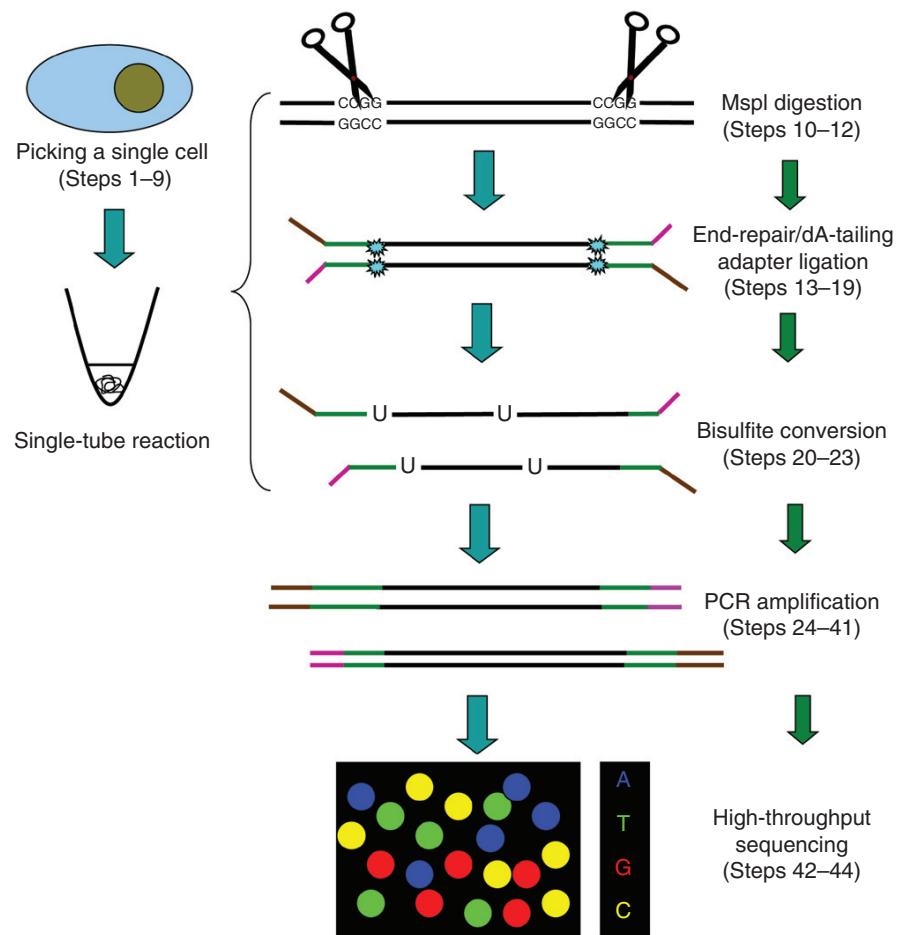
Step 1: bisulfite treatment -> DNA fragmentation & conversion of *unmethylated cytosines to thymine*

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

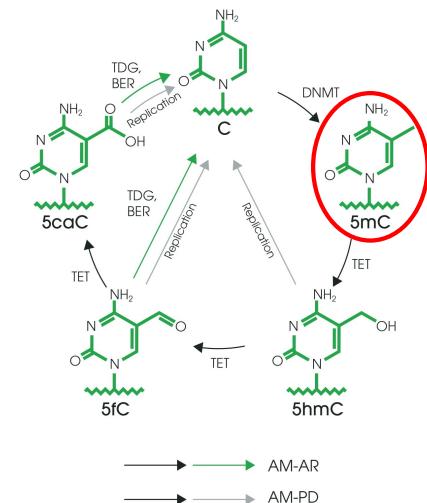
Step 3: After capturing the tagged strands, a second adaptor is similarly integrated, and PCR amplification is performed with indexed primers



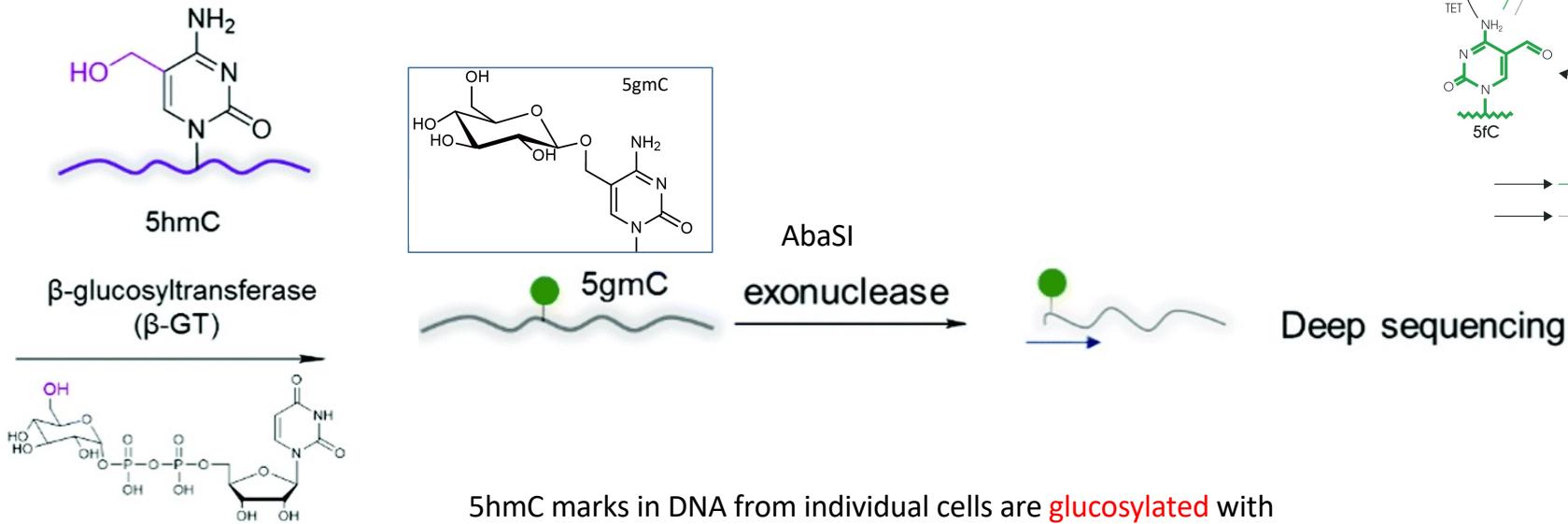
scRRBS-seq: single-cell reduced-representation bisulfite sequencing



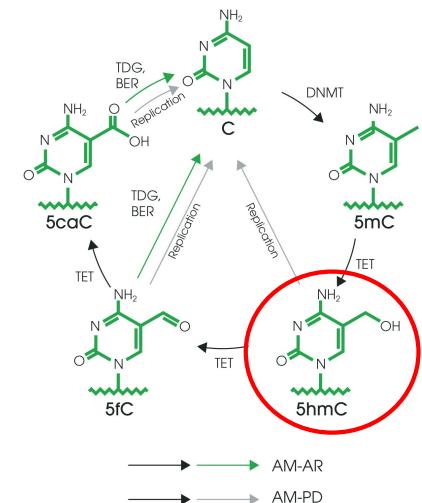
Mainly focus on the **CpG island** (related to gene expression regulation -> methylation of "C" in CpG island silences gene expression)



scAba-seq : single-cell 5hmC sequencing



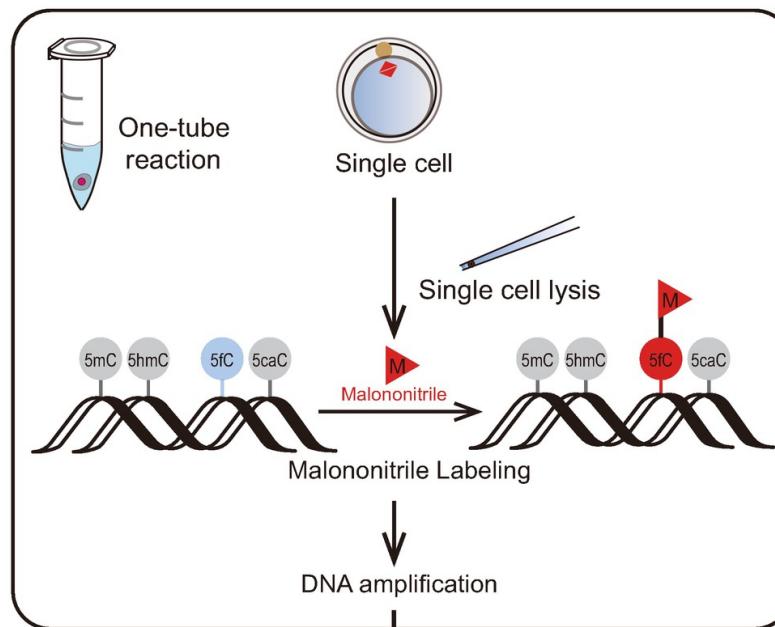
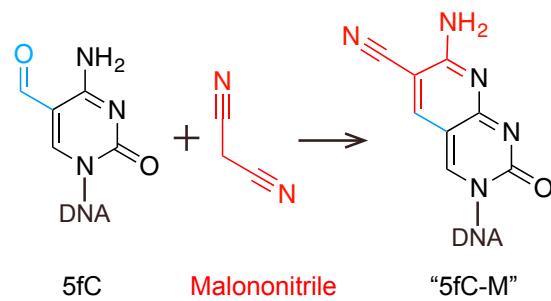
5hmC marks in DNA from individual cells are **glucosylated** with **T4 phage- β -glucosyltransferase (T4 β -GT)**, and the DNA is digested with the restriction endonuclease **AbaSI**. The digested DNA is ligated to an adapter containing a cell-specific barcode, an Illumina 5' adapter, and a T7 promoter.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



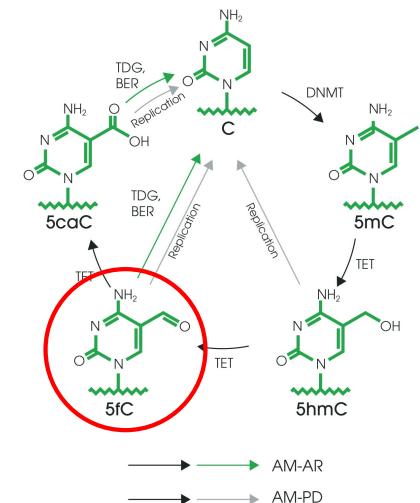
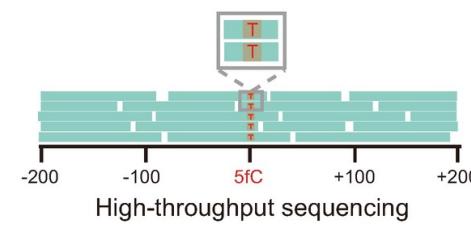
CLEVER-seq

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

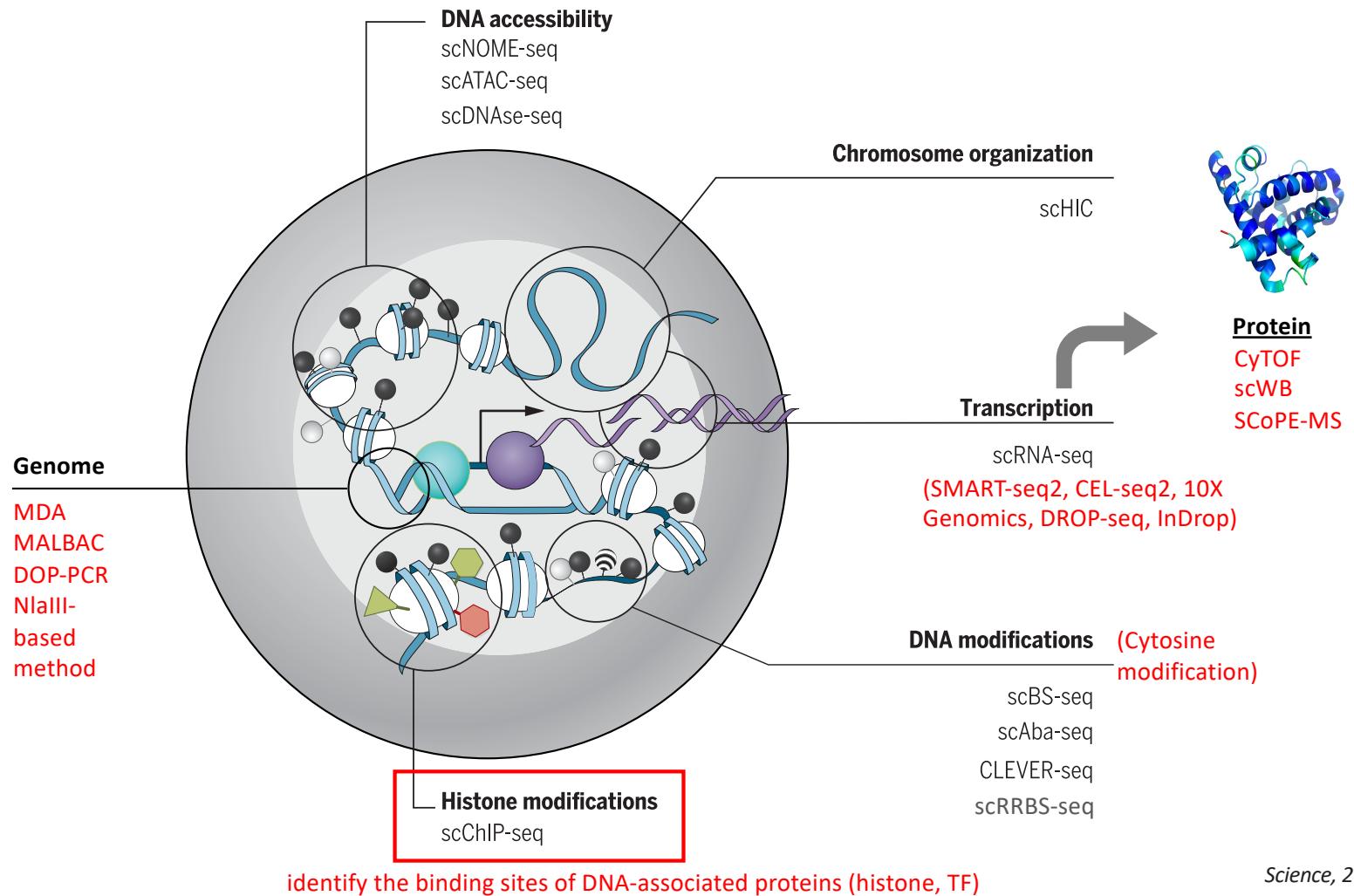
- A single-cell, single-base resolution whole-genome **5fC**-sequencing technology



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



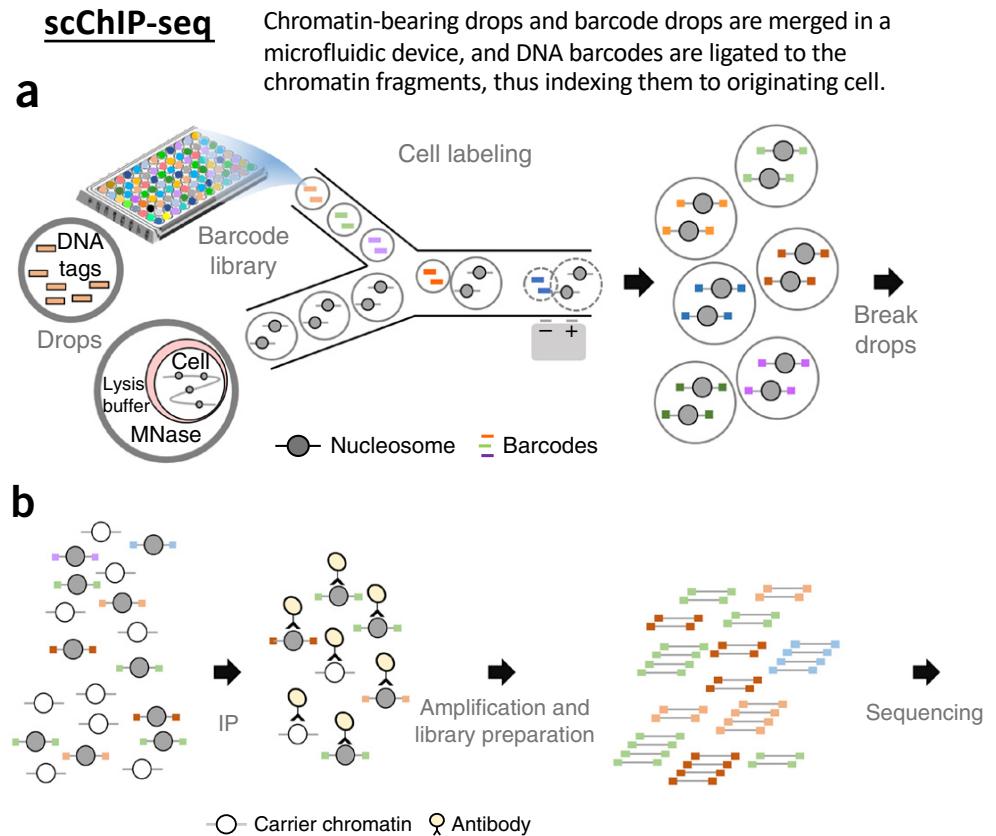
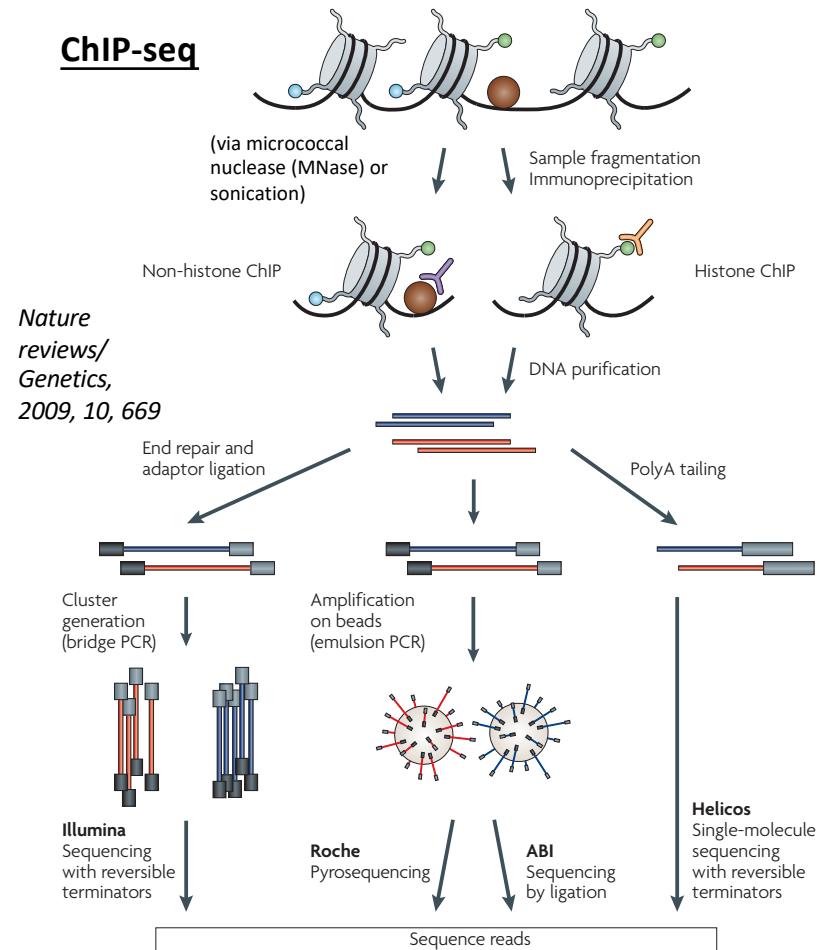
Overview of single cell -omics



Single cell chromatin immunoprecipitation (CHIP) sequencing

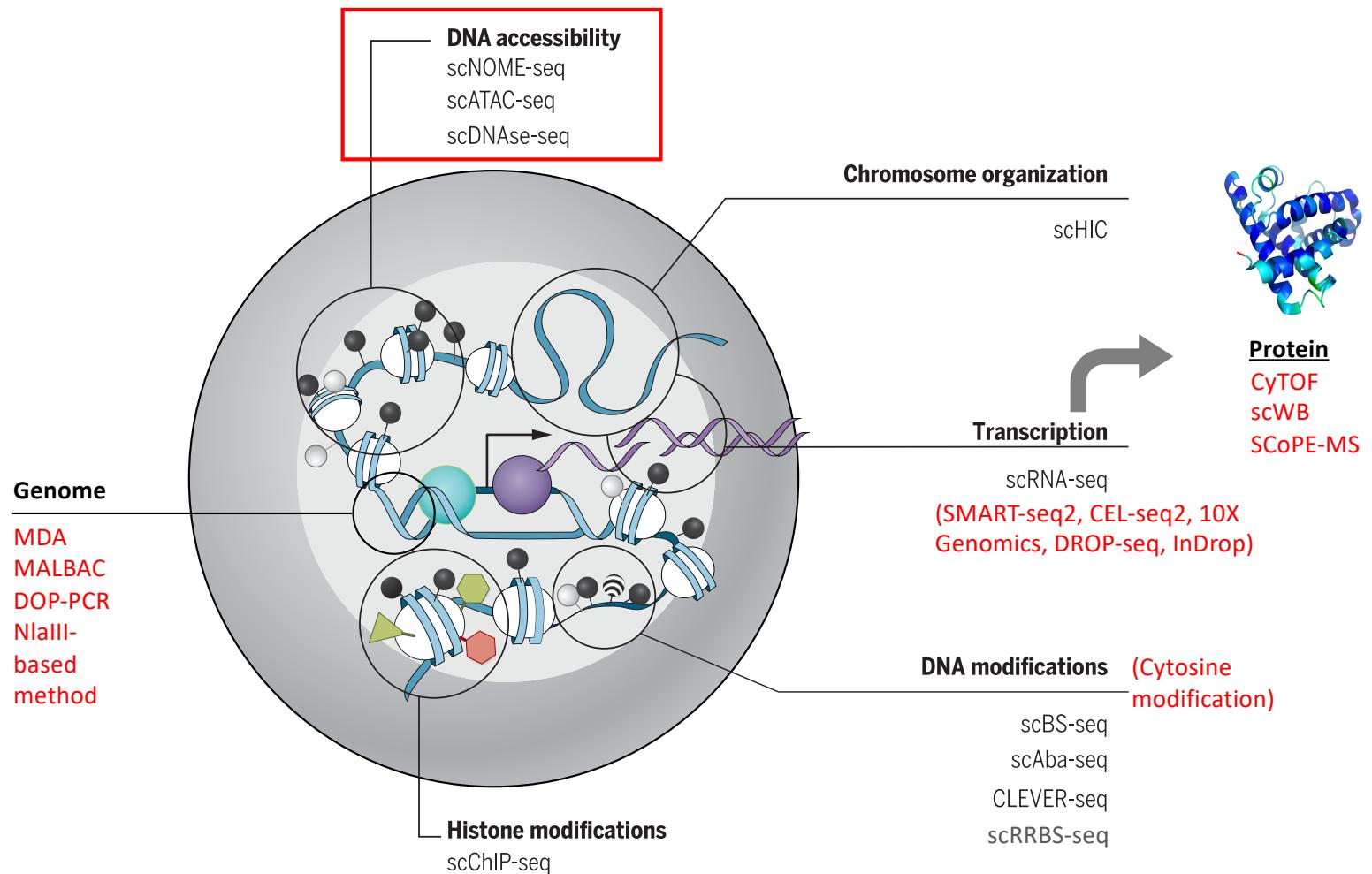
Single cell chromatin immunoprecipitation (ChIP) sequencing

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



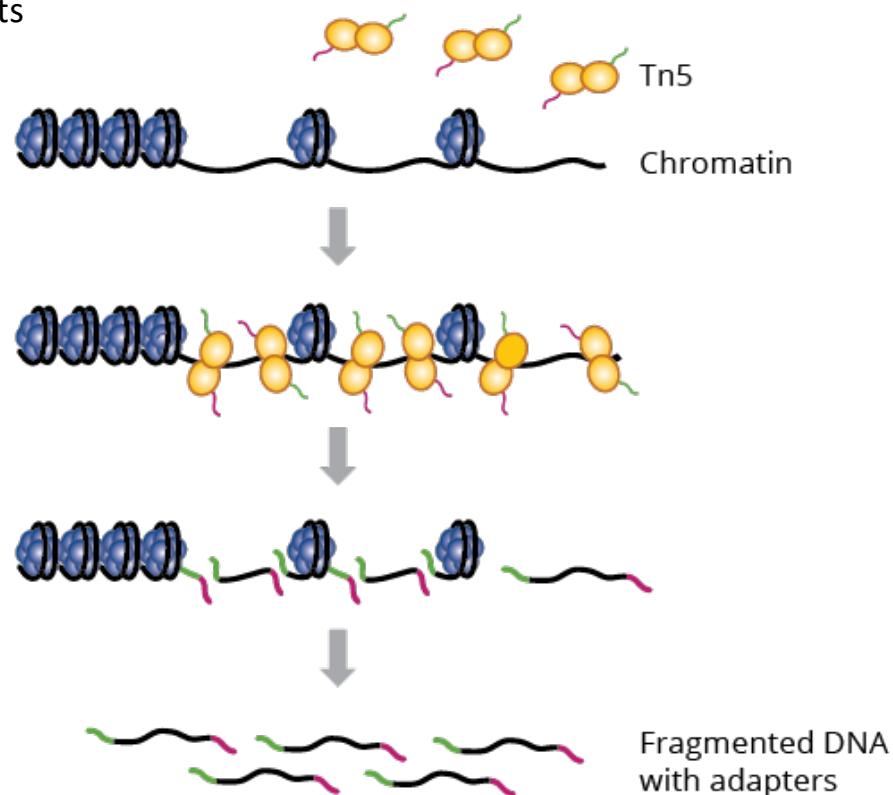
Nature Biotechnology, 2015, 33, 1165

Overview of single cell -omics

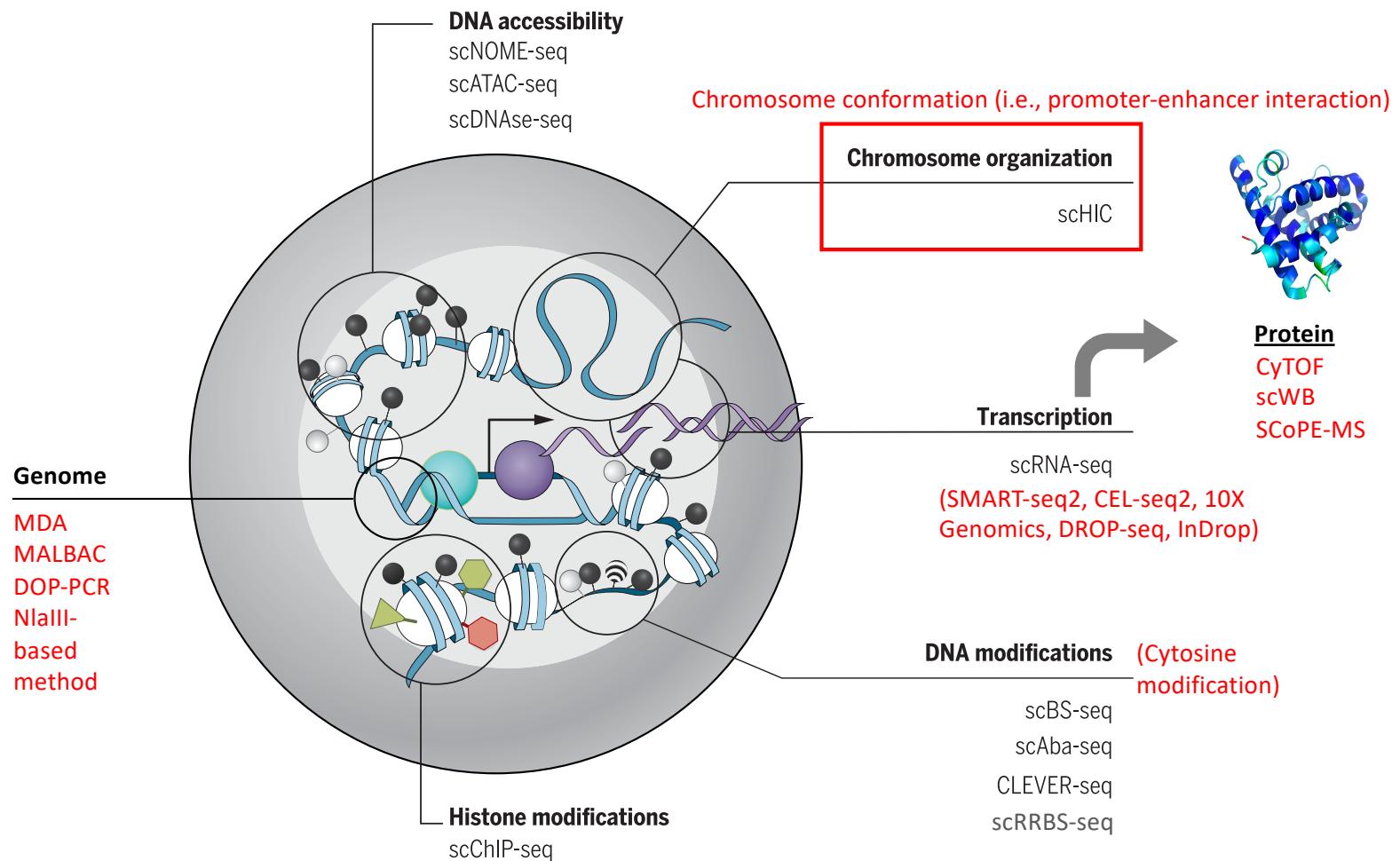


scATAC-seq: Assay for transposase-accessible chromatin

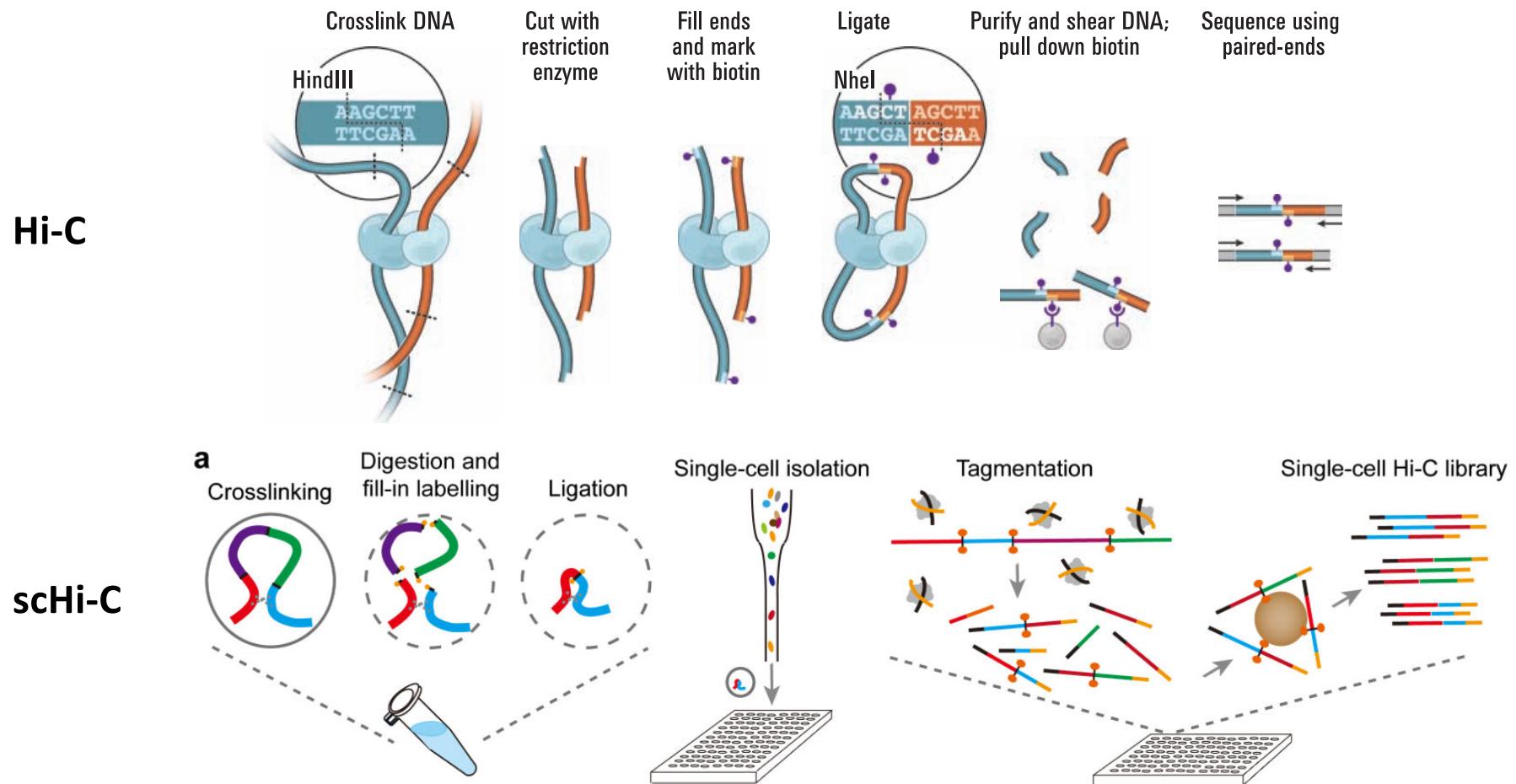
- scATACseq can be applied to
 - nucleosome mapping experiments
 - map transcription factor binding sites
 - map DNA methylation sites



Overview of single cell -omics

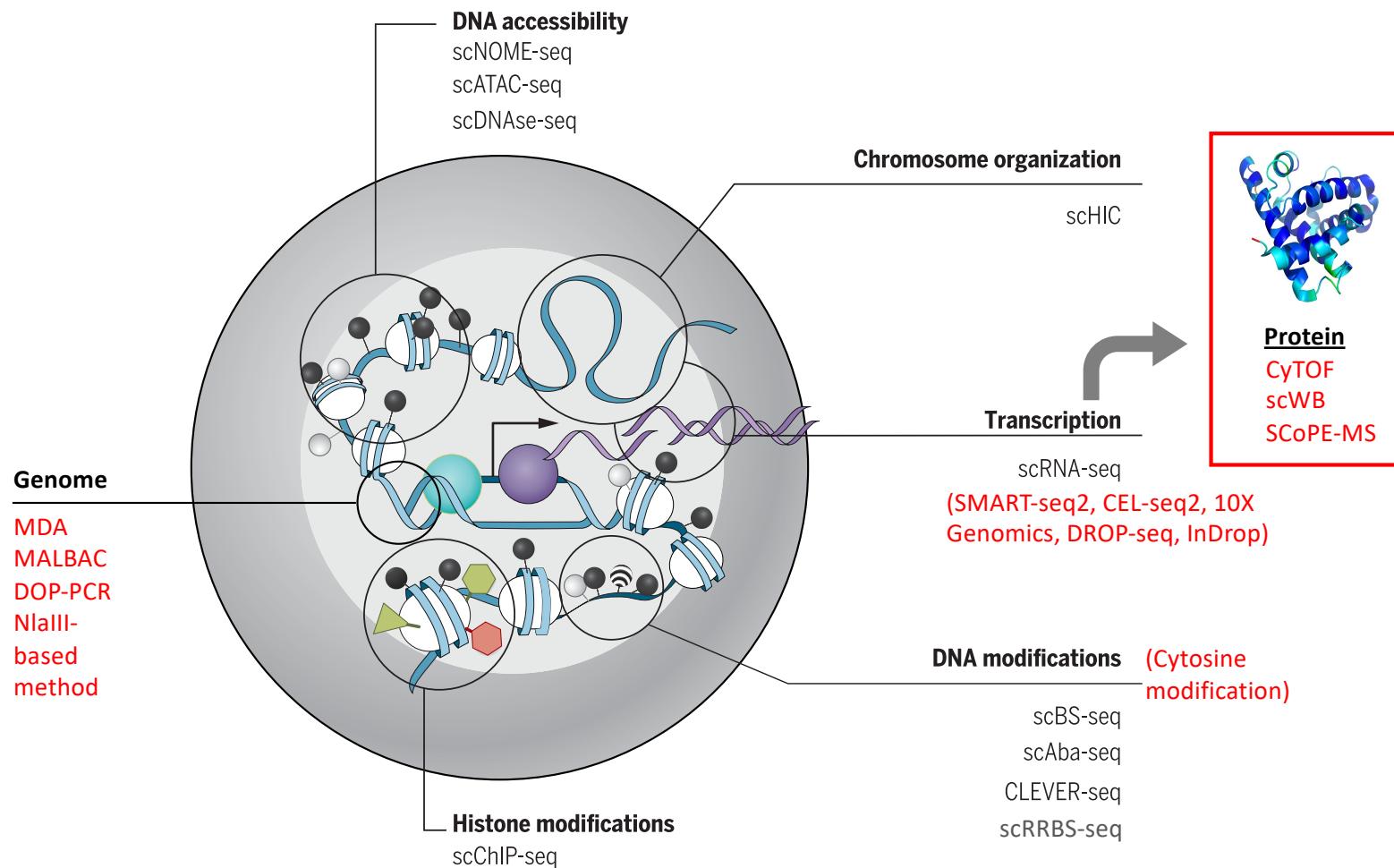


Single cell Hi-C (high-resolution chromosome conformation capture)



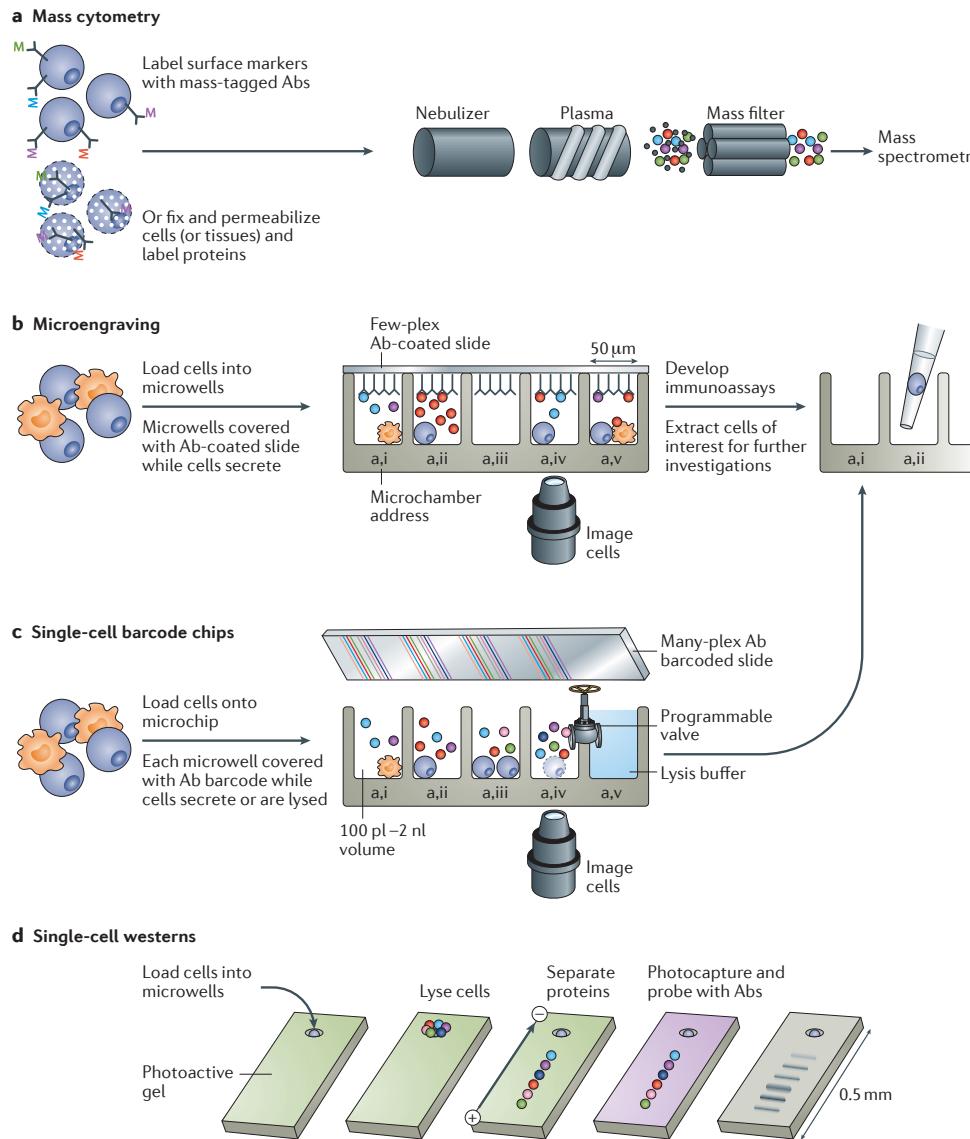
Science, 2009, 326, 289; Nature, 2017, 547, 61

Overview of single cell -omics



Single cell proteomics

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



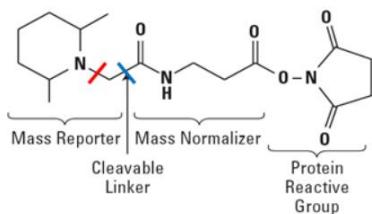
Antibody-independent approaches

-> Mass spectrometry (Mass spec)-based method

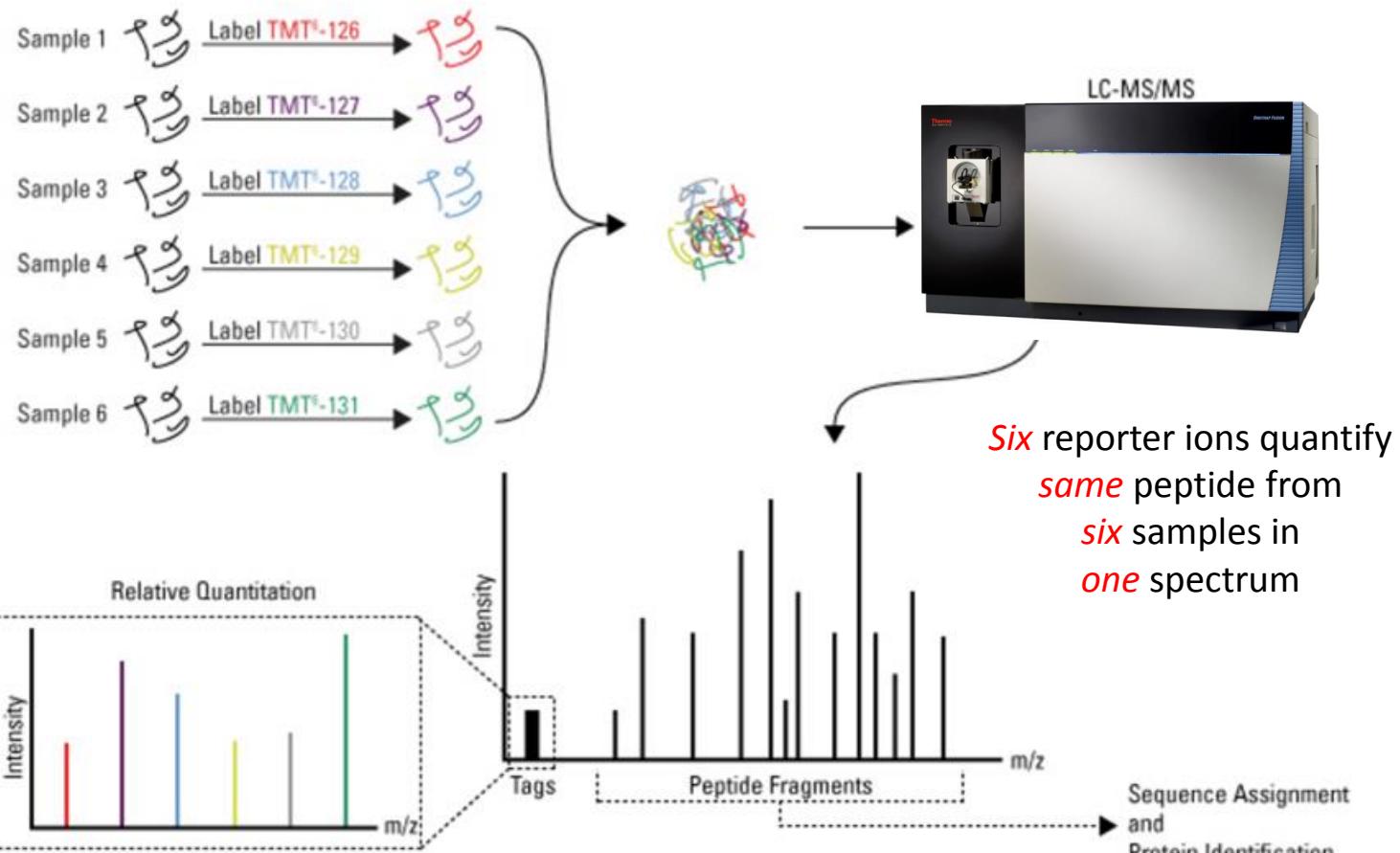
(tandem mass tags)

TMT⁰

Method Development & SRM



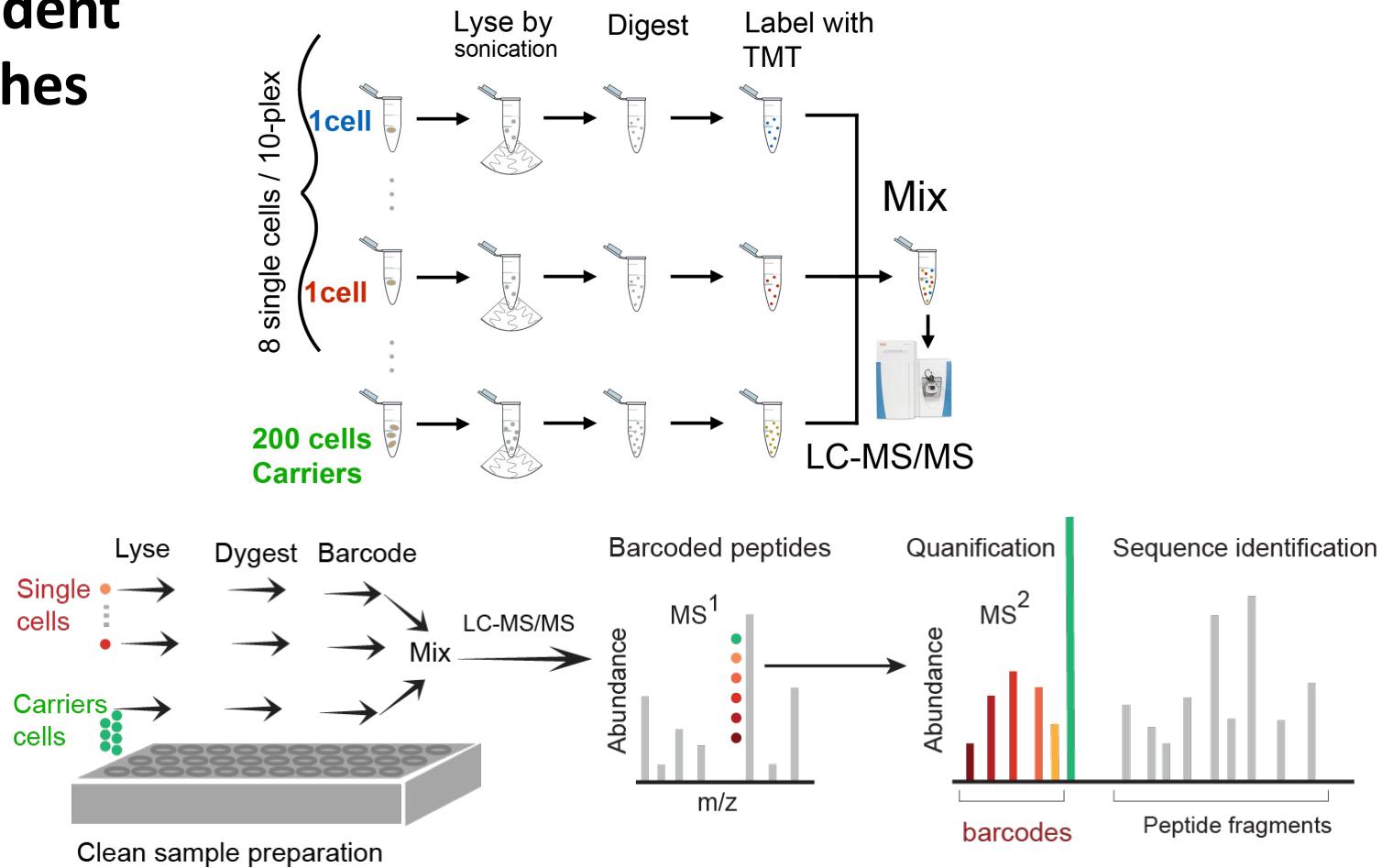
SCoPE-MS (Single Cell ProtEomics by Mass Spectrometry)



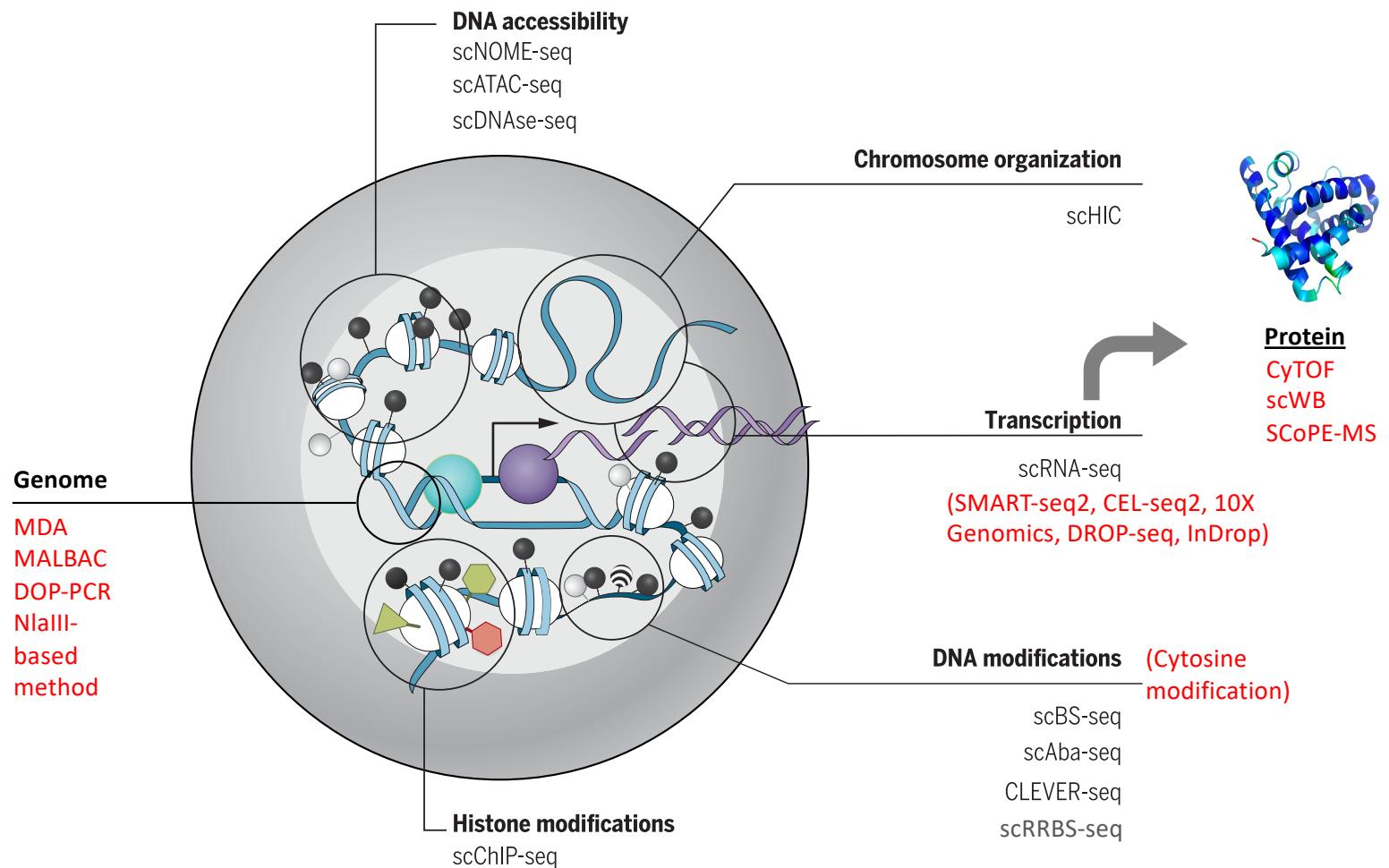
Nikolai Slavov group
Genome Biology, 2018, 19, 161

Antibody-independent approaches

SCoPE-MS (Single Cell ProtEomics by Mass Spectrometry)

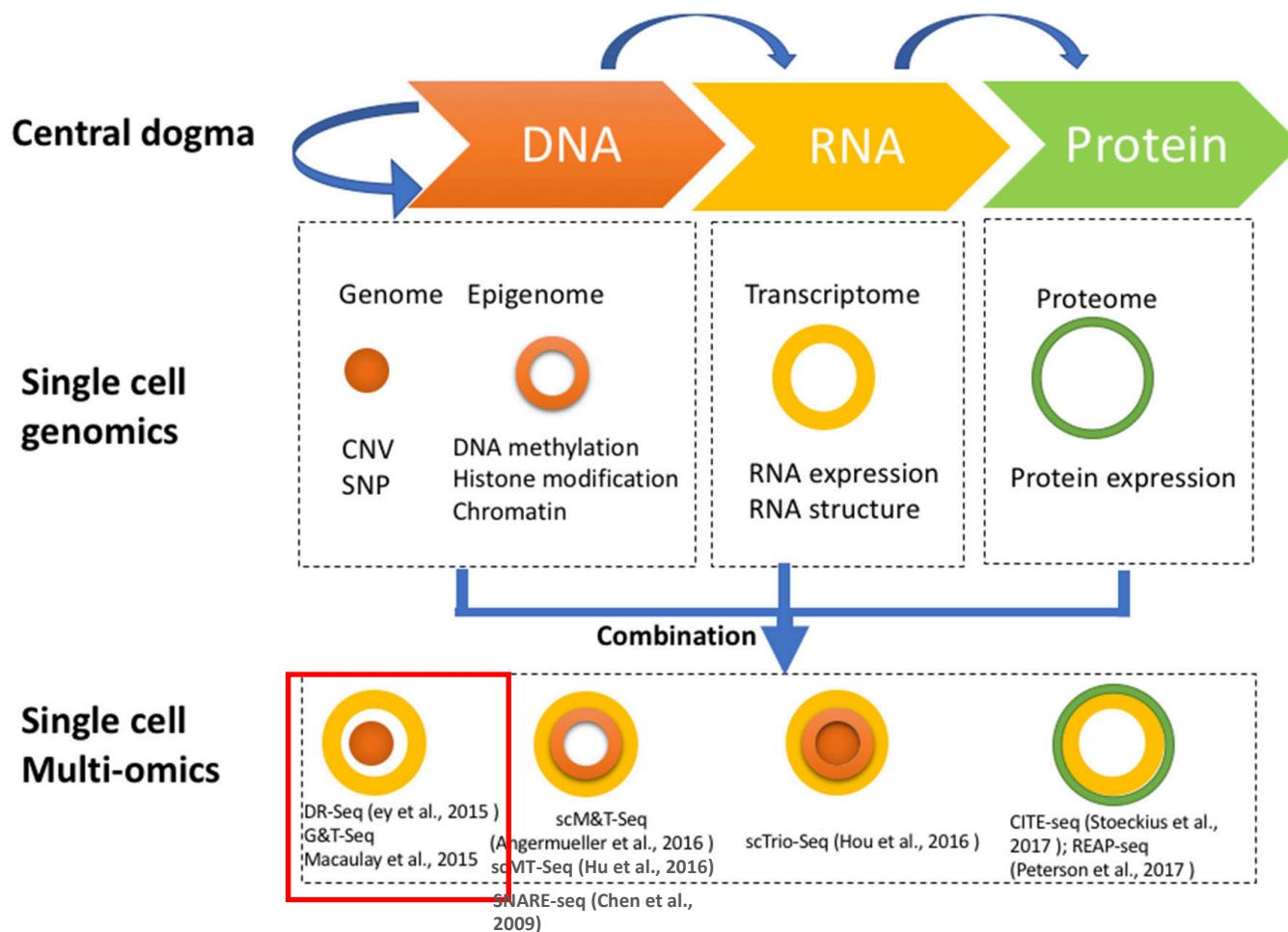


Overview of single cell -omics



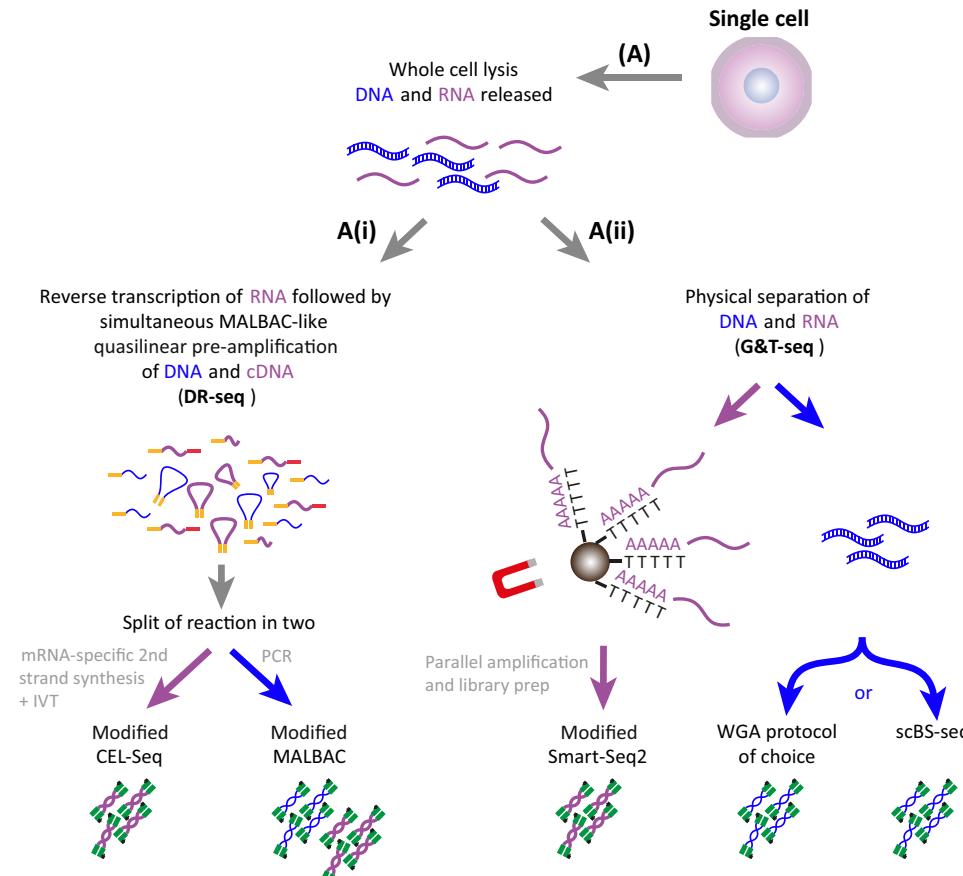
Single cell multi–omics

Strategies for multi-omics profiling of single cells

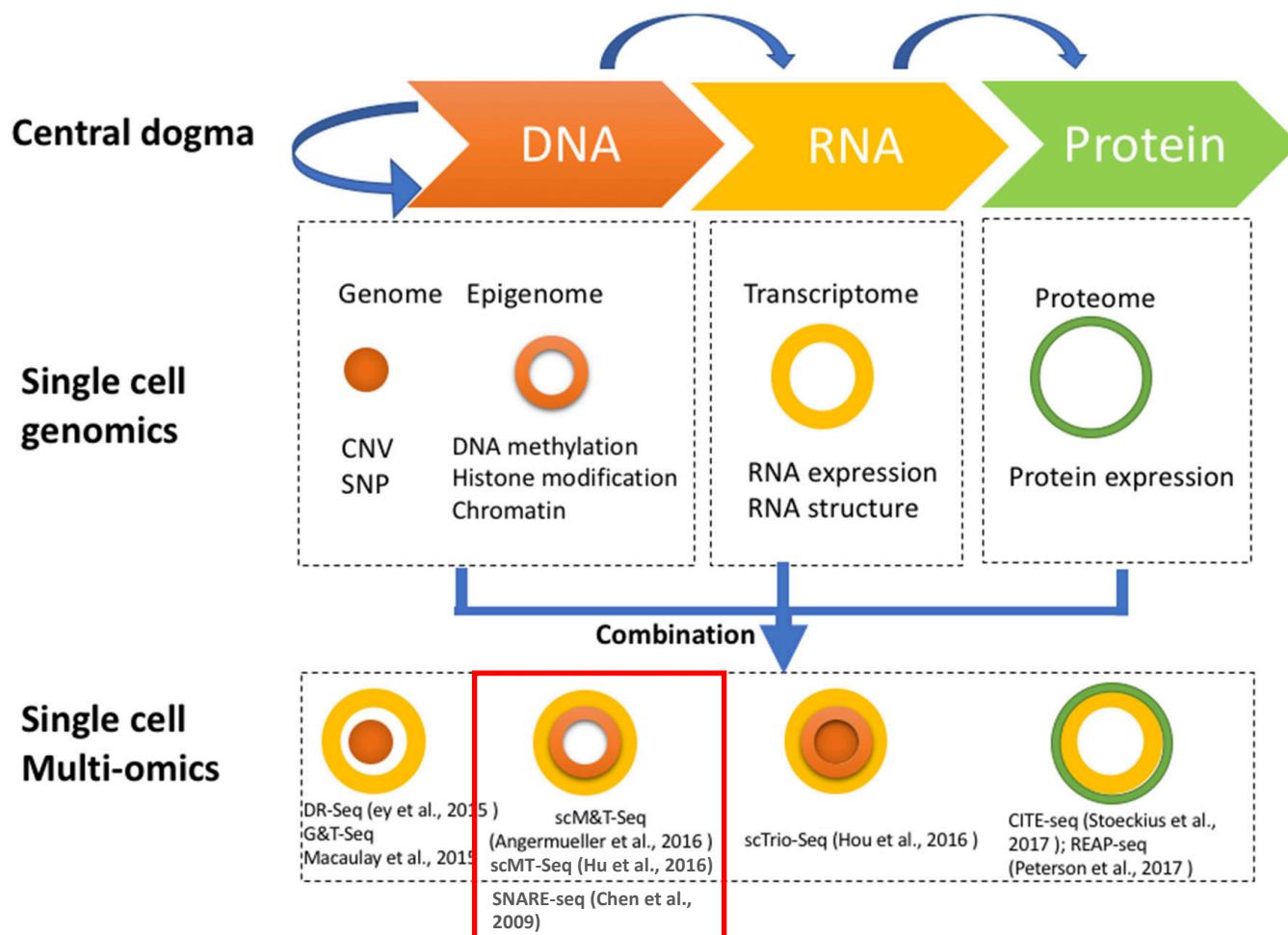


Single cell DNA- and RNA-Sequencing

Single cell DNA- and RNA-Sequencing



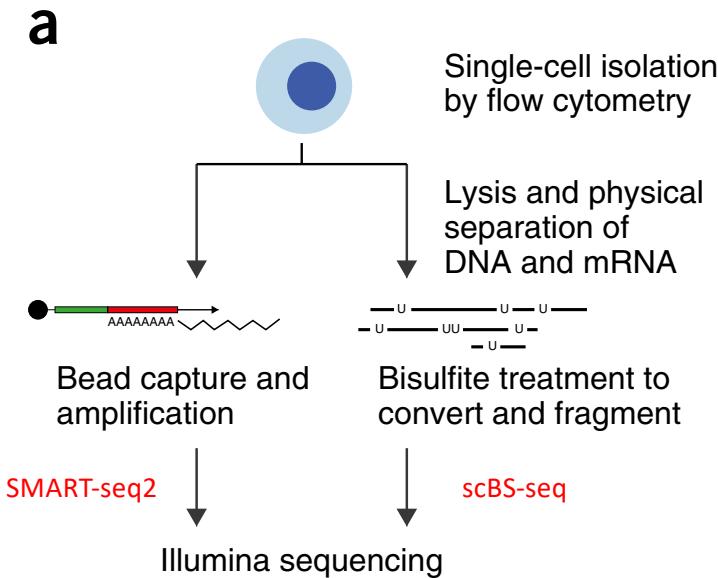
Strategies for multi-omics profiling of single cells



Single cell RNA- and methylation- Sequencing

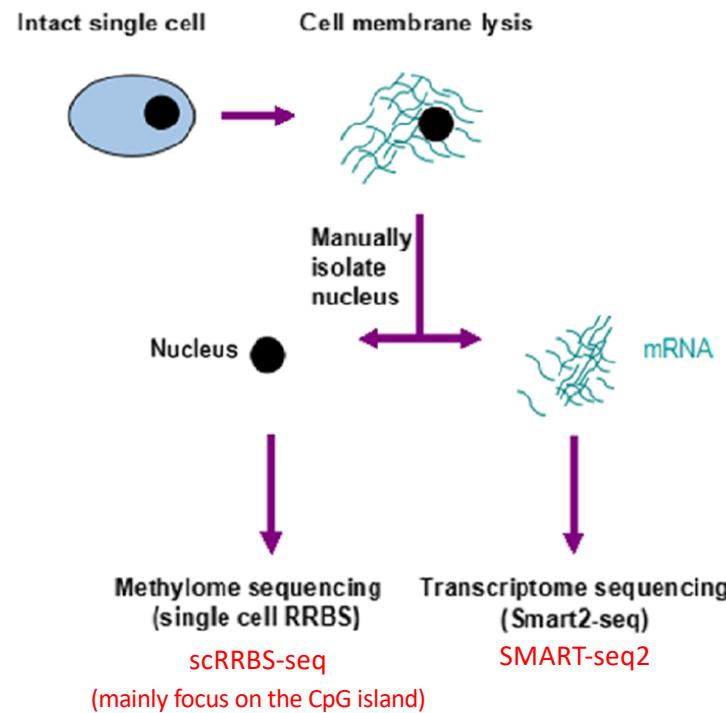
scM&T-seq

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)

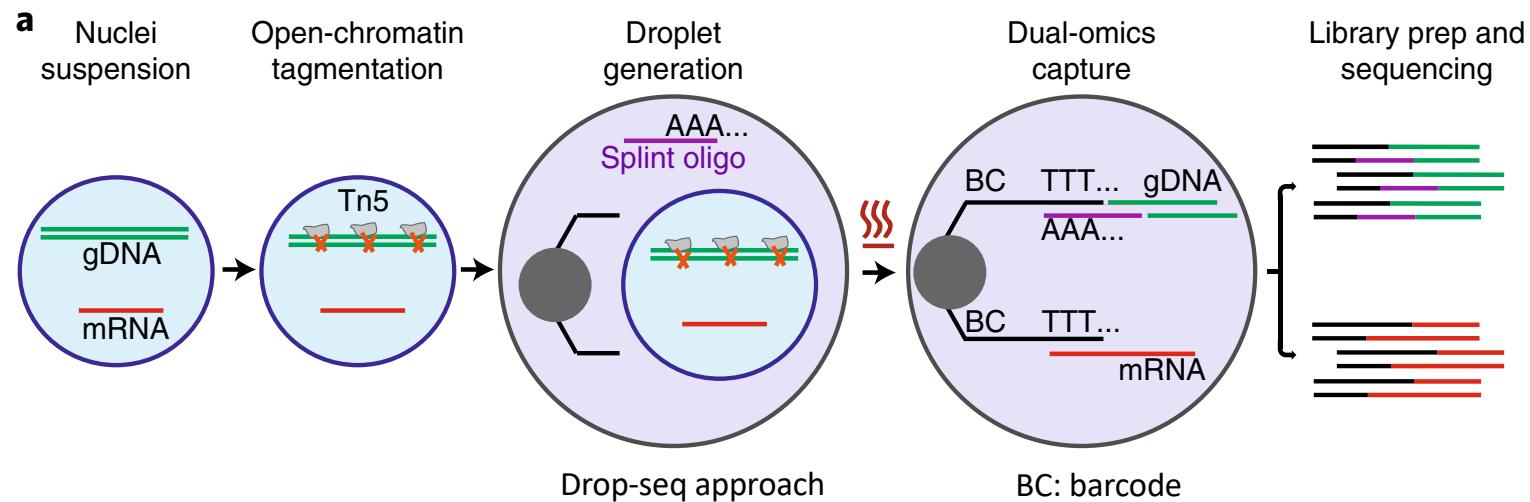
nature
biotechnology

LETTERS

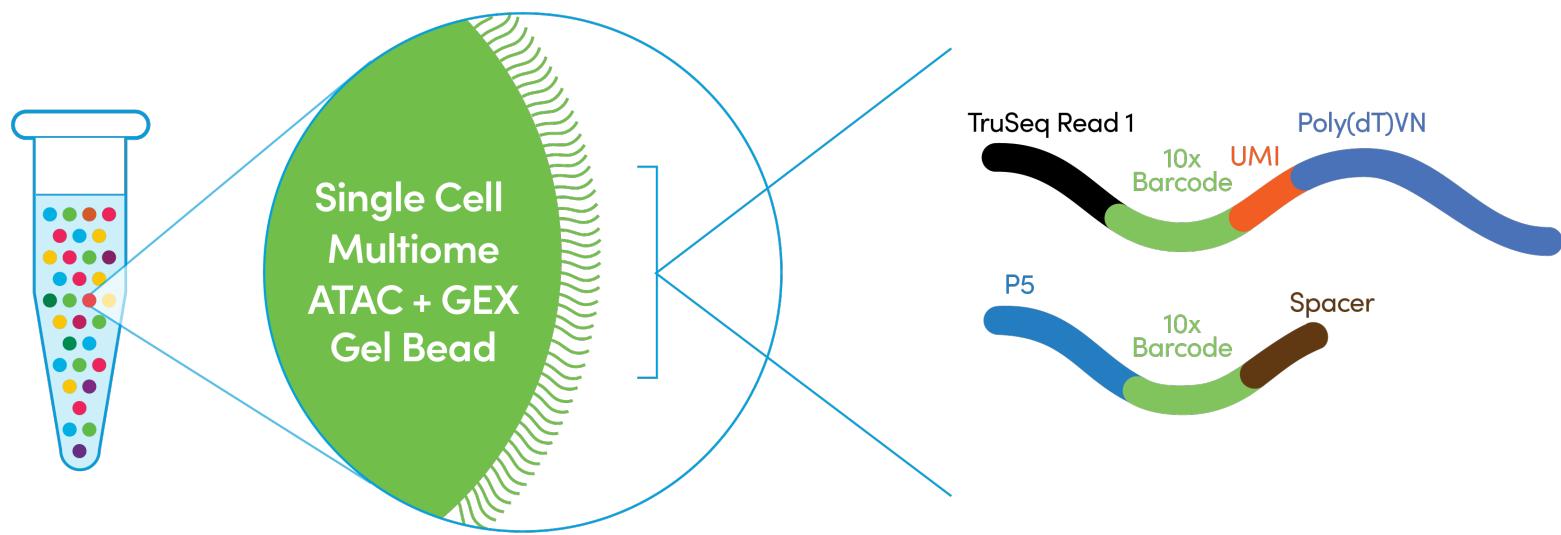
<https://doi.org/10.1038/s41587-019-0290-0>

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

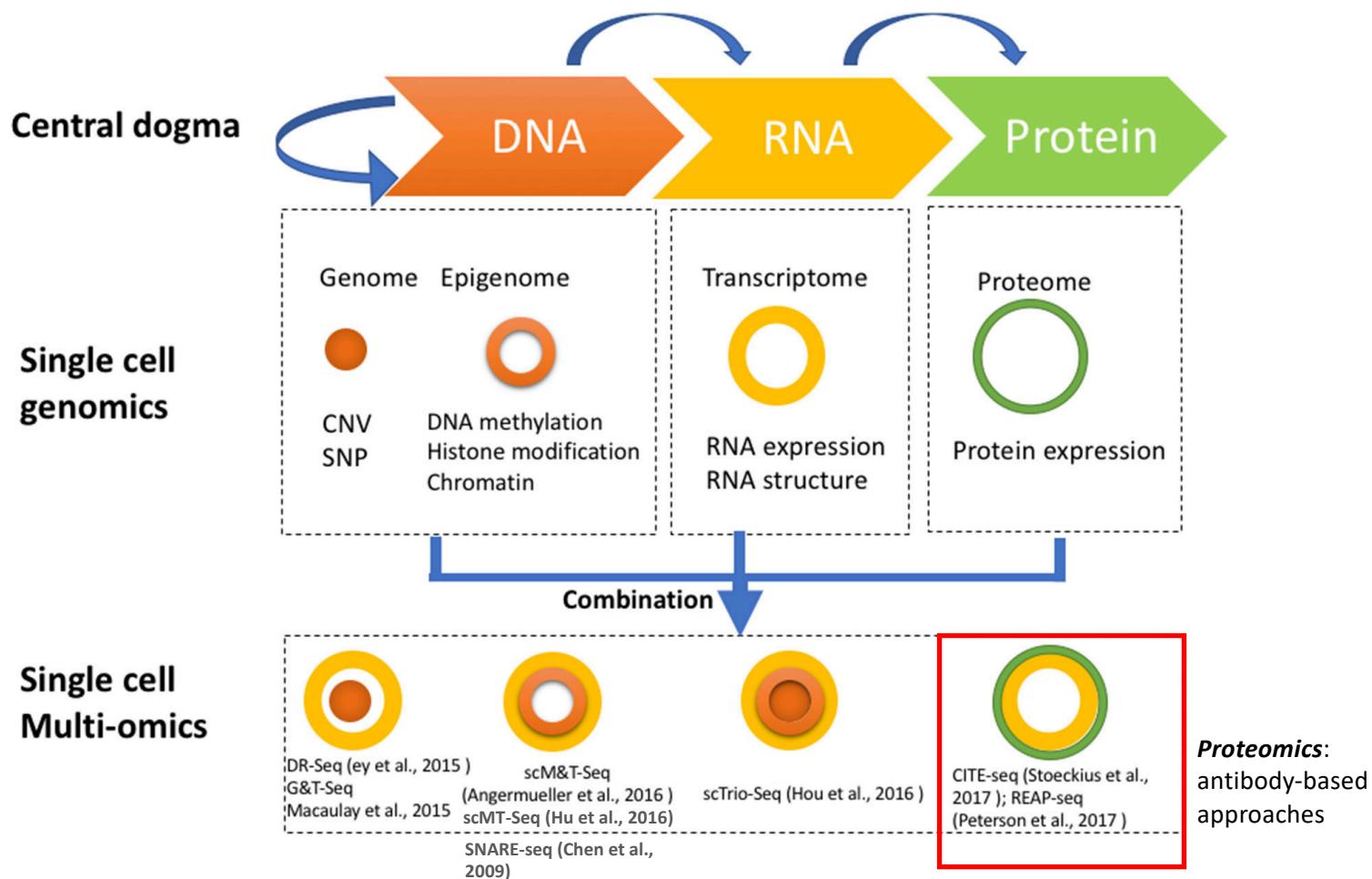
Song Chen^{ID}, Blue B. Lake^{ID} and Kun Zhang^{ID*}



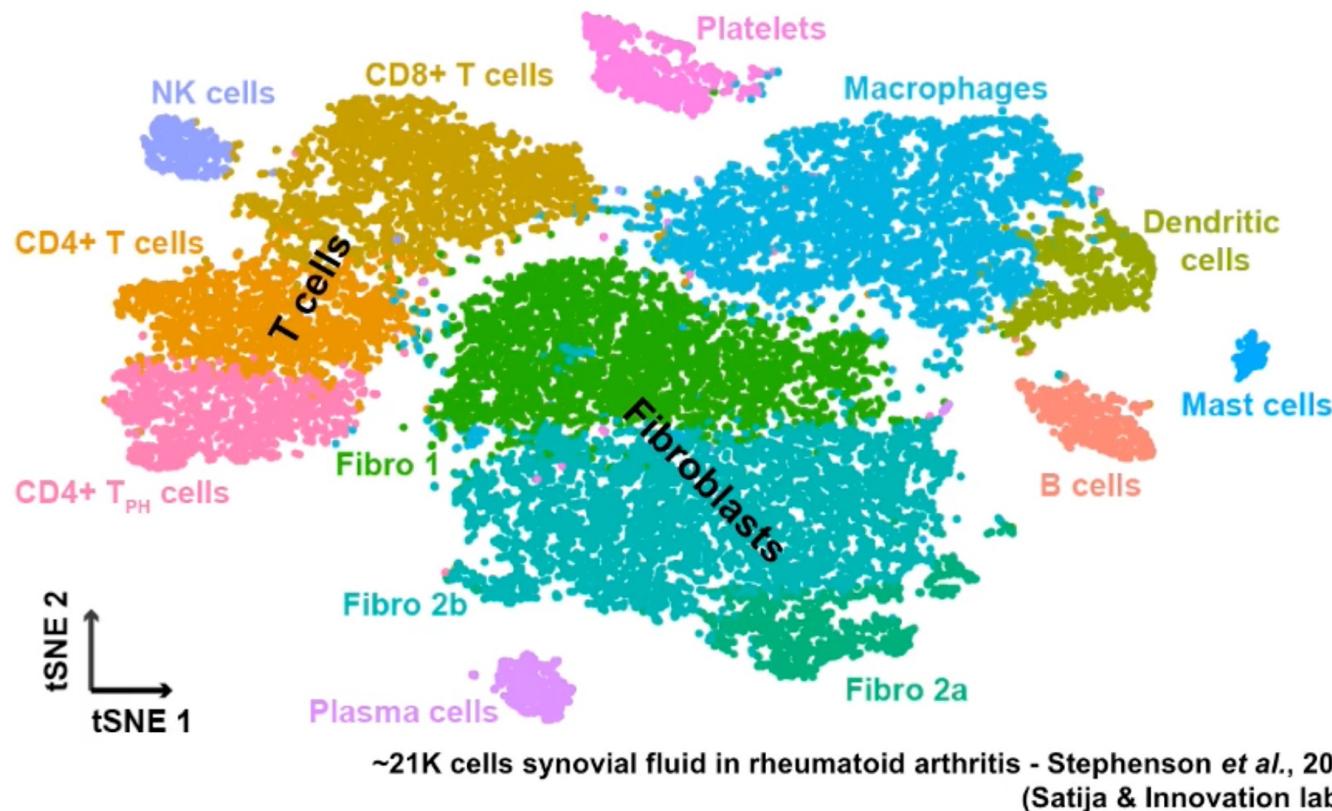
10X Single Cell Multiome (scATAC + scRNA)



Strategies for multi-omics profiling of single cells

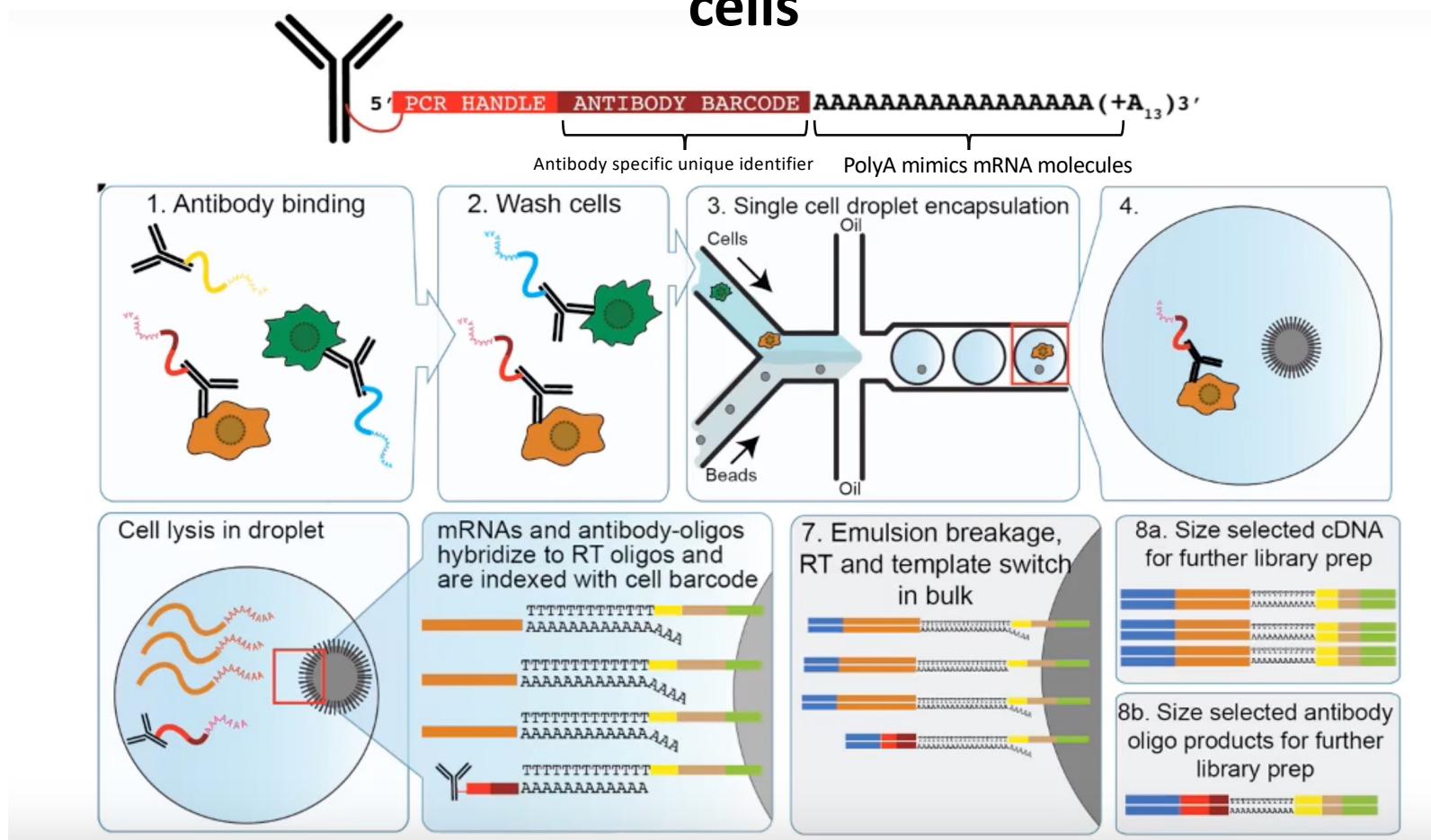


Distinguishing cells/states that are transcriptomically similar is difficult in scRNA-seq



- 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

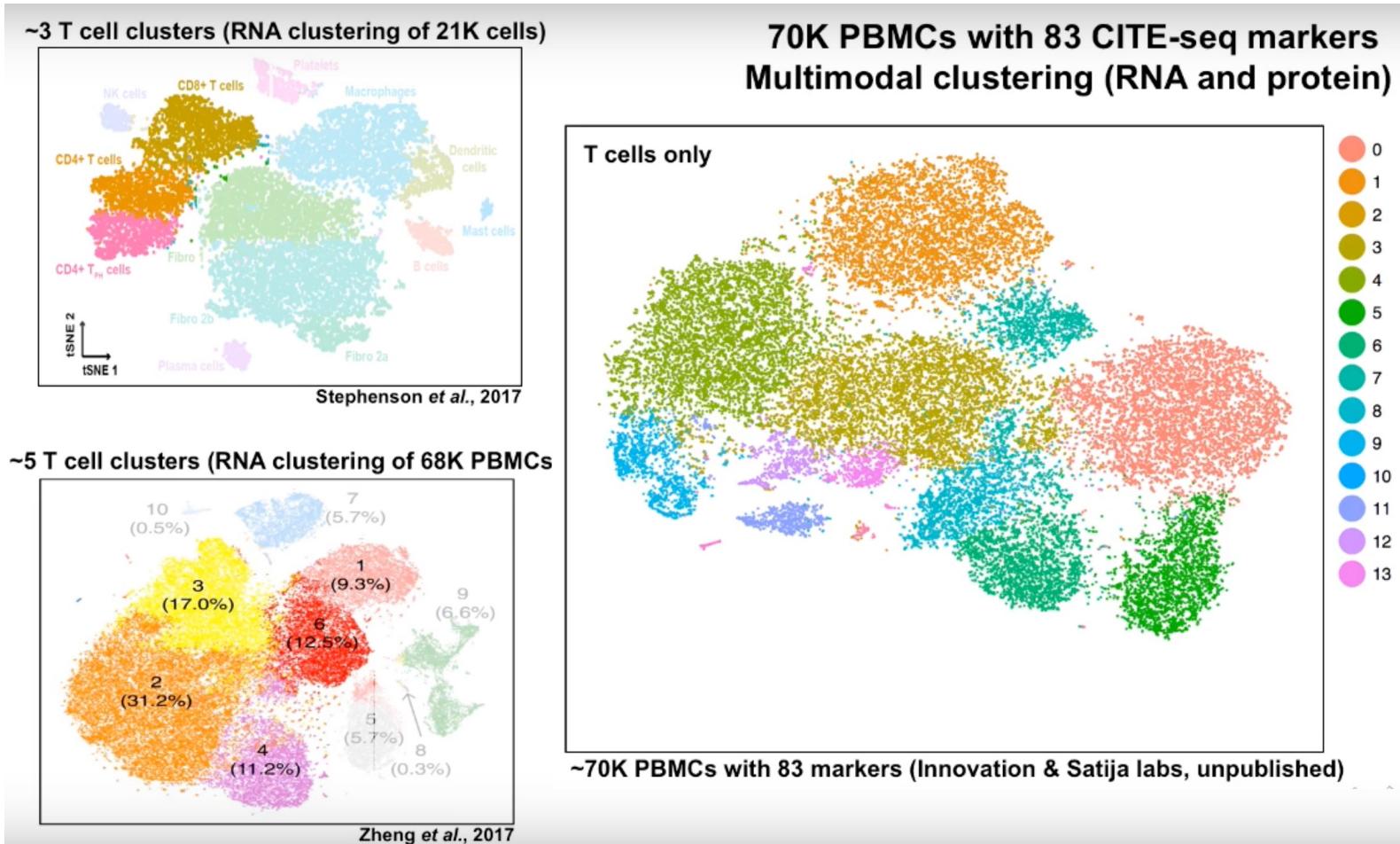


Marlon Stoeckius group

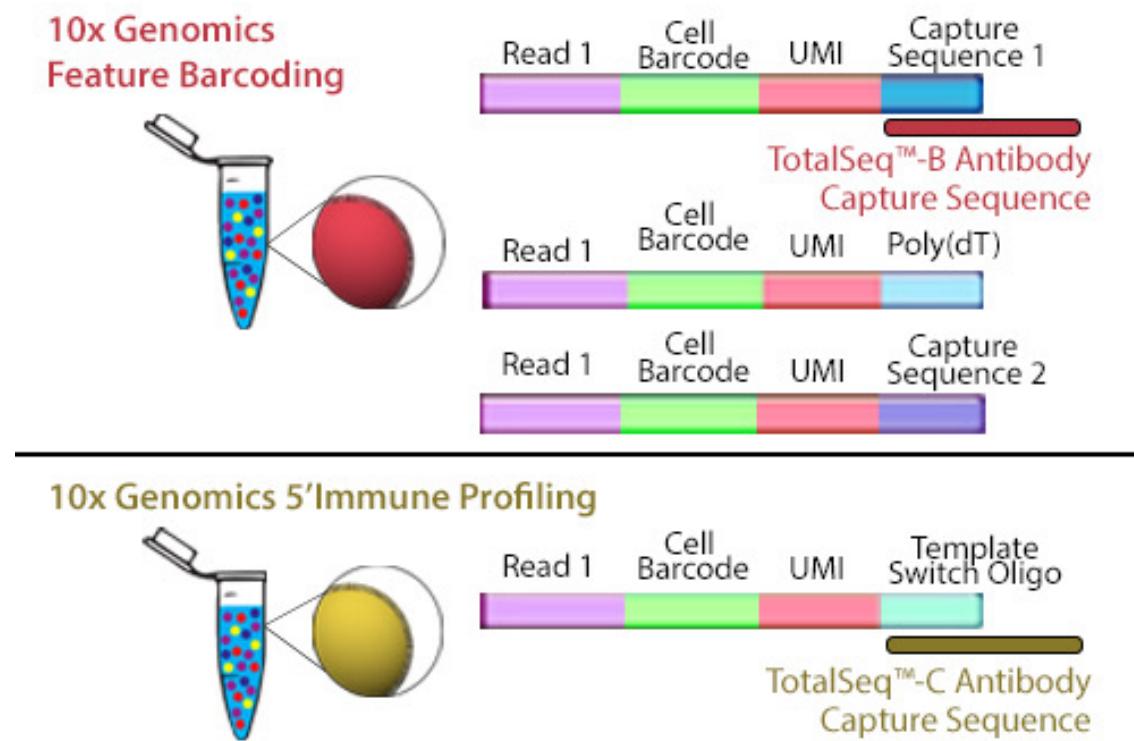
Nature Methods, 2017, 14, 865–868

Multimodal clustering resolves more T cell clusters

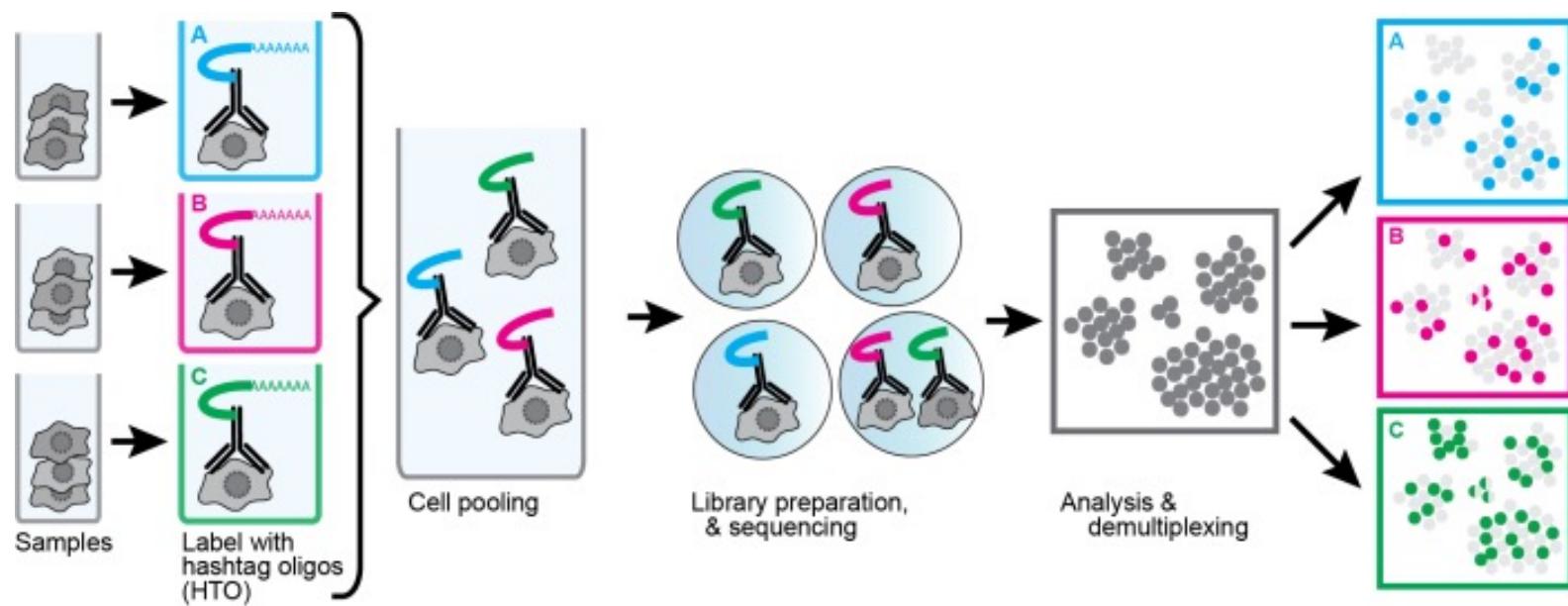
13 T cell clusters with distinct protein and RNA markers



CITE-seq method is also available via 10X Genomics

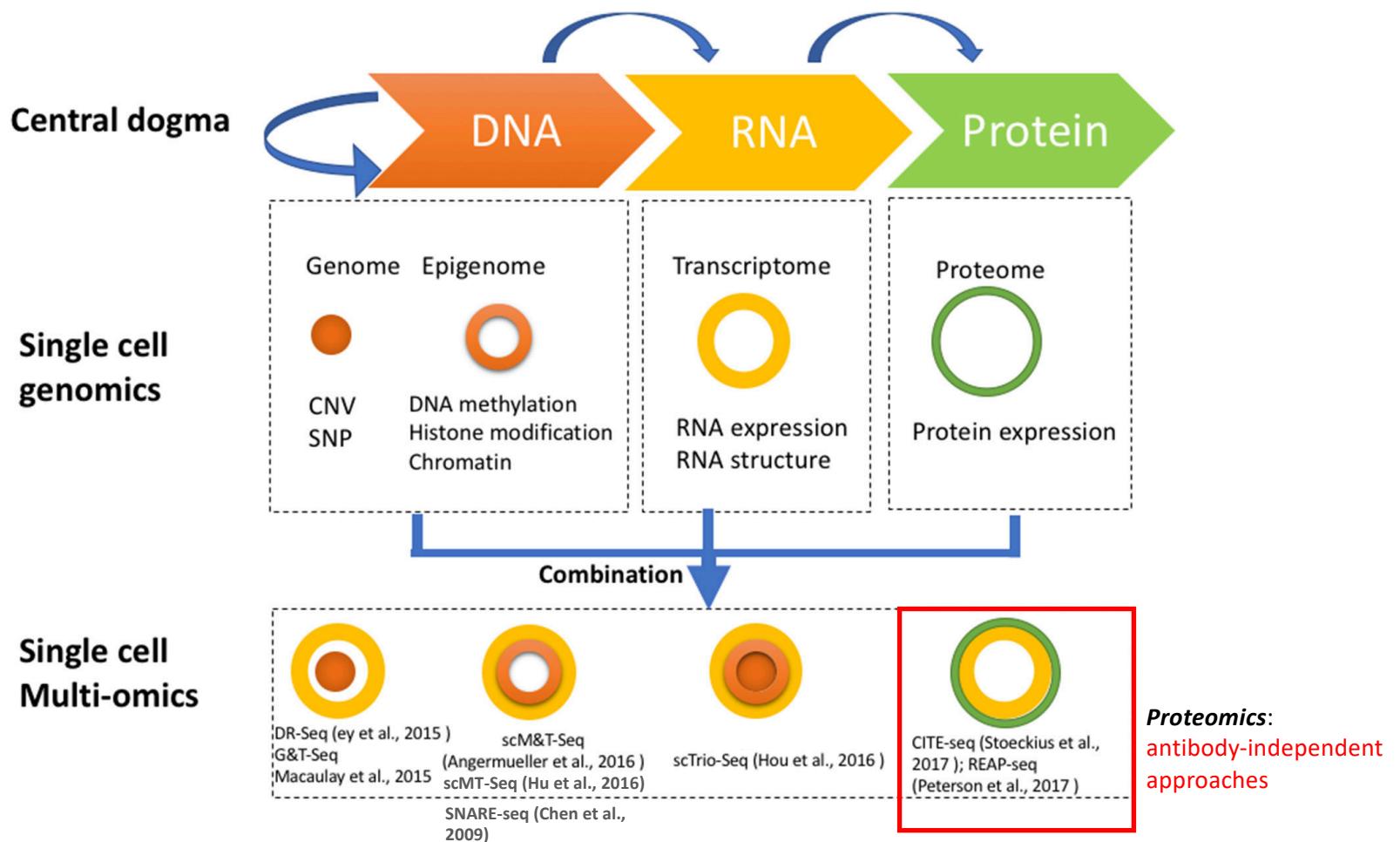


Cell Hashing: apply CITE-seq to multiplex multiple samples



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

Strategies for multi-omics profiling of single cells



Single cell whole transcriptome & proteome analysis

RESEARCH

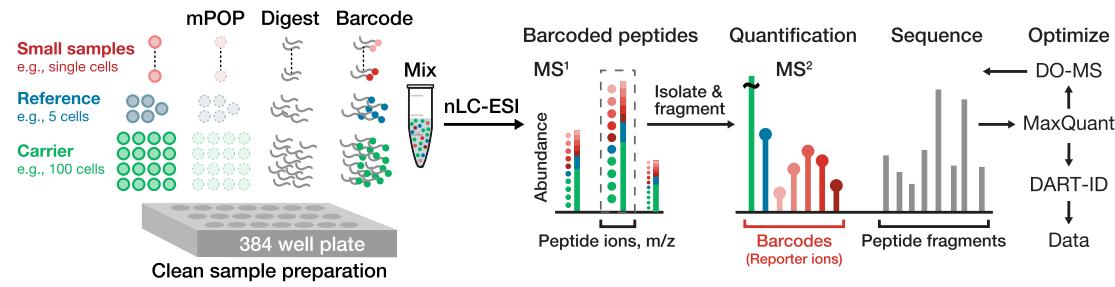
Open Access



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