

Single cell multi-omics

Miao-Ping Chien

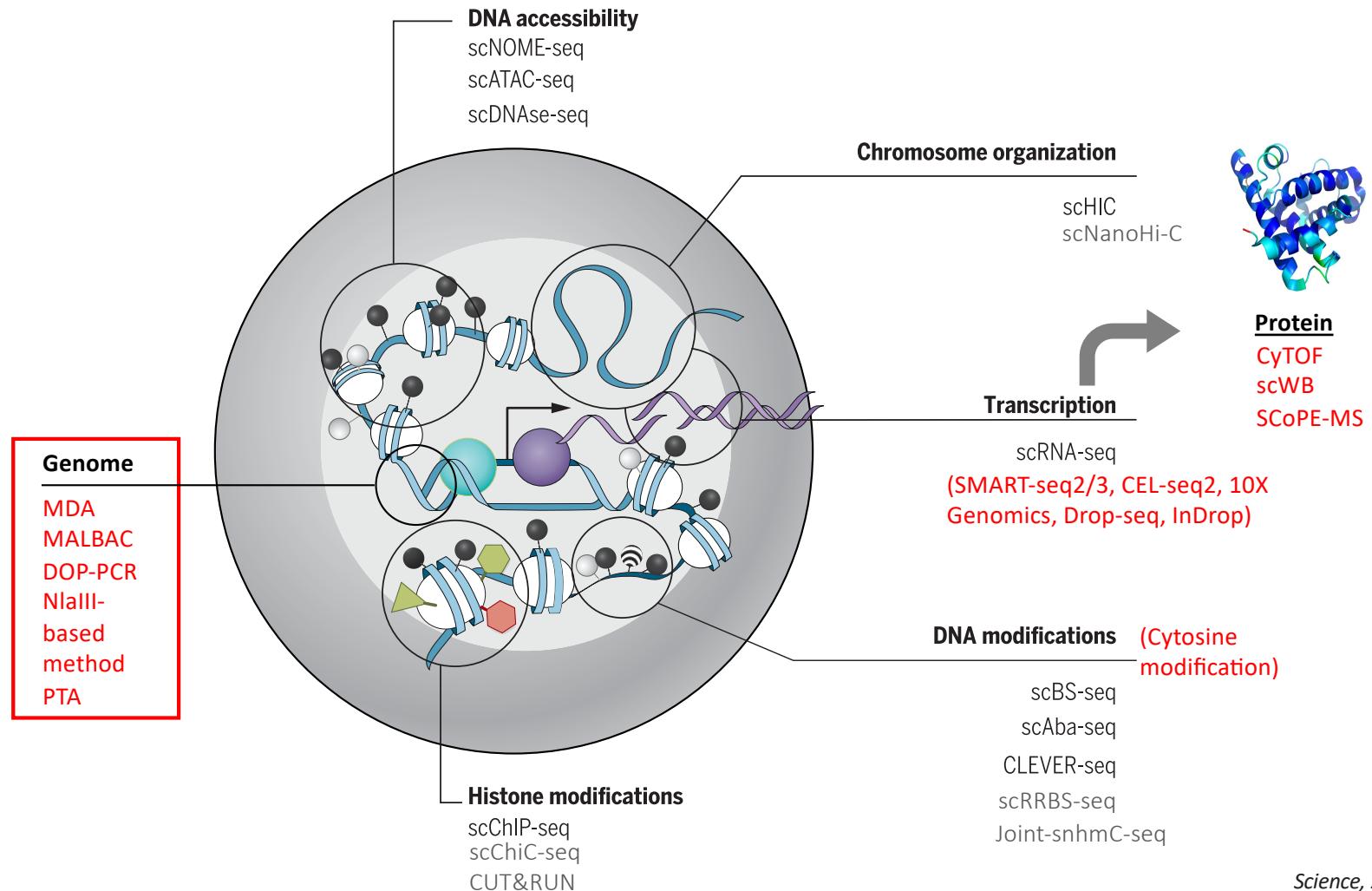
Erasmus MC, Associate professor

2024 Single Cell Analysis Workshop, 2024/10/30

Outline

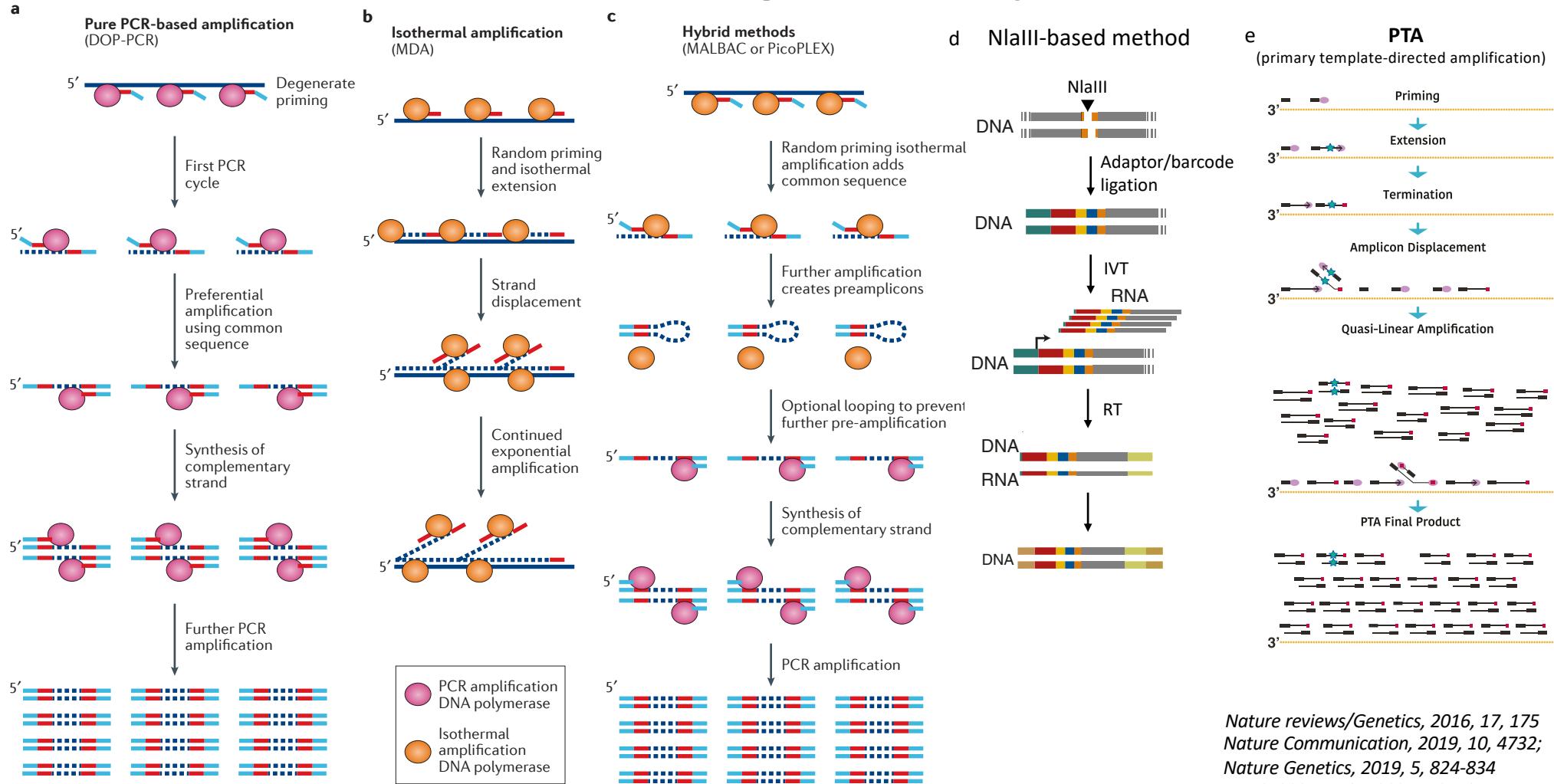
- (Quick) overview of different single cell –omics methods
- Different single cell multi–omics methods

Overview of single cell -omics



Single cell genomic sequencing

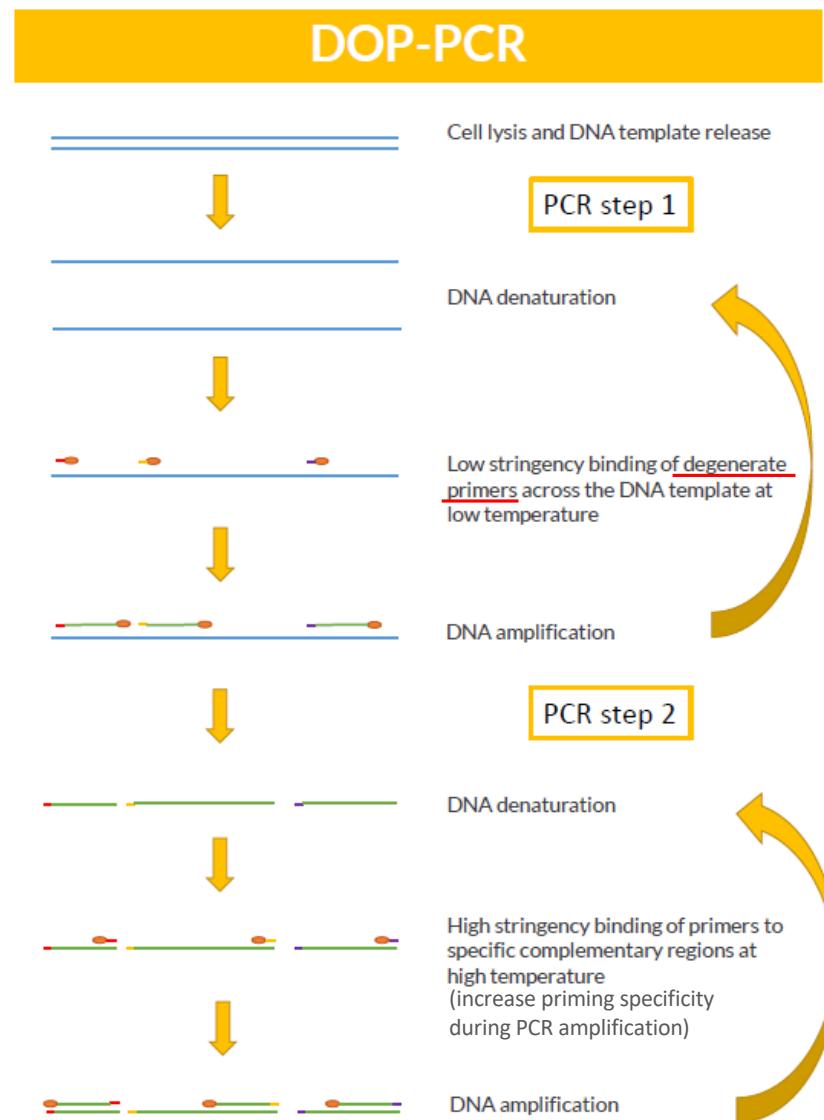
Overview of the main whole-genome amplification methods



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



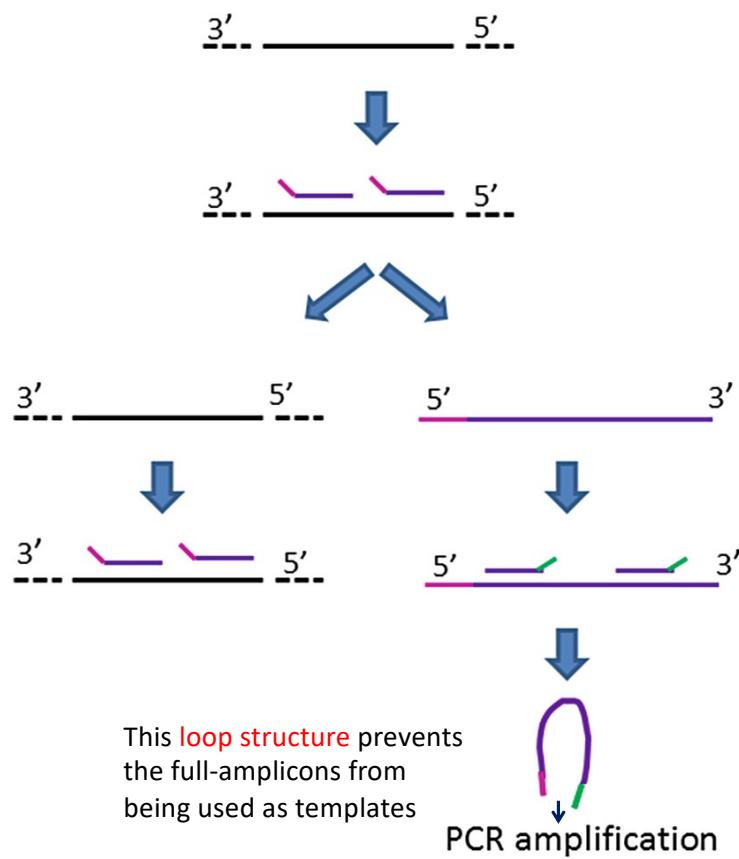
MALBAC vs MDA

Isothermal amplification

MDA: multiple displacement amplification

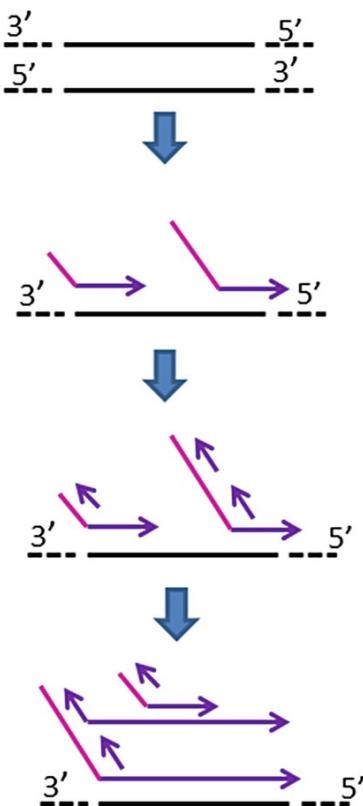
A

MALBAC



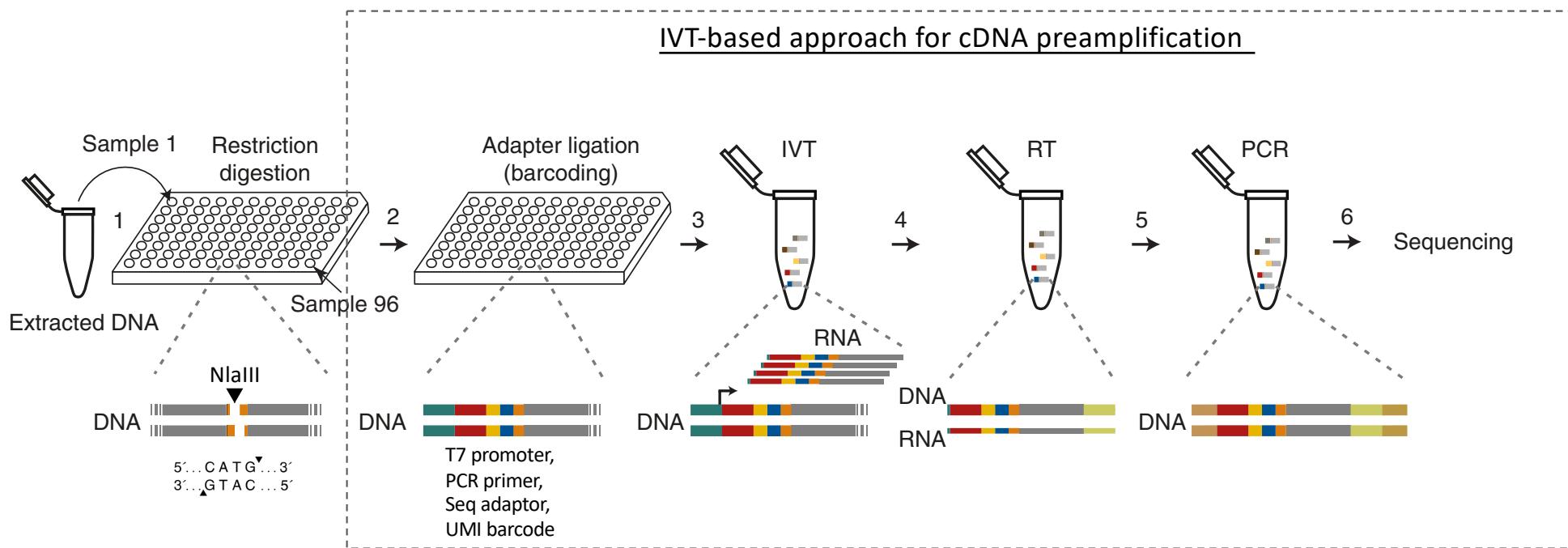
B

MDA

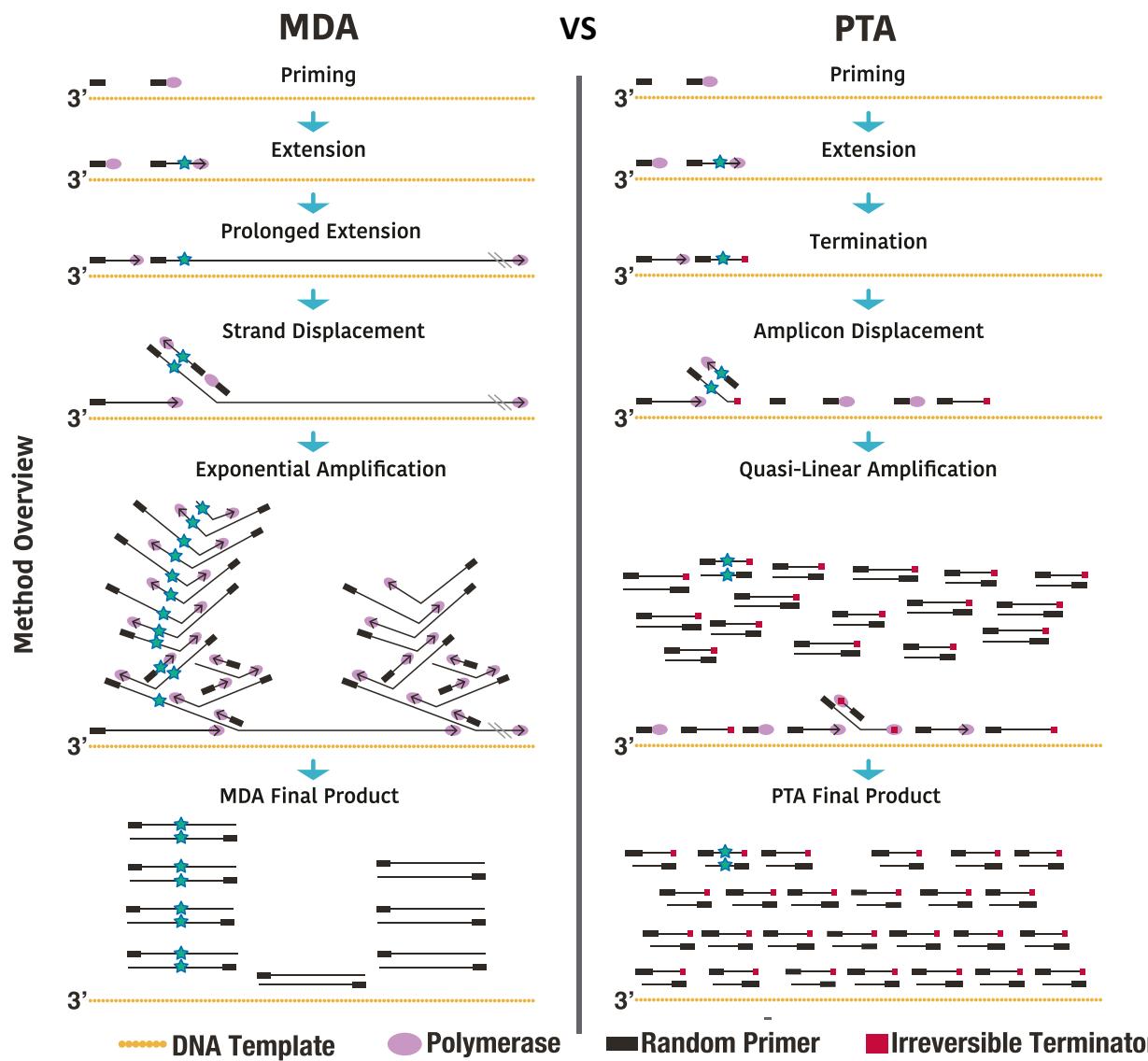


- The **phi29 DNA polymerase** enables the newly synthesized strand to displace the formerly synthesized one on the same template.
- Free primers will anneal to these displaced single-strands and continue such cycles of "displace and anneal".

NlaIII-based scDNAseq method



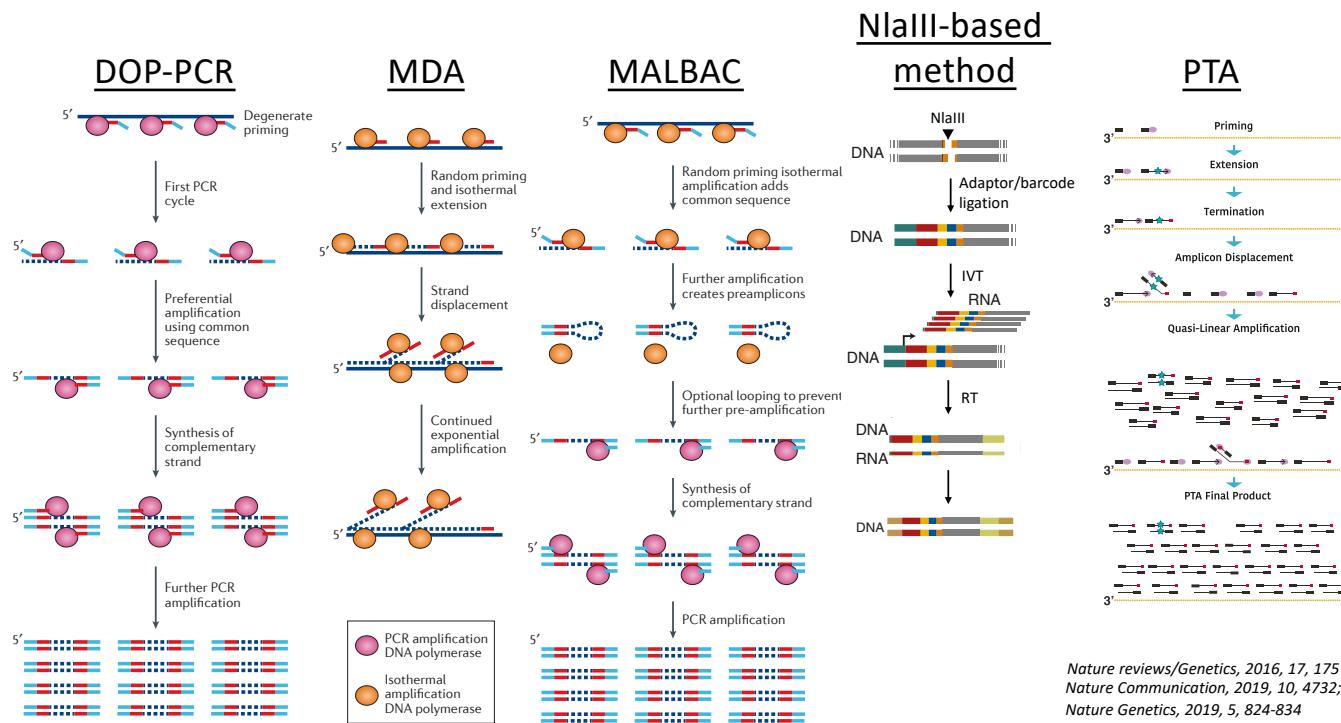
*Nature Communication, 2019, 10, 4732;
Nature Genetics, 2019, 5, 824-834*



PTA (primary template-directed amplification):

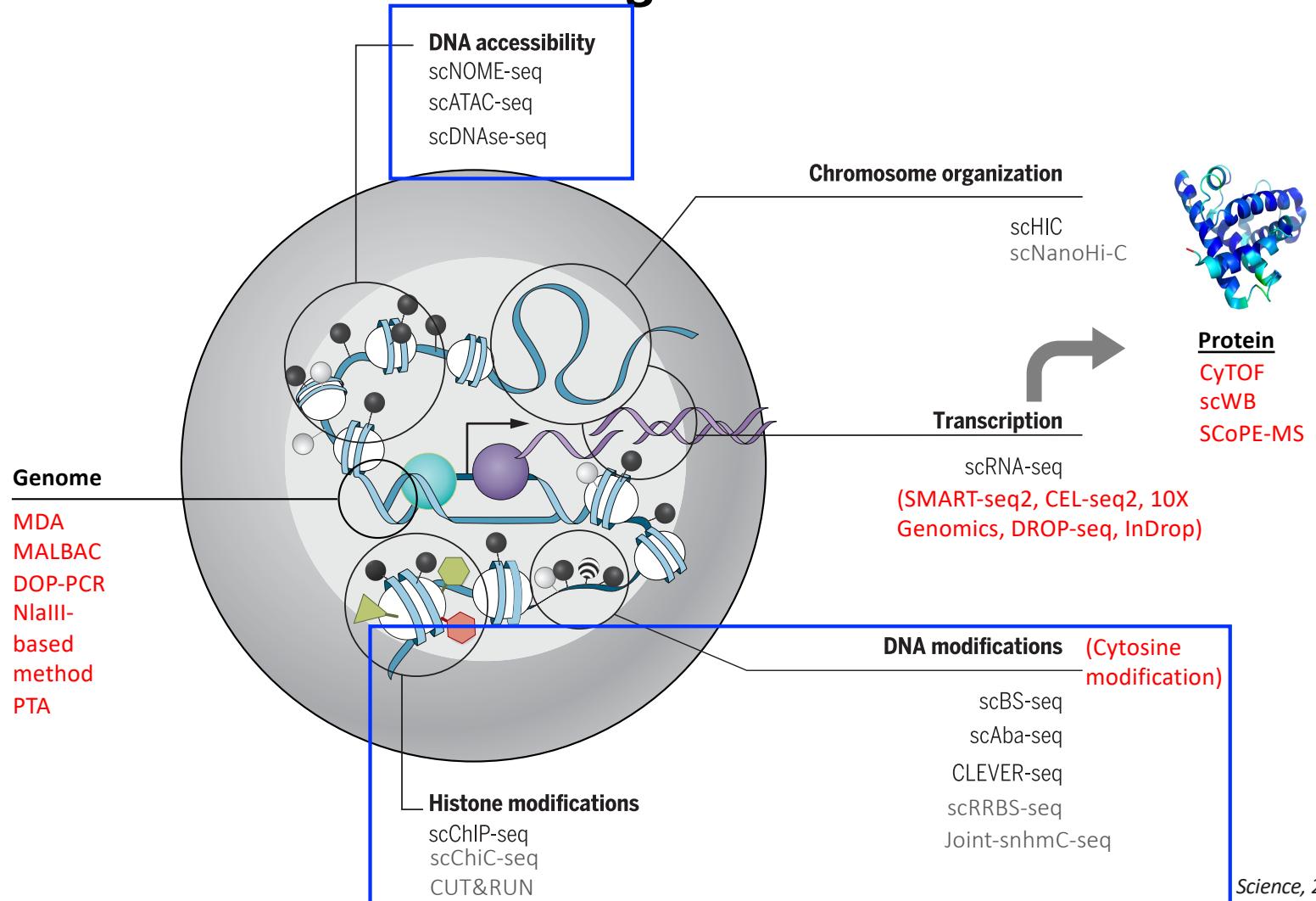
- incorporation of **exonuclease-resistant terminators** in the reaction result in smaller double-stranded amplification products
- This approach undergoes **limited subsequent amplification**, resulting in a quasilinear process with more amplification **originating from the primary template**.
- With 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



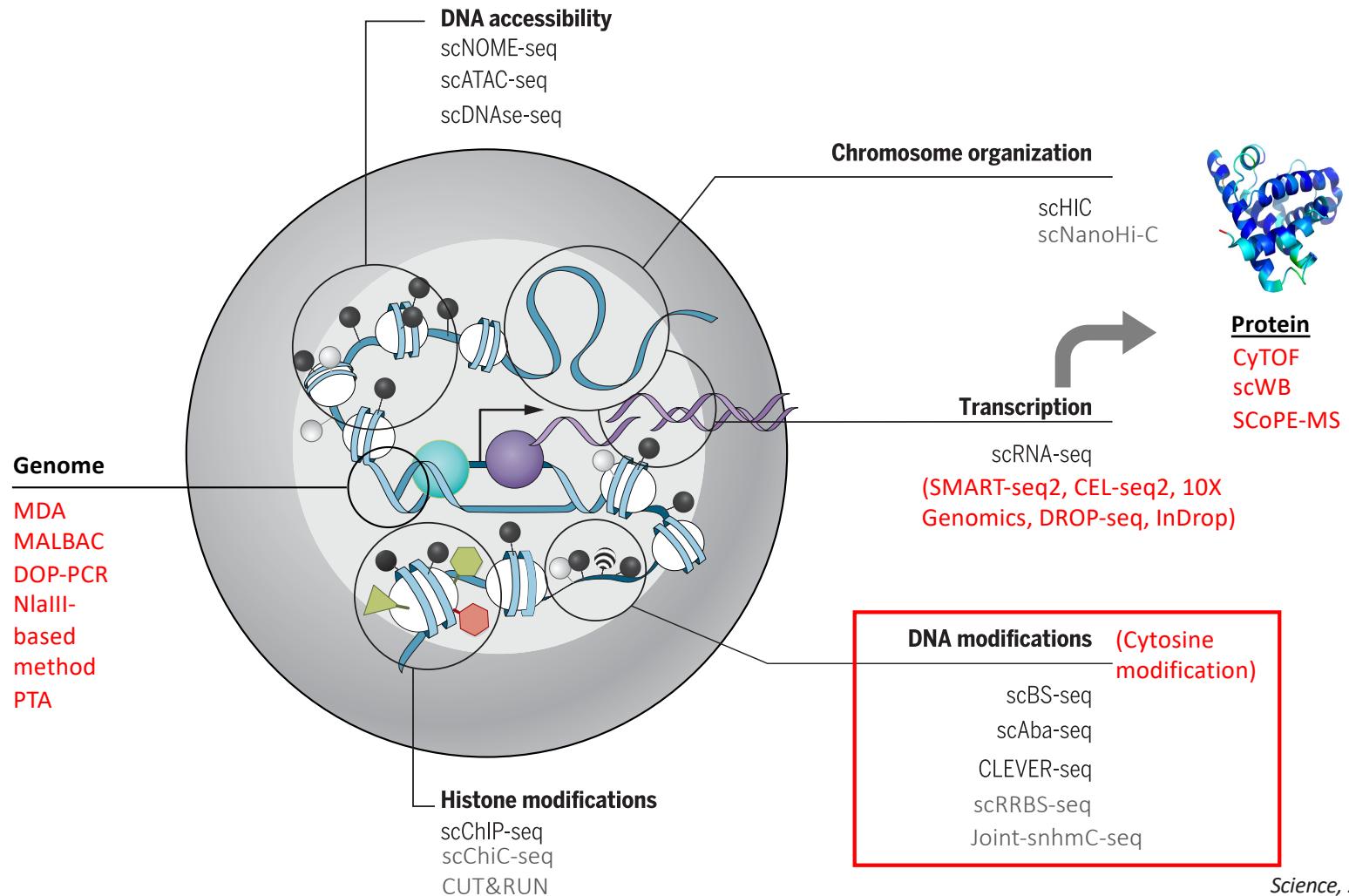
- Highest to lowest genome recovery rate?
PTA (>95%) > MDA (~80%) > MALBAC (~50%) > DOP-PCR (~6%) / NlaIII-based method (~5-10%)
- Highest to lowest duplication ratio (duplicate the product more than the original template)?
DOP-PCR >> MDA > MALBAC, NlaIII-based method > PTA
- Homogeneous amplification (uniformity)?
PTA > MALBAC, NlaIII-based method > MDA > DOP-PCR

Overview of single cell -omics



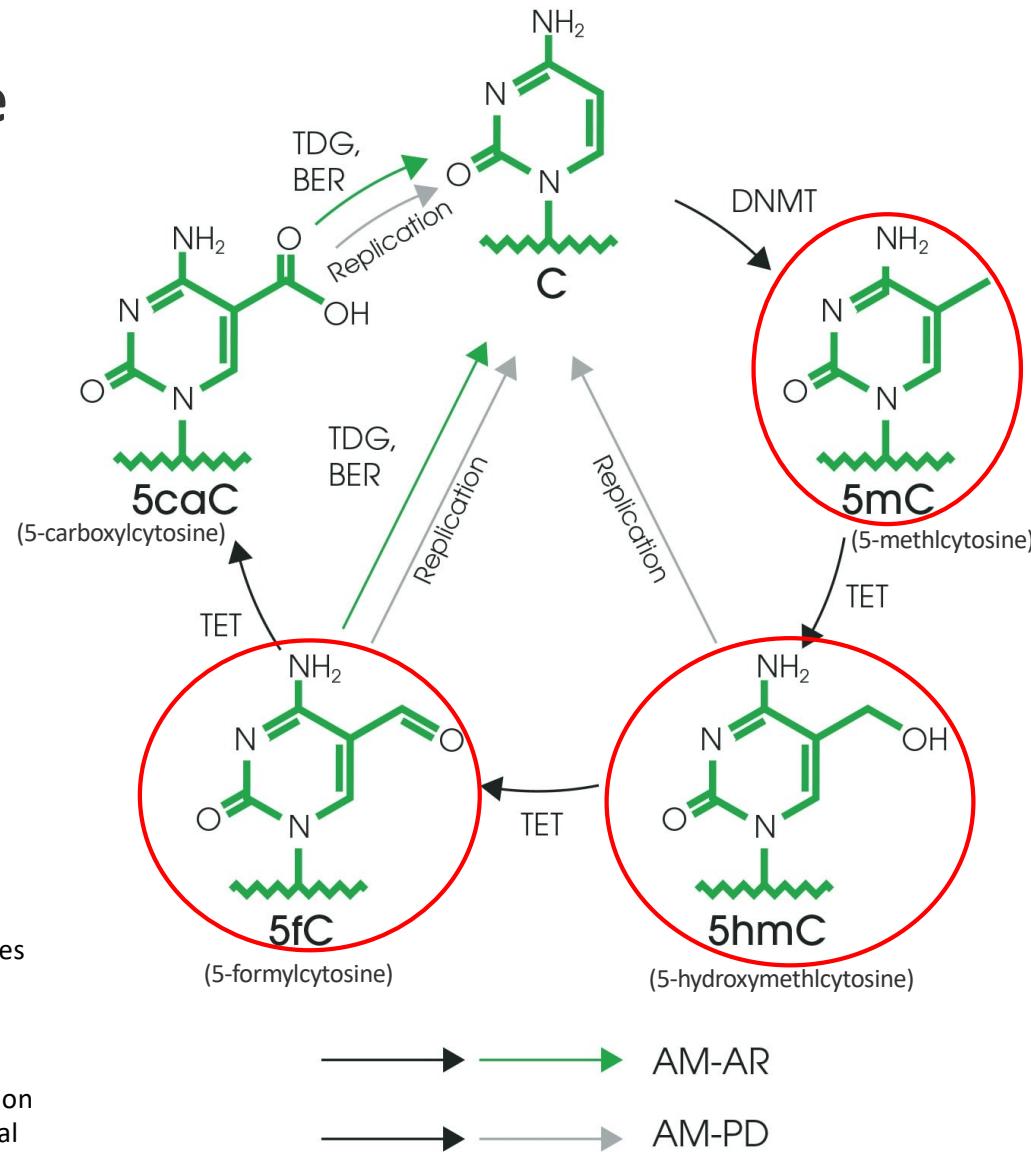
Single cell epigenomic sequencing

Overview of single cell -omics



Science, 2017, 358, 69–75

The cycle of cytosine (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

Fragmented Genomic DNA Samples

Step 2

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

Cytosine

CN1C=CC2=C1C(=O)N(C2=O)[Na+].NaHSO3.[H] >> CN1C=CC2=C1C(O)C(C2=O)[Na+].NaHSO3.[H]

Step 3

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

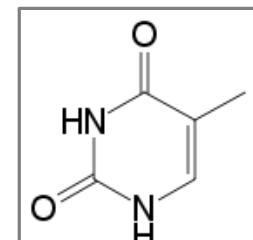
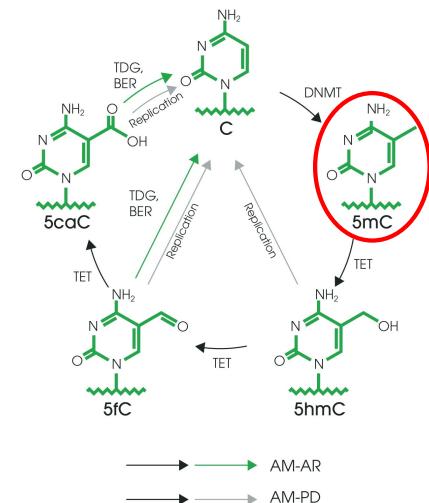
Uracil

CN1C=CC2=C1C(=O)N(C2=O)[Na+].NaHSO3.[OH] >> CN1C=CC2=C1C(O)C(C2=O)N([OH])[Na+].NaHSO3.

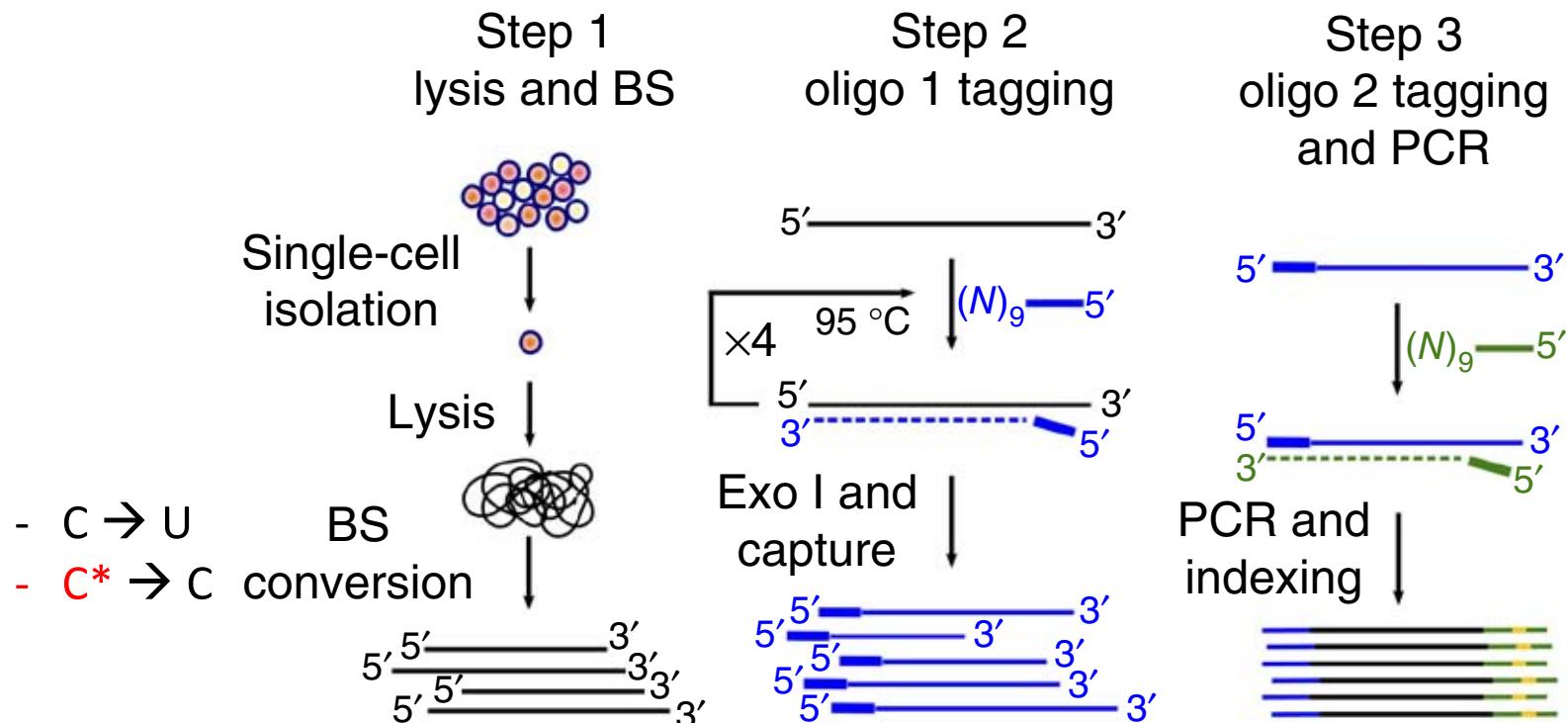
5-Methylcytosine (5-mC)

CN1C=CC2=C1C(=O)N(C2=O)CH3.[NaHSO3].[H] >> X

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



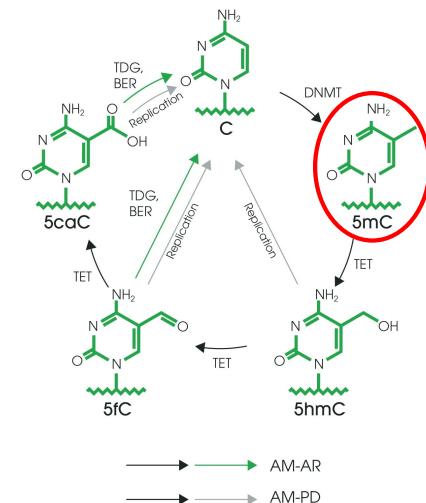
Single cell bisulfite sequencing (scBS-seq)



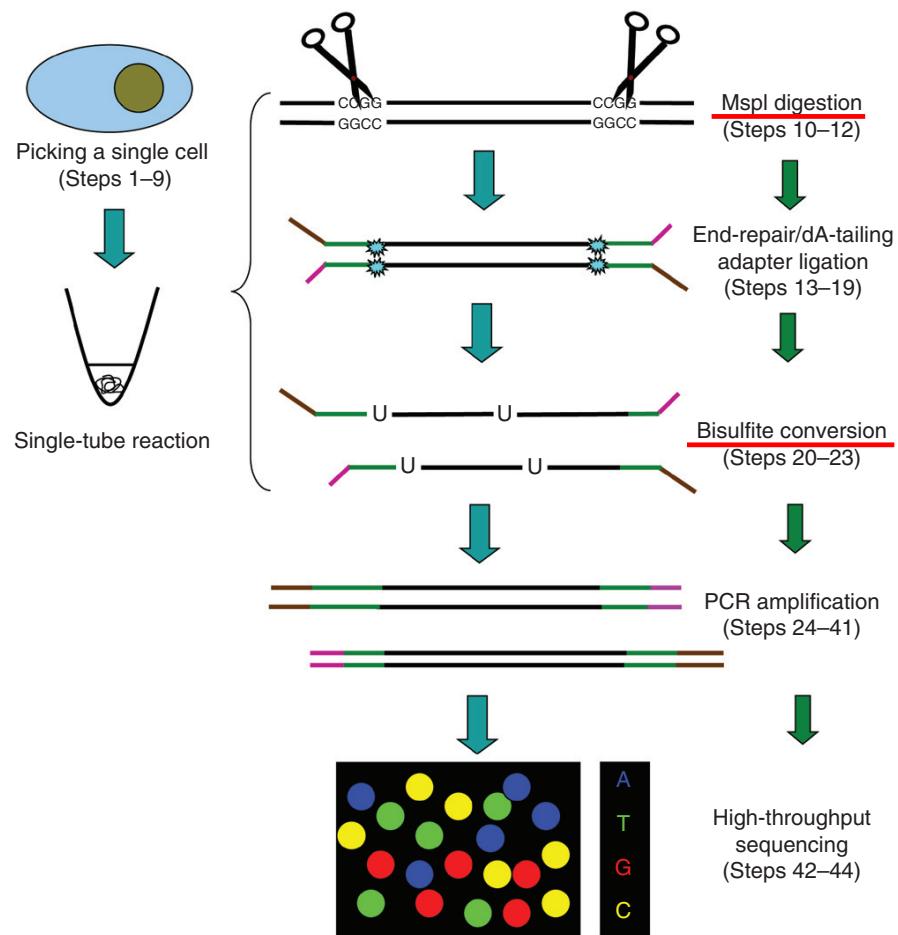
Step 1: Bisulfite (BS) treatment:
DNA fragmentation & conversion of unmethylated cytosines to thymine

Step 2: Synthesis of complementary strands is primed with random priming and extension using oligo 1.

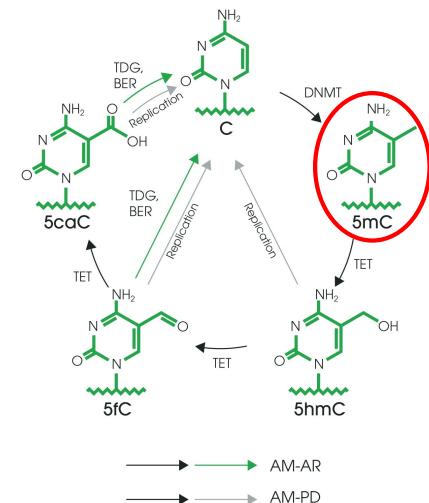
Step 3: Second random priming and extension step using oligo 2



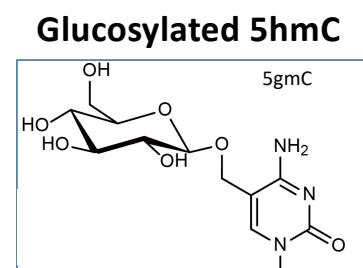
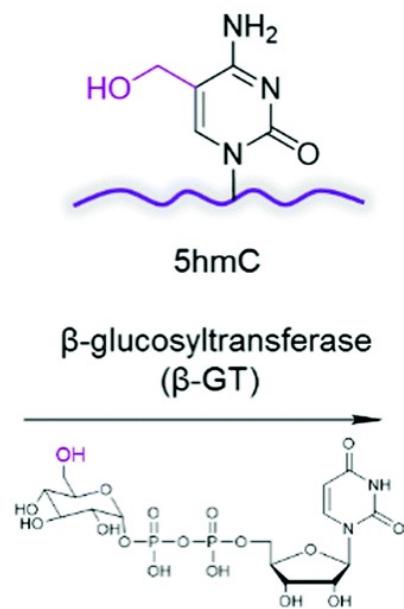
scRRBS-seq: single-cell reduced-representation bisulfite sequencing



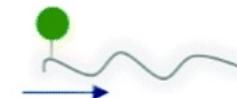
Mainly focus on the **CpG island**
 (related to gene expression regulation
 → methylation of “C” in CpG island silences gene expression)



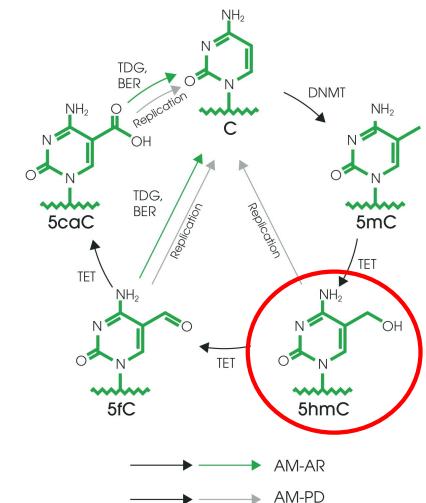
scAba-seq : single-cell 5hmC sequencing

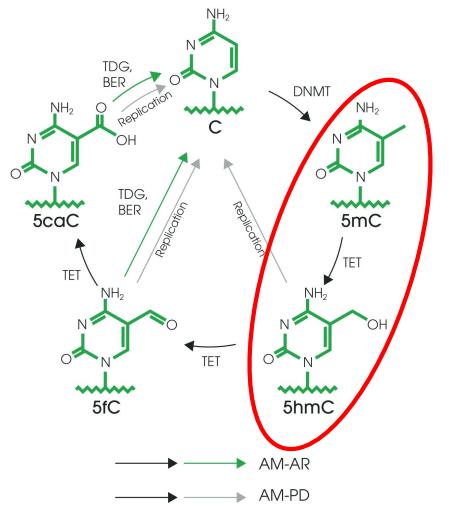


AbaSI
exonuclease



Deep sequencing



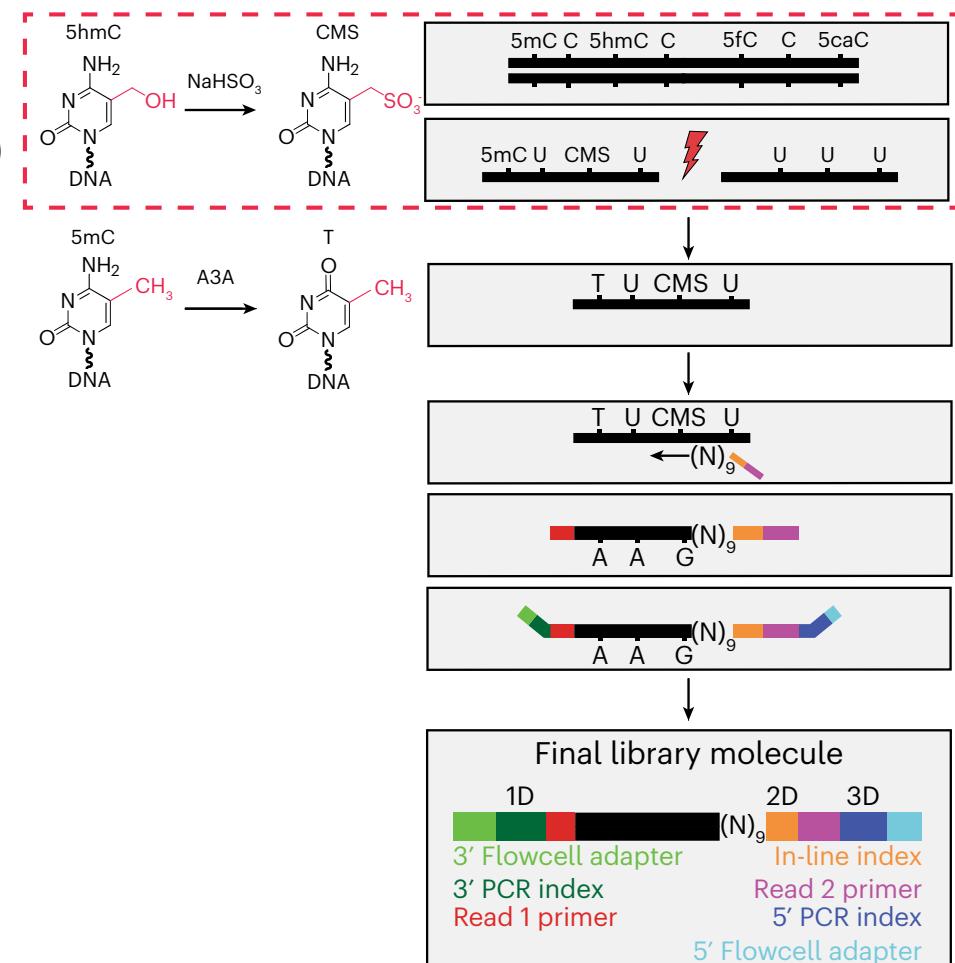


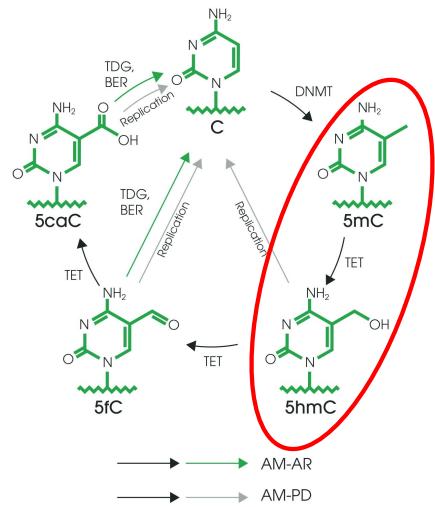
Simultaneously profiles 5hmC & 5mC in single cells

- joint single-nucleus (hydroxy)methylcytosine sequencing

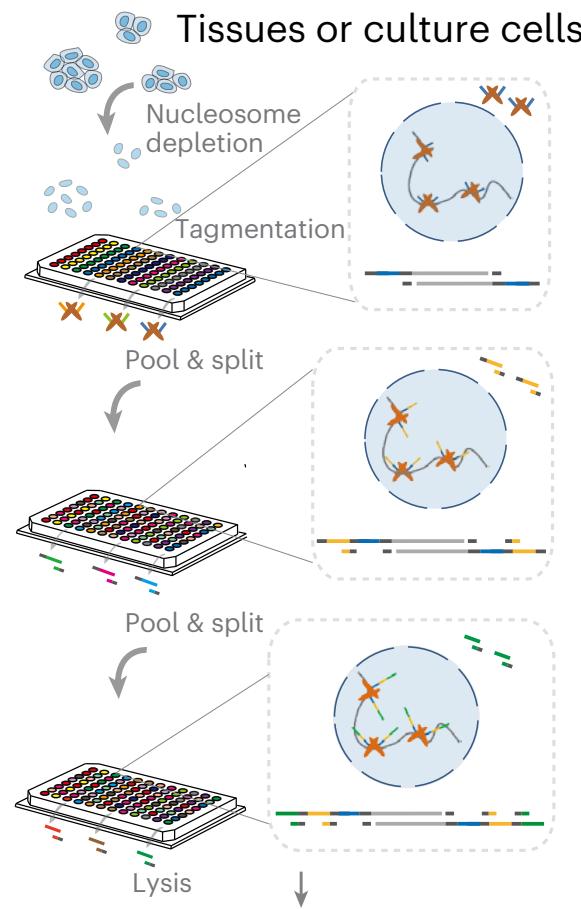
I) 5hmC to CMS
(Cytosine-5-methylenesulfonate)
– read as “C”

II) 5mC to T using
A3A (APOBEC3A deaminase)



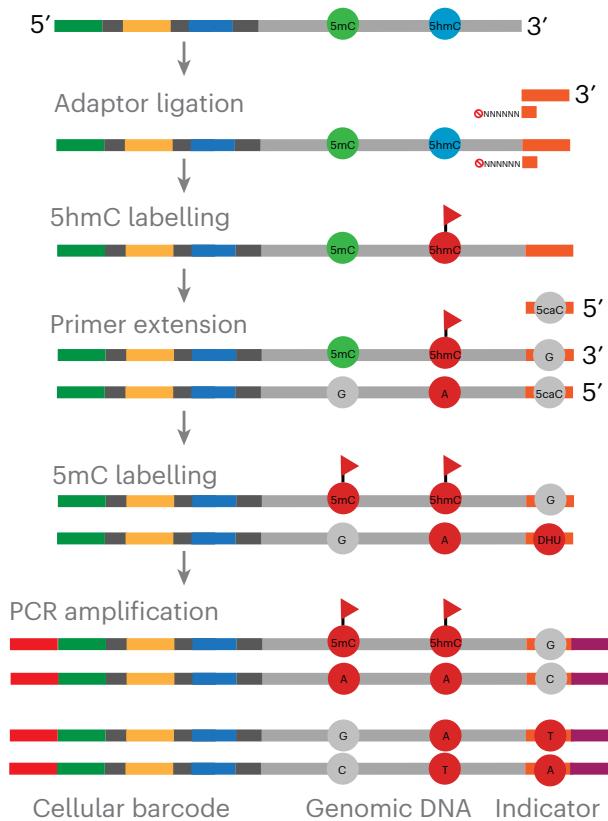


Simultaneously profiles 5hmC & 5mC in single cells



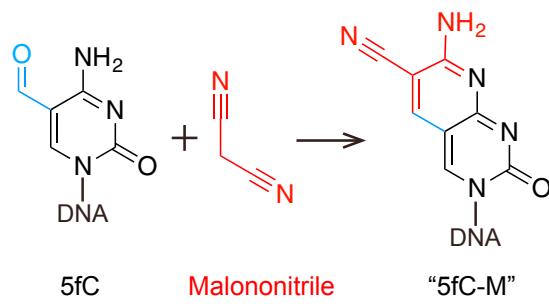
SIMPLE-seq

- Simultaneous single-cell analysis of 5mC & 5hmC

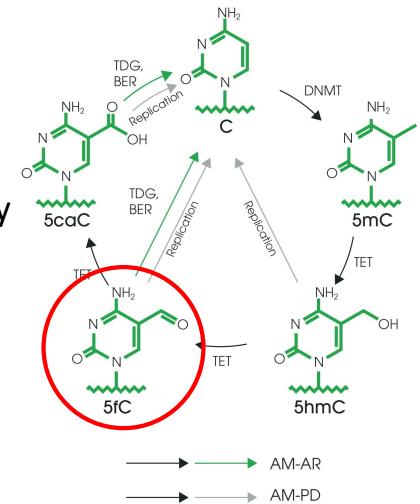
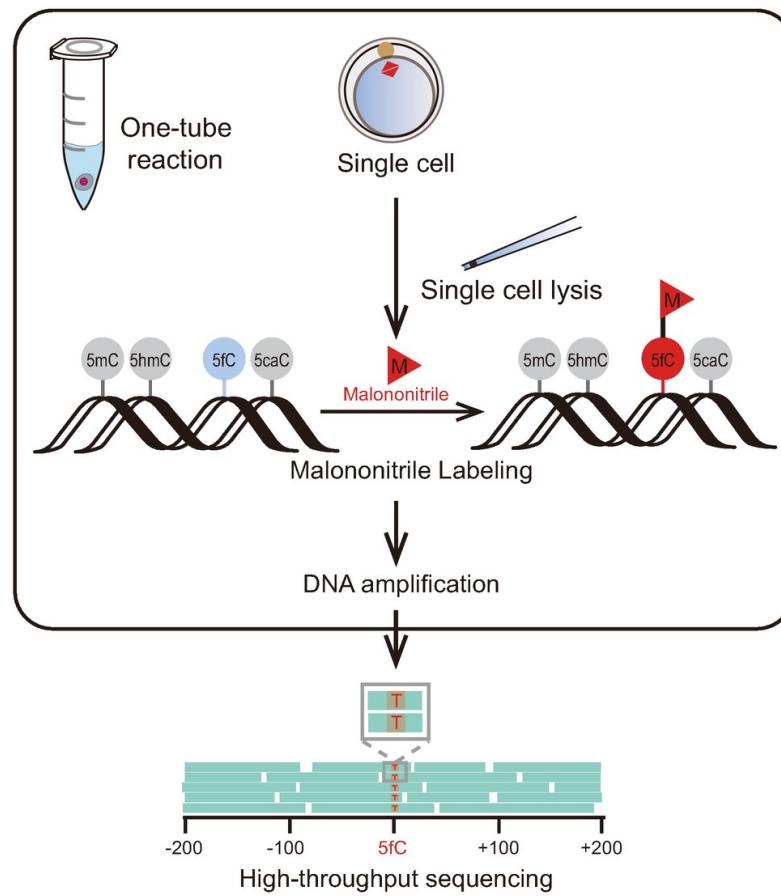


CLEVER-seq

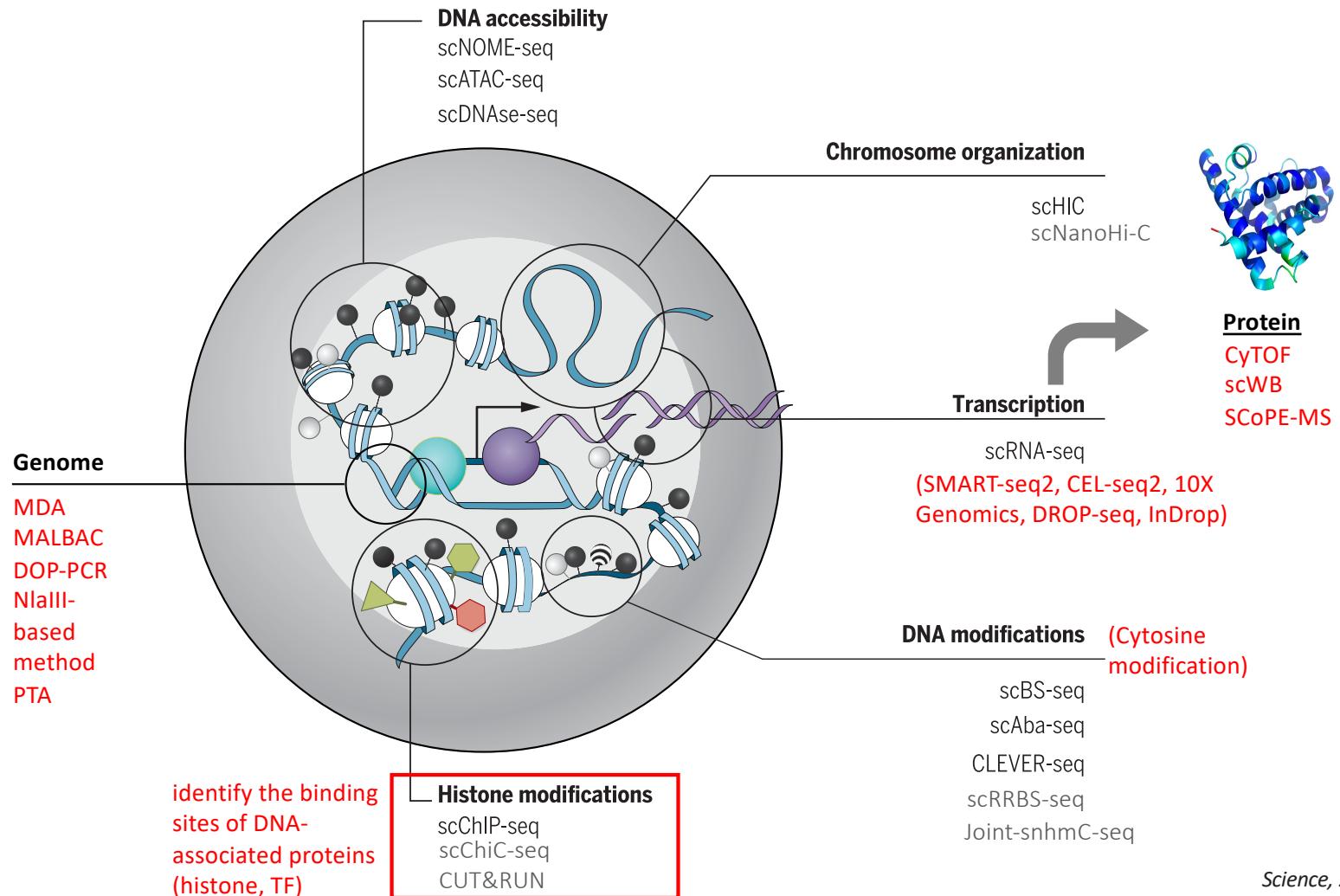
- Chemical-labeling-enabled C-to-T conversion sequencing
- A single-cell, single-base resolution whole-genome **5fC**-sequencing technology



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



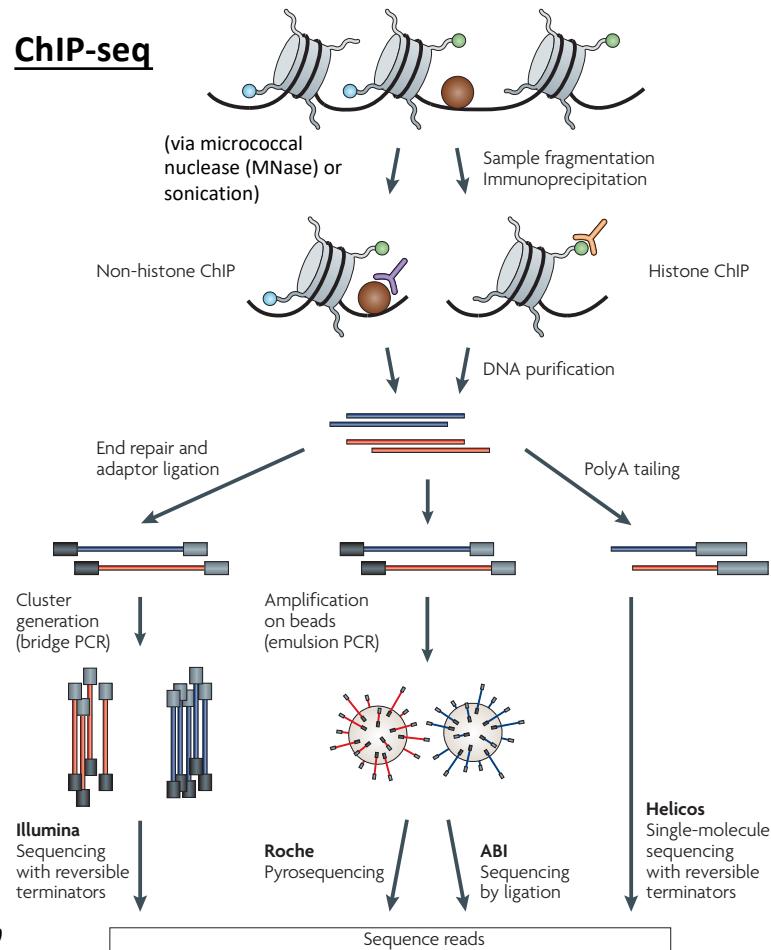
Overview of single cell -omics



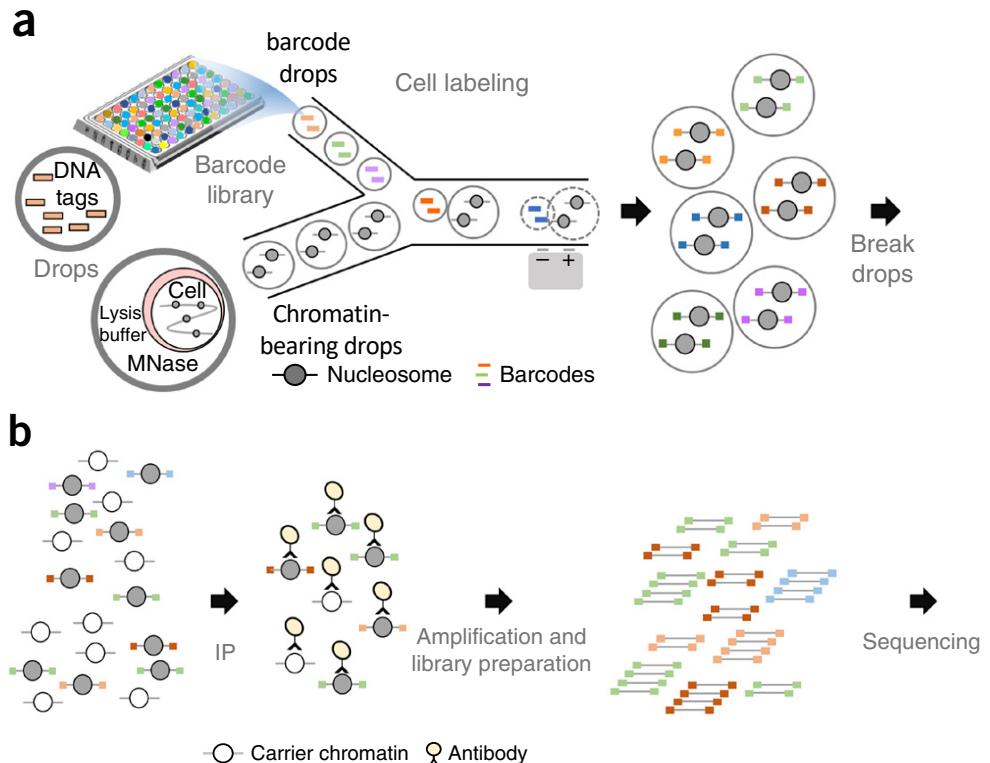
Single cell sequencing for histone modifications

Single cell chromatin immunoprecipitation (ChIP) sequencing

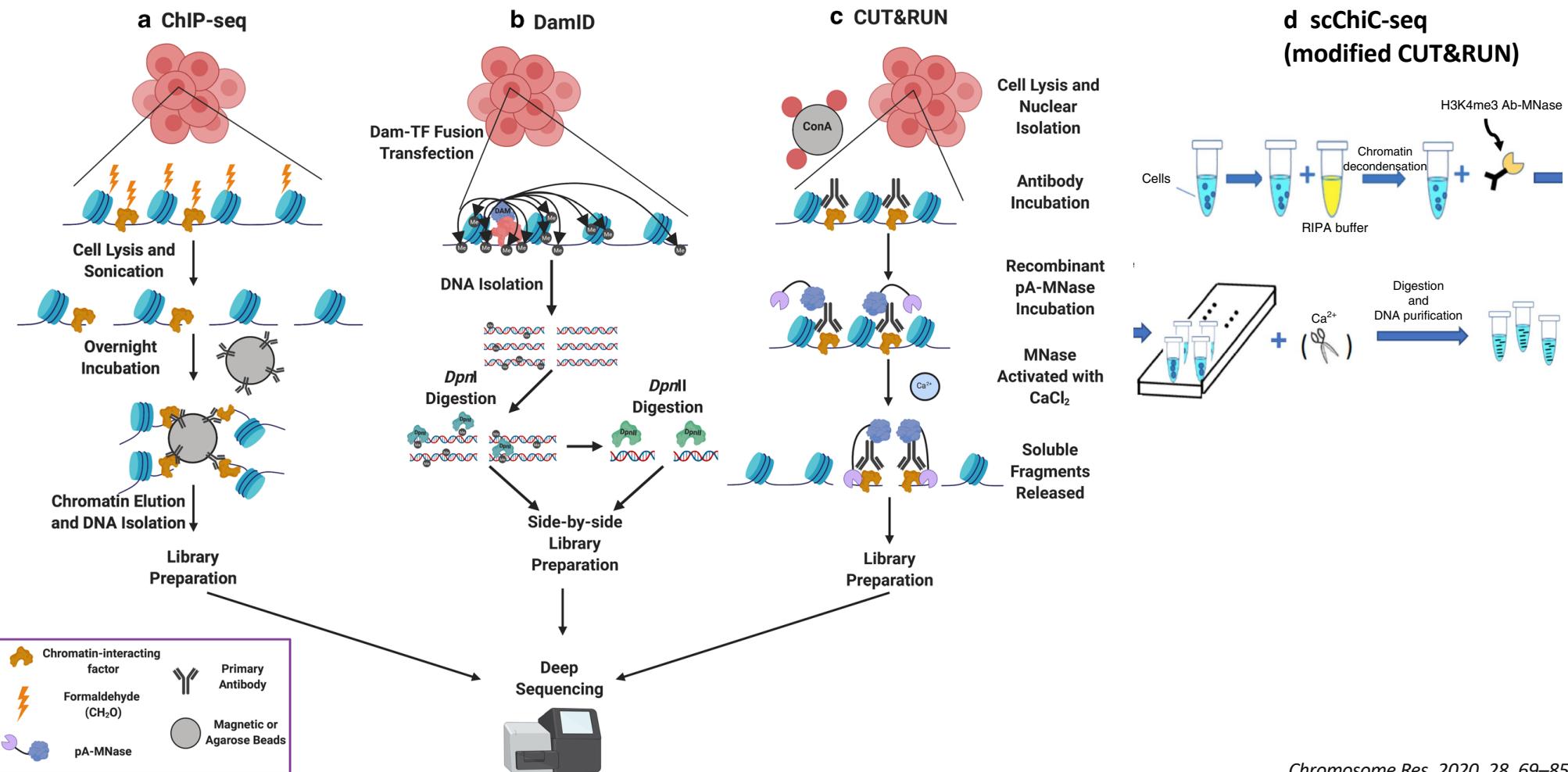
ChIP-seq is a widely used method for mapping histone modifications, transcription factors and other protein-DNA interactions genome-wide



scChIP-seq

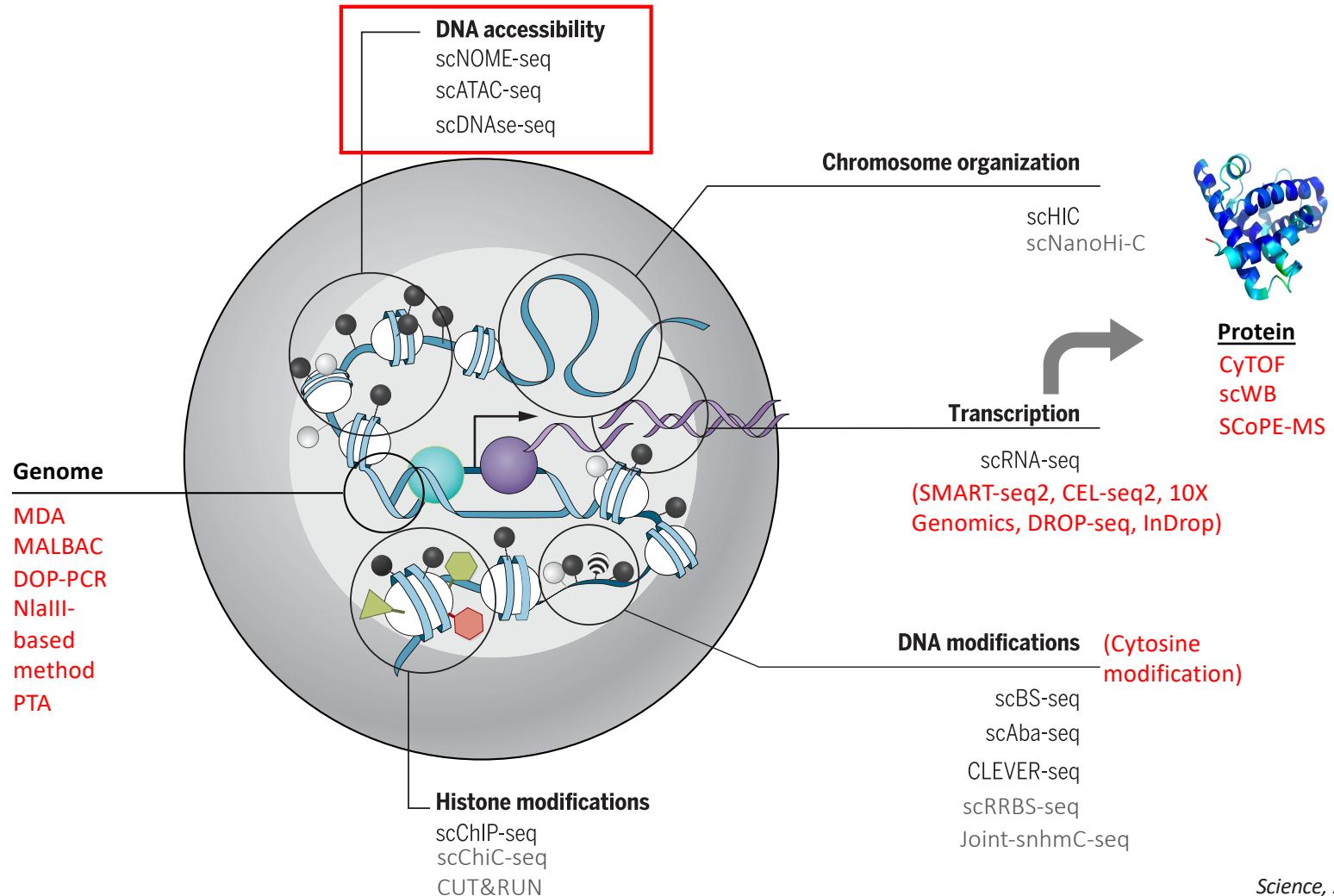


Method comparison for histone modifications

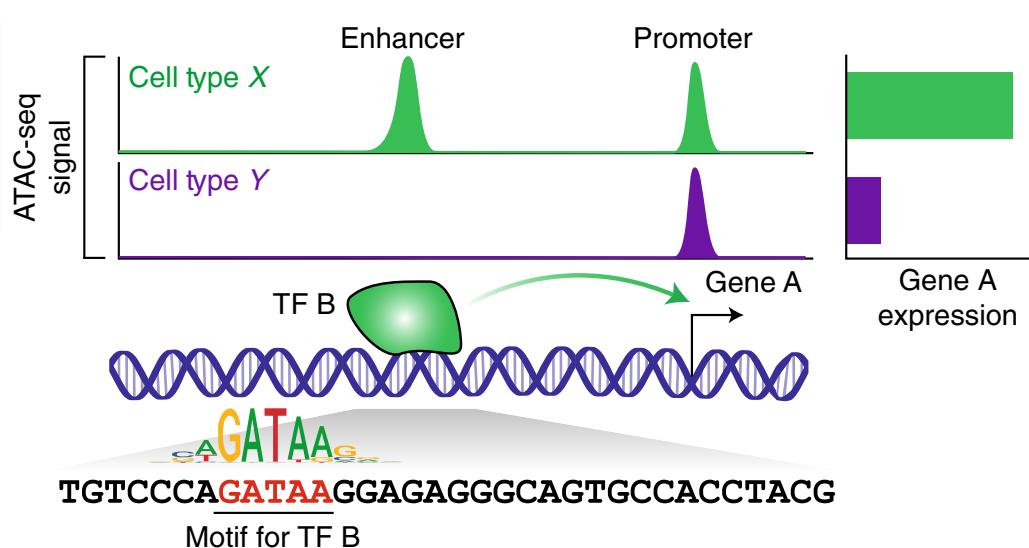
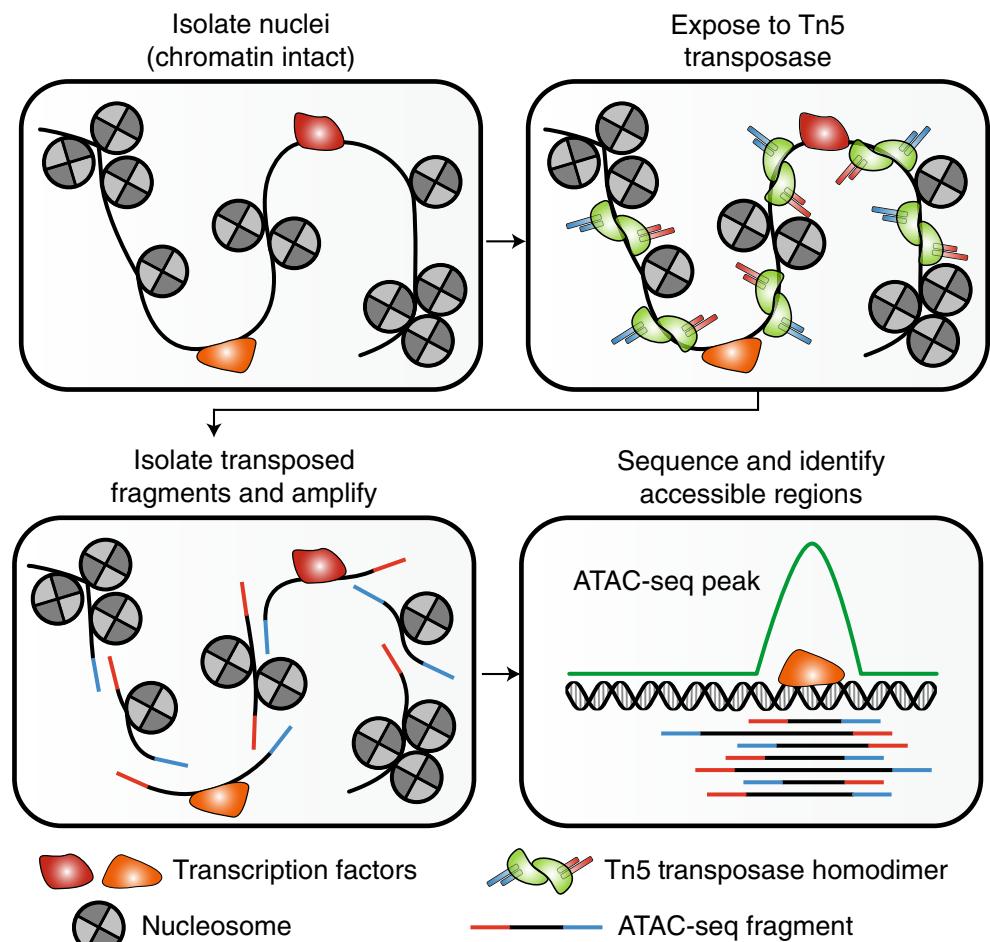


Chromosome Res, 2020, 28, 69–85
Nature Methods, 2019, 16, 323–325

Overview of single cell -omics

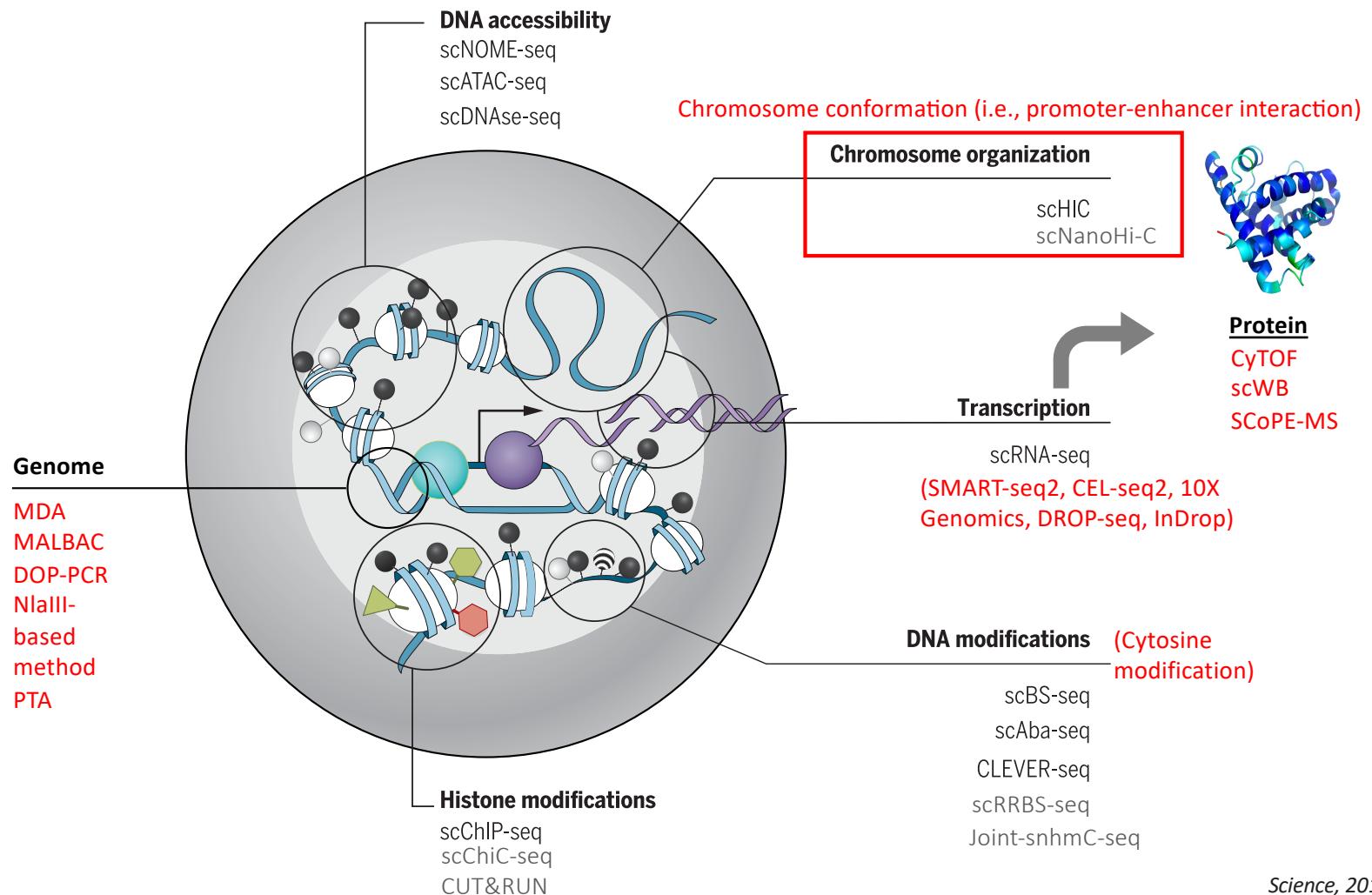


scATAC-seq: Assay for transposase-accessible chromatin

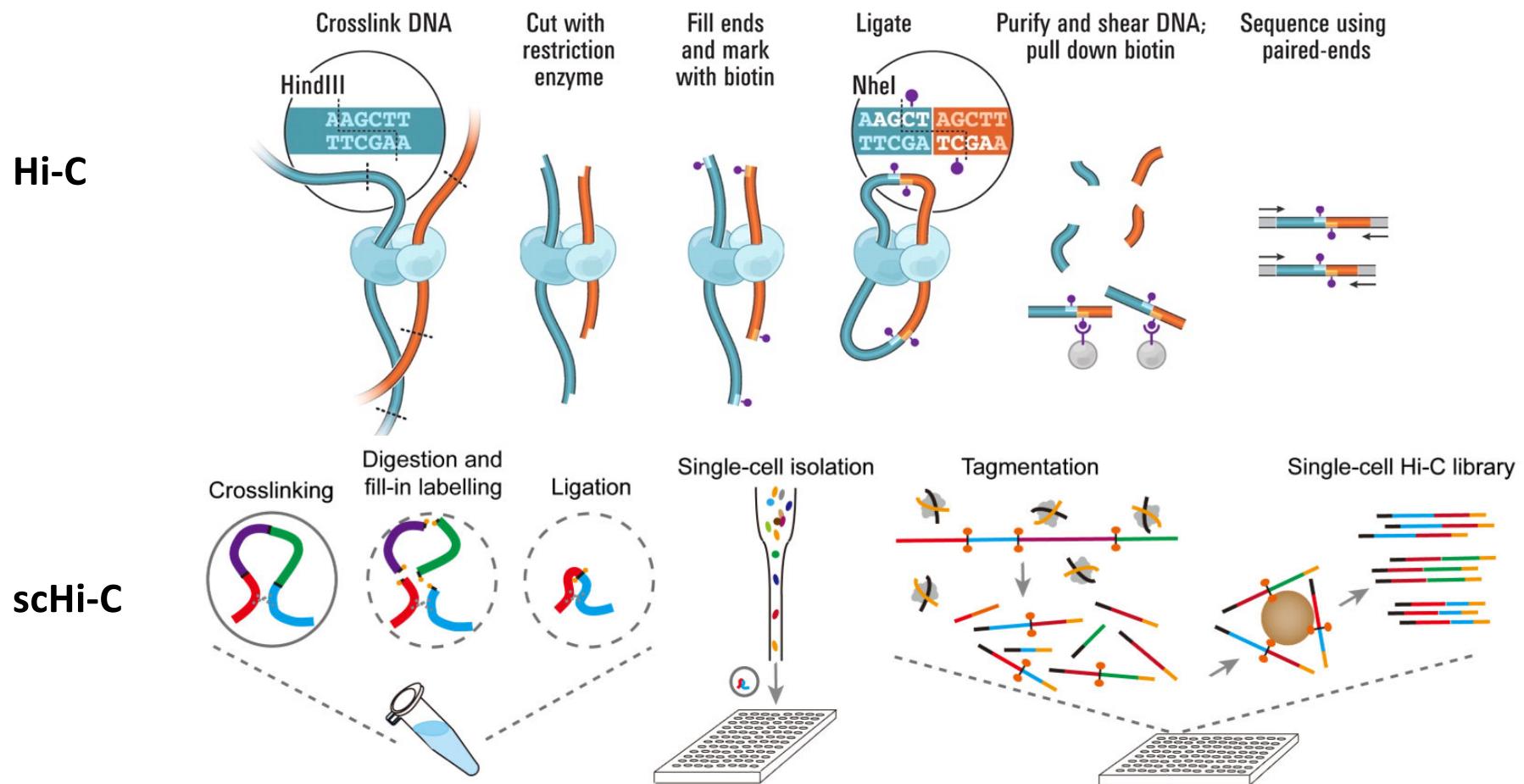


- scATAC-seq can be applied to:
 - nucleosome mapping experiments
 - map transcription factor binding sites

Overview of single cell -omics



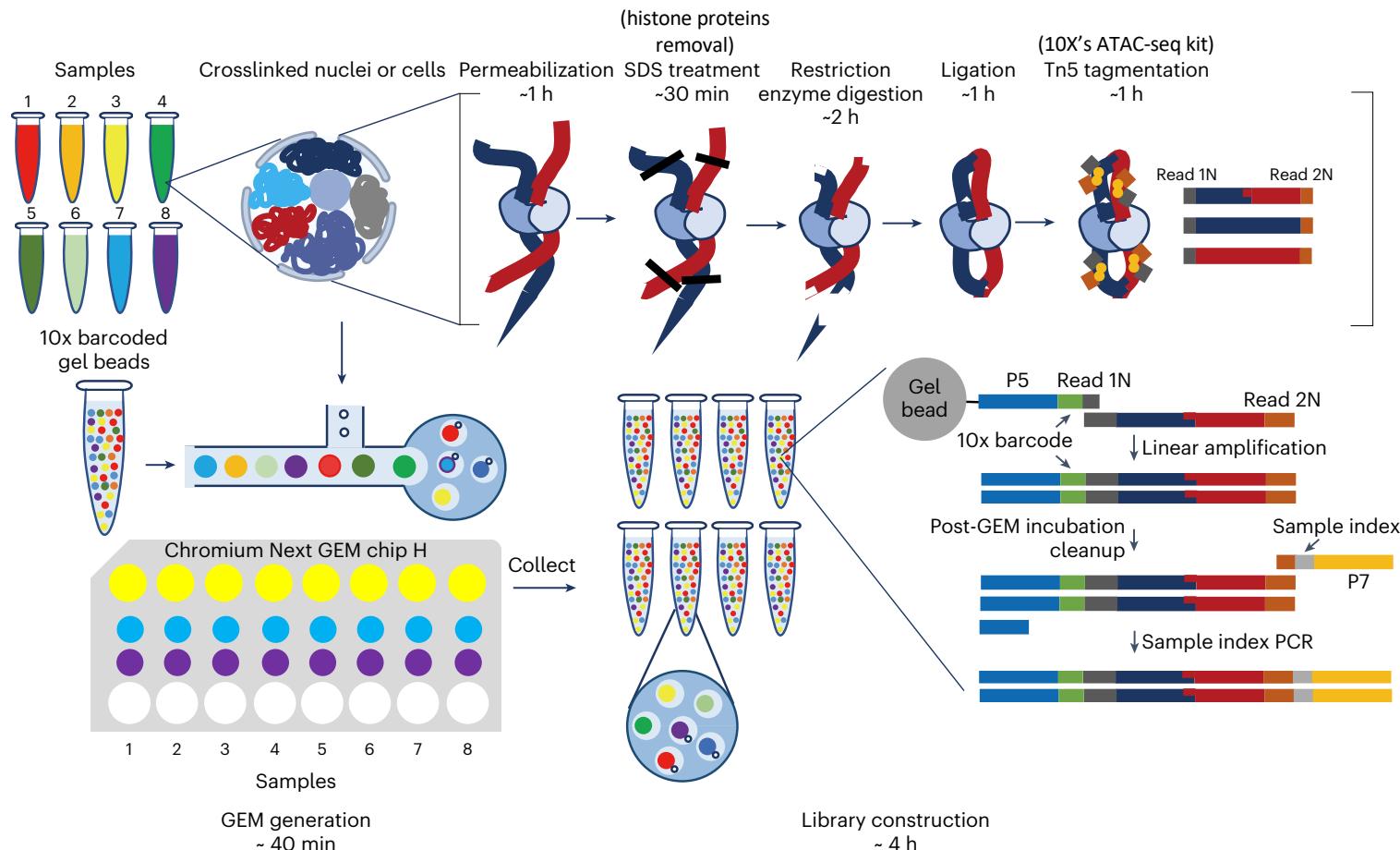
Single cell Hi-C (high-resolution chromosome conformation capture)



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

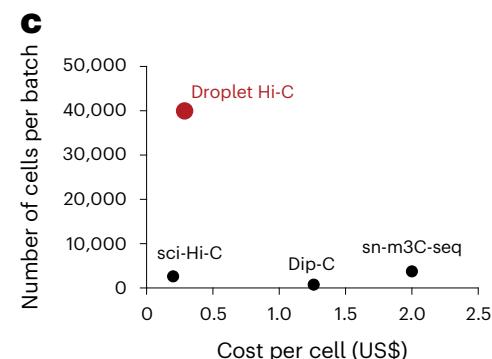
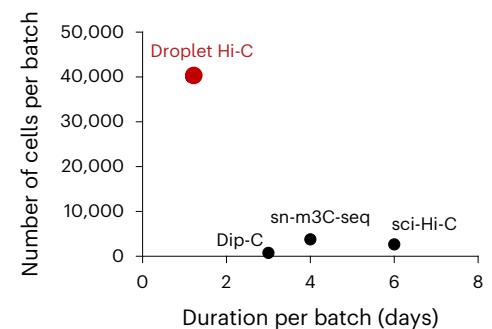
Droplet Hi-C

- Scalable, single-cell profiling of **chromatin architecture** in heterogeneous tissues

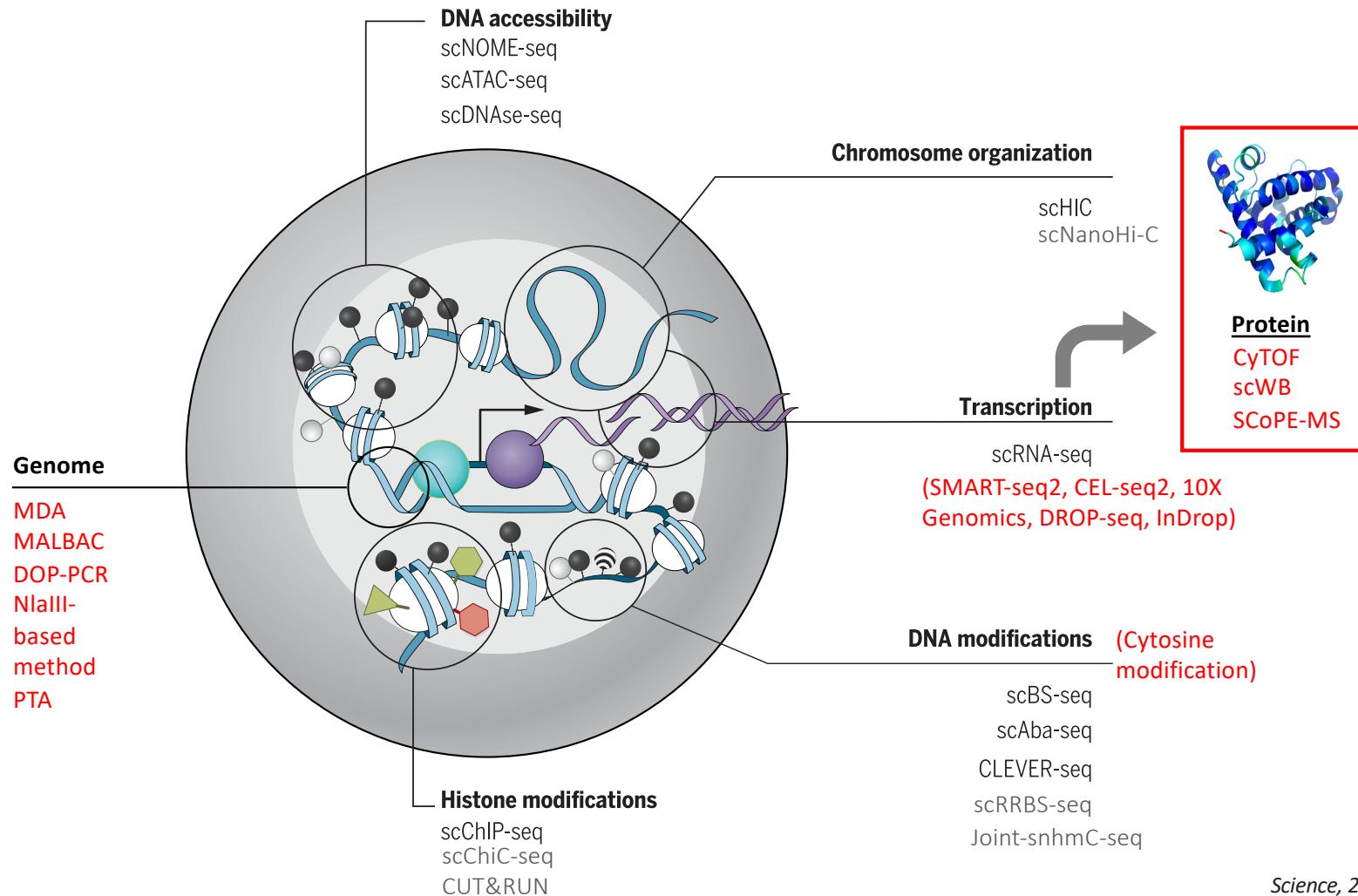


(enabling the profiling of **40,000** or more cells simultaneously)

Nat Biotechnol, 2024, <https://doi.org/10.1038/s41587-024-02447-1>



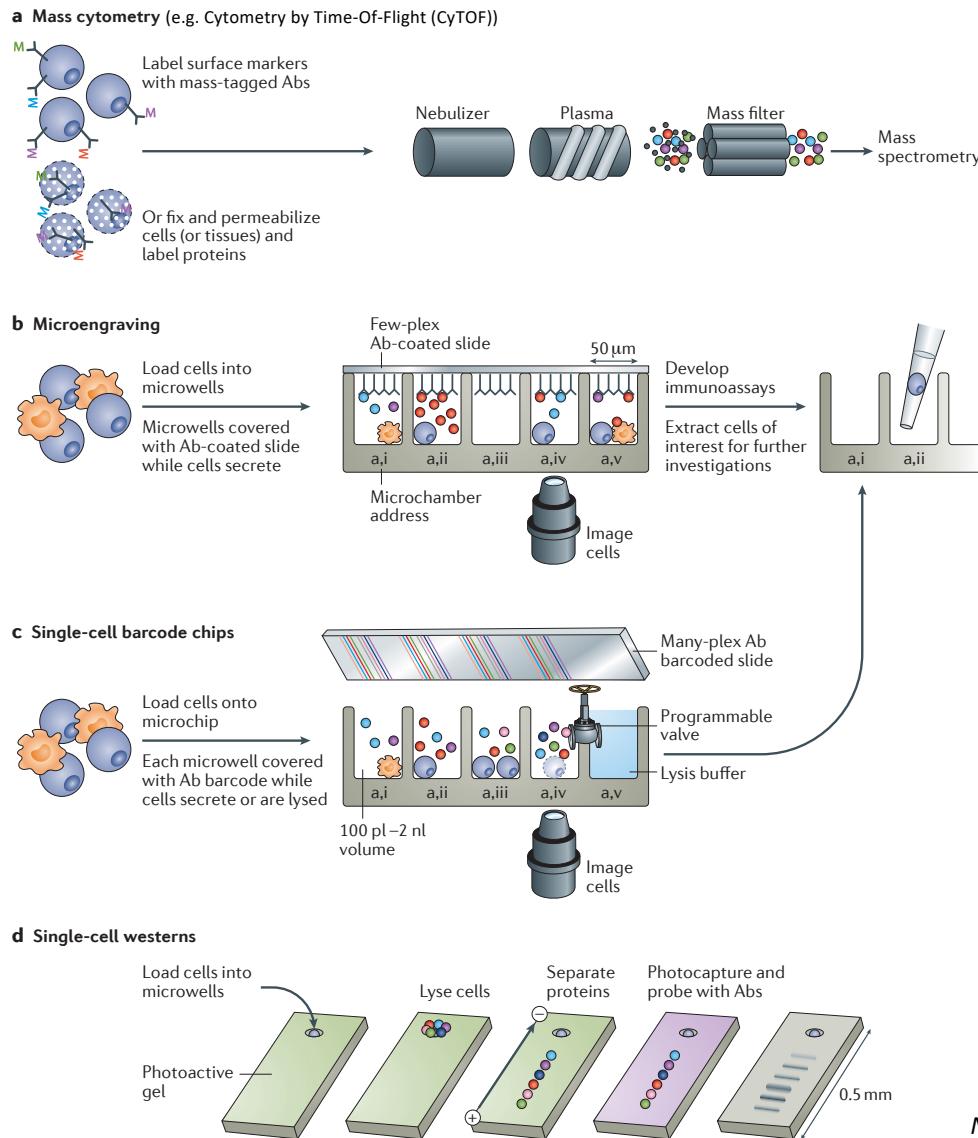
Overview of single cell -omics



Science, 2017, 358, 69–75

Single cell proteomics

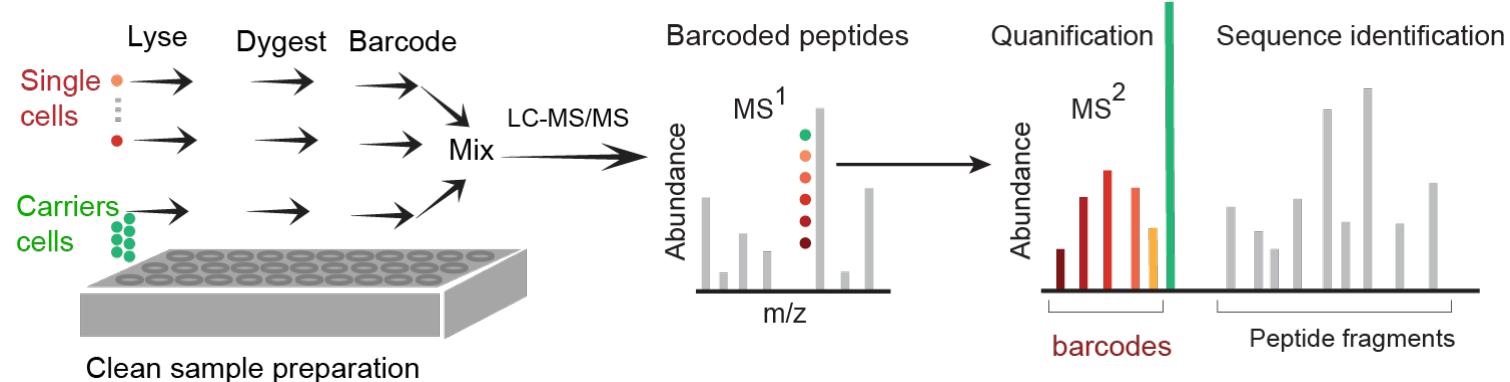
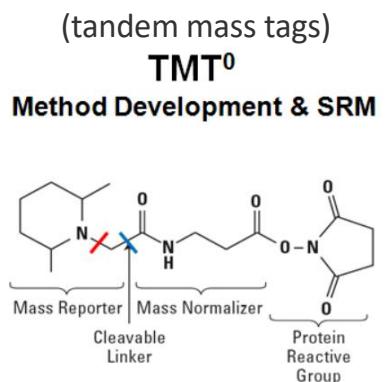
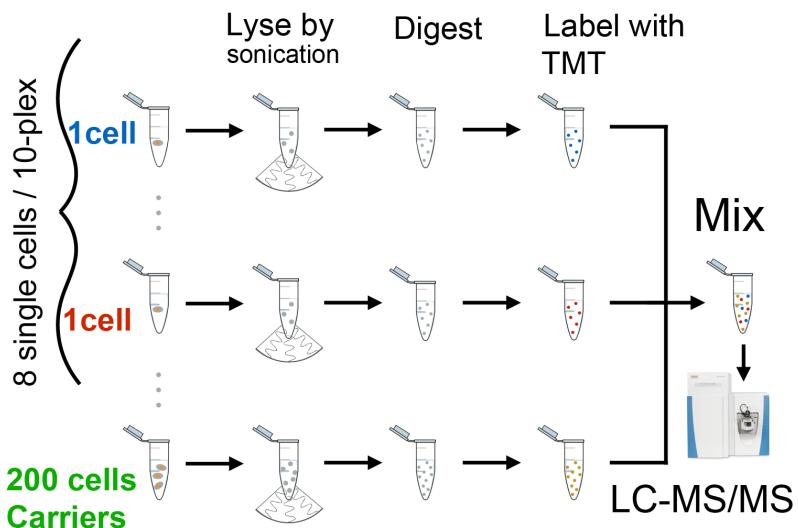
Emerging single-cell proteomics methods (antibody-dependent approaches)



Antibody-independent approaches

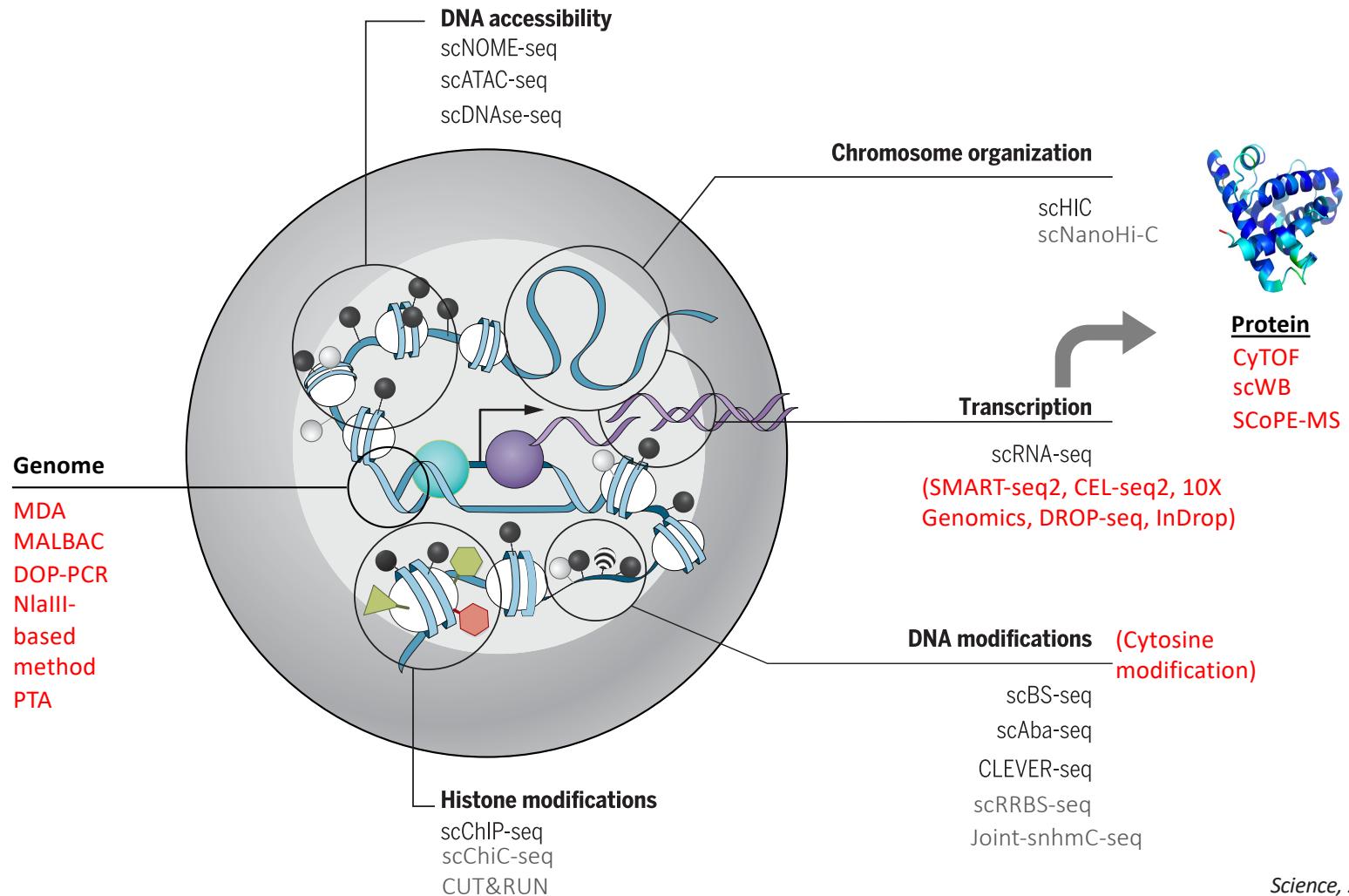
-> Mass spectrometry (Mass spec)-based method

SCoPE-MS (Single Cell ProtEomics by Mass Spectrometry)



Nikolai Slavov group
Genome Biology, 2018, 19, 161

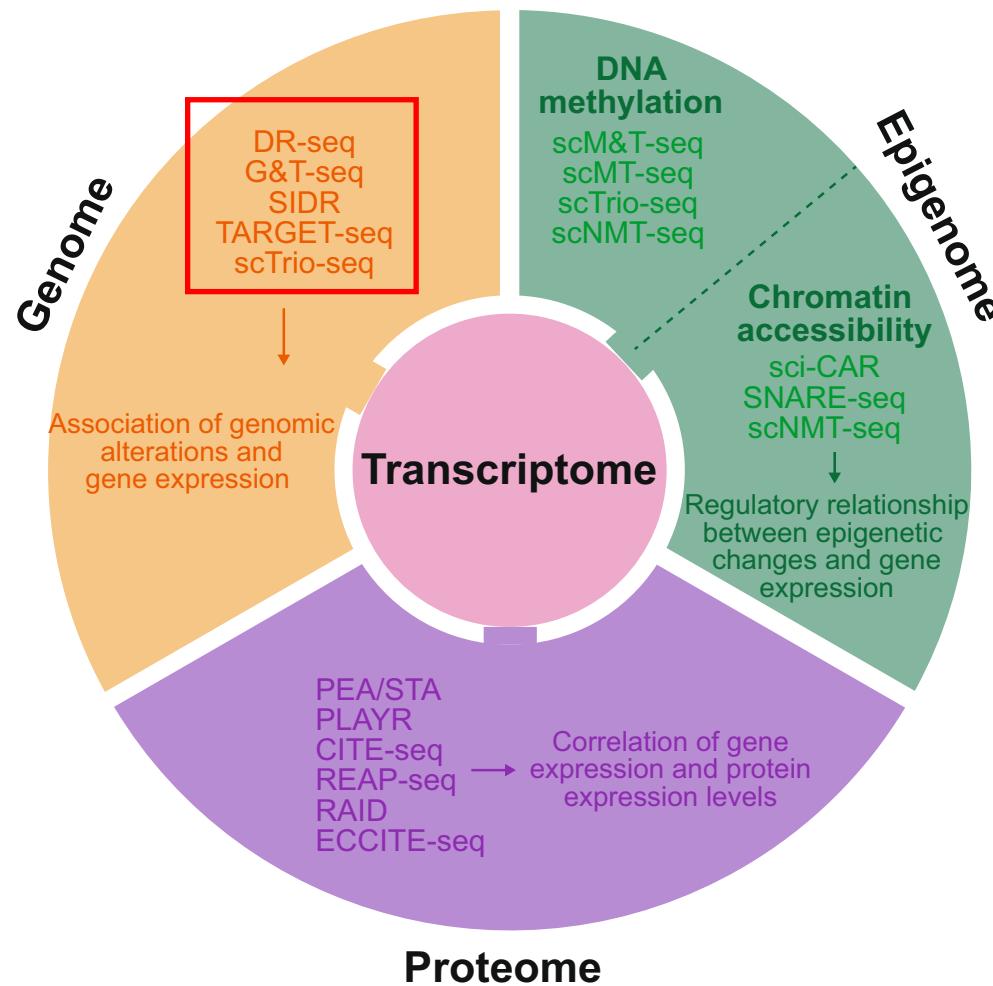
Overview of single cell -omics



Science, 2017, 358, 69–75

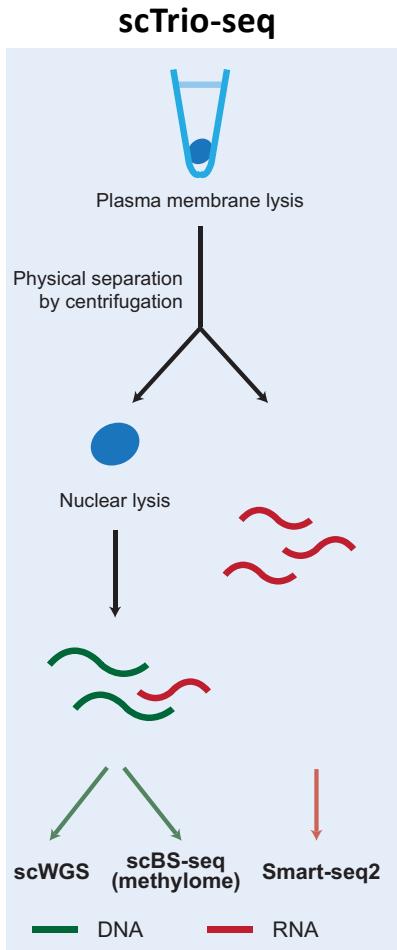
Single cell multi–omics

Multi-omics profiling of single cells

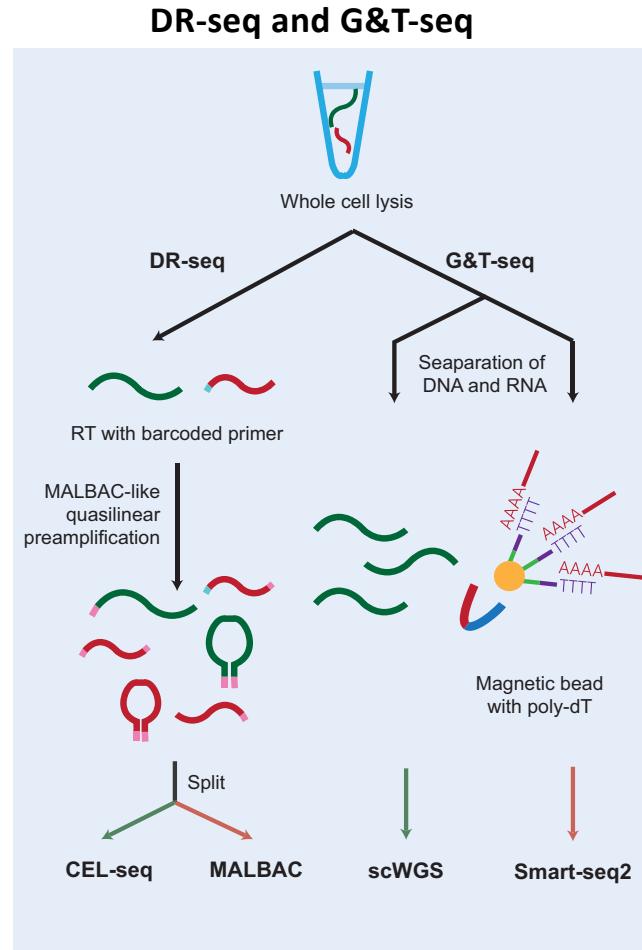


Single cell DNA- and RNA-Sequencing

Single cell DNA- and RNA-Sequencing



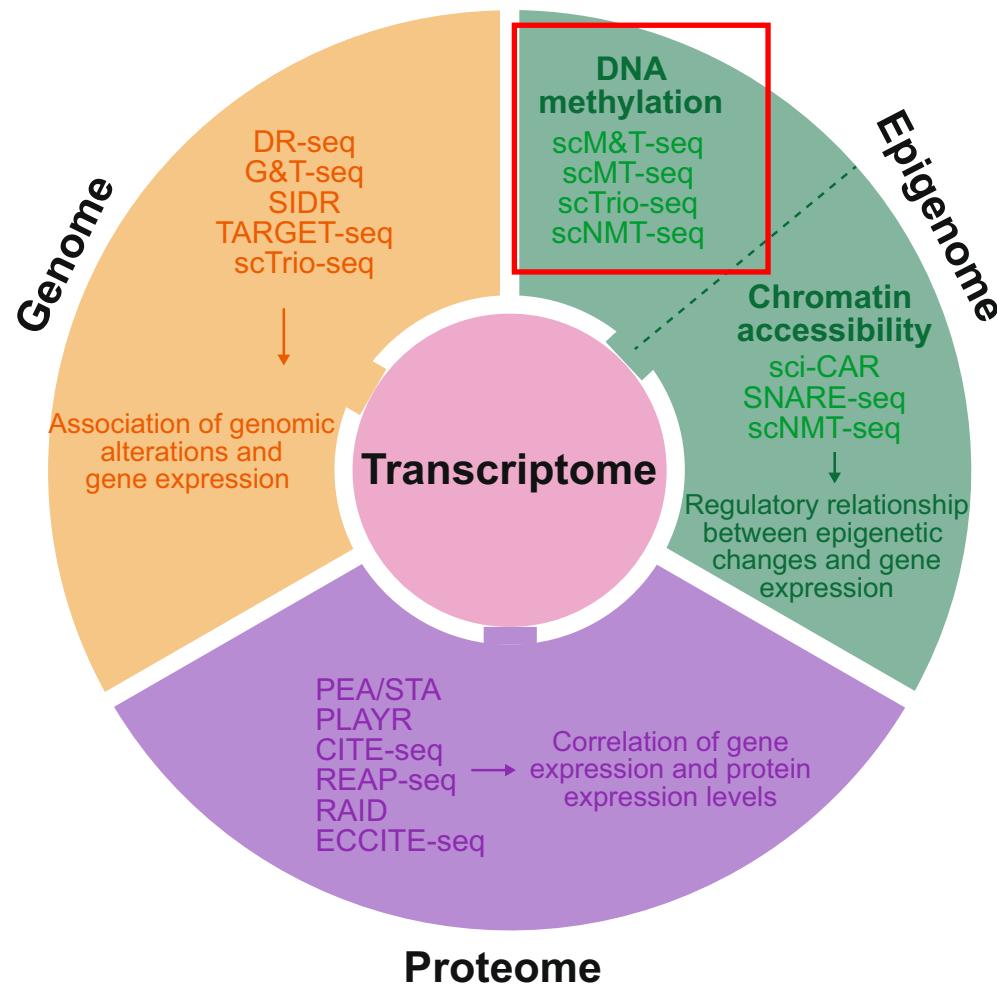
Cell Res, 2016, 26, 304–319



Nat Biotechnol, 2015, 33, 285–289;
Nat Methods, 2015, 12, 519–522

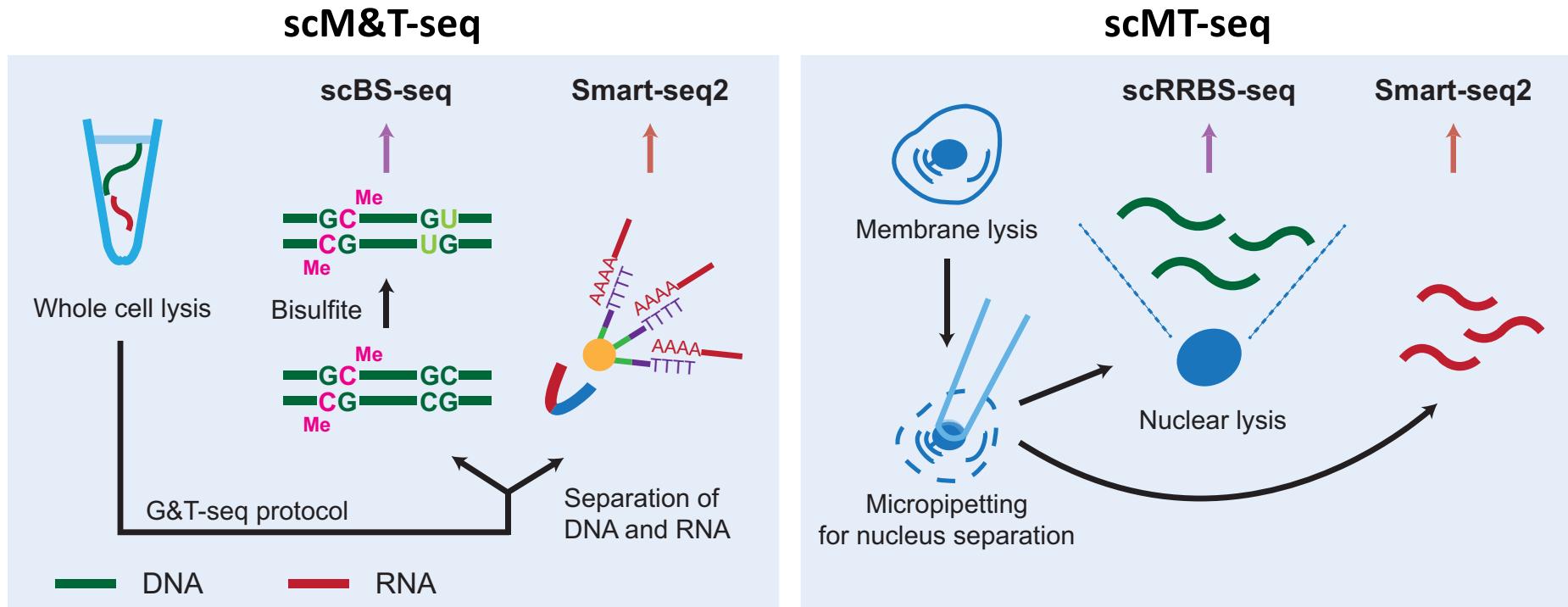
Exp Mol Med, 2020, 52, 1428–1442

Multi-omics profiling of single cells

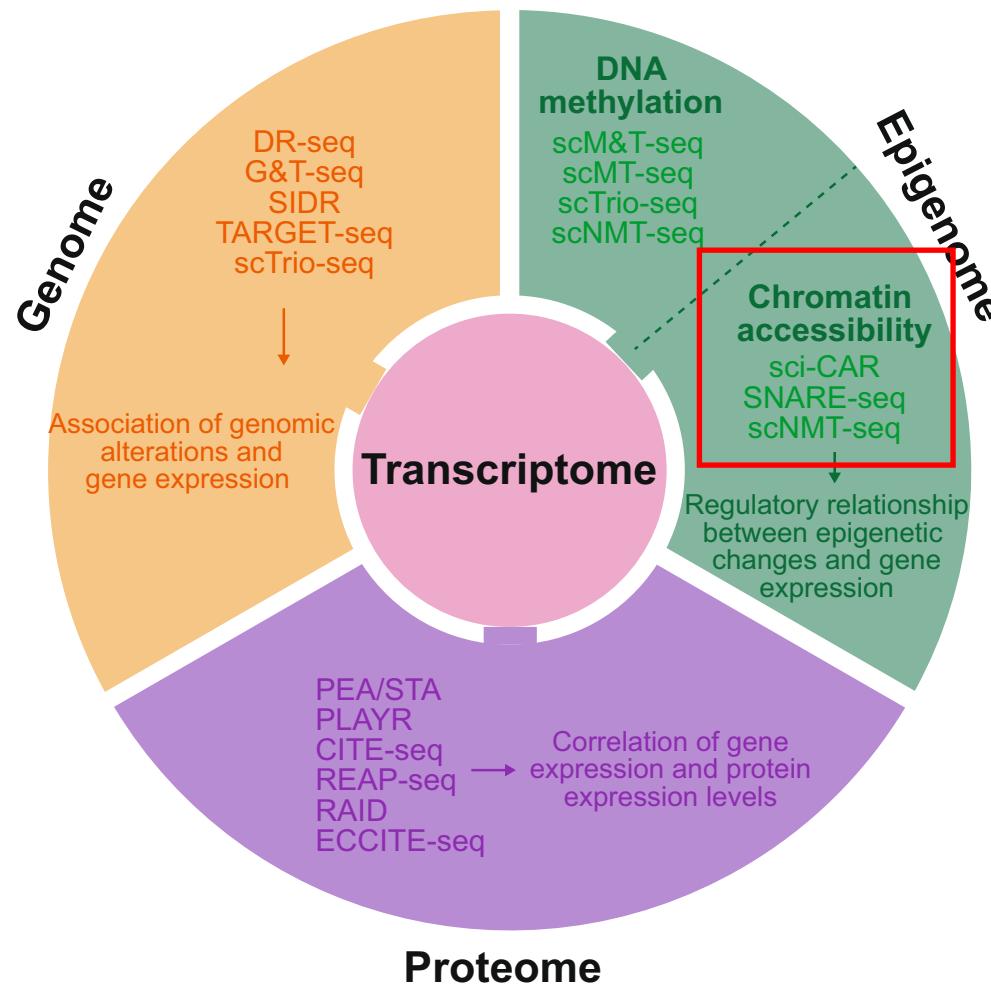


Single cell RNA- and methylation- Sequencing

Single cell RNA- and methylation-Sequencing



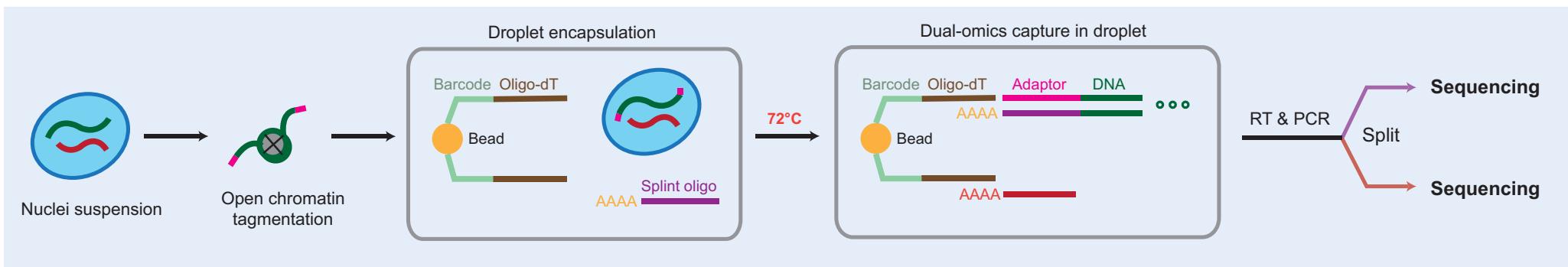
Multi-omics profiling of single cells



Single cell RNA-seq and chromosomal accessibility

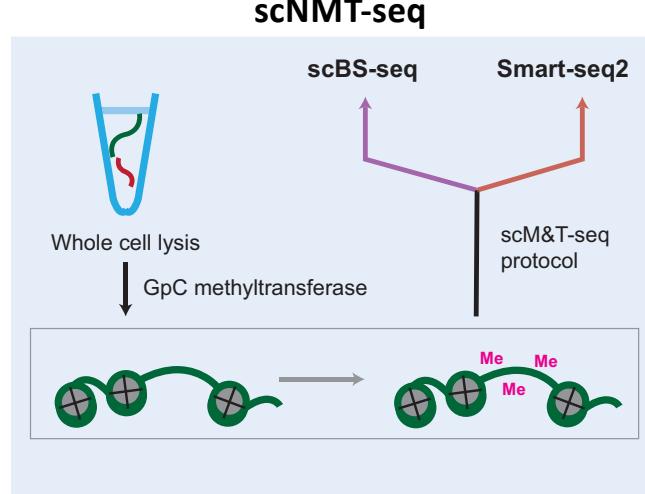
Single cell RNAseq and chromosomal accessibility

SNARE-seq (single-nucleus chromatin accessibility and mRNA expression sequencing)



Nat Biotechnol, 2019, 37, 1452–1457

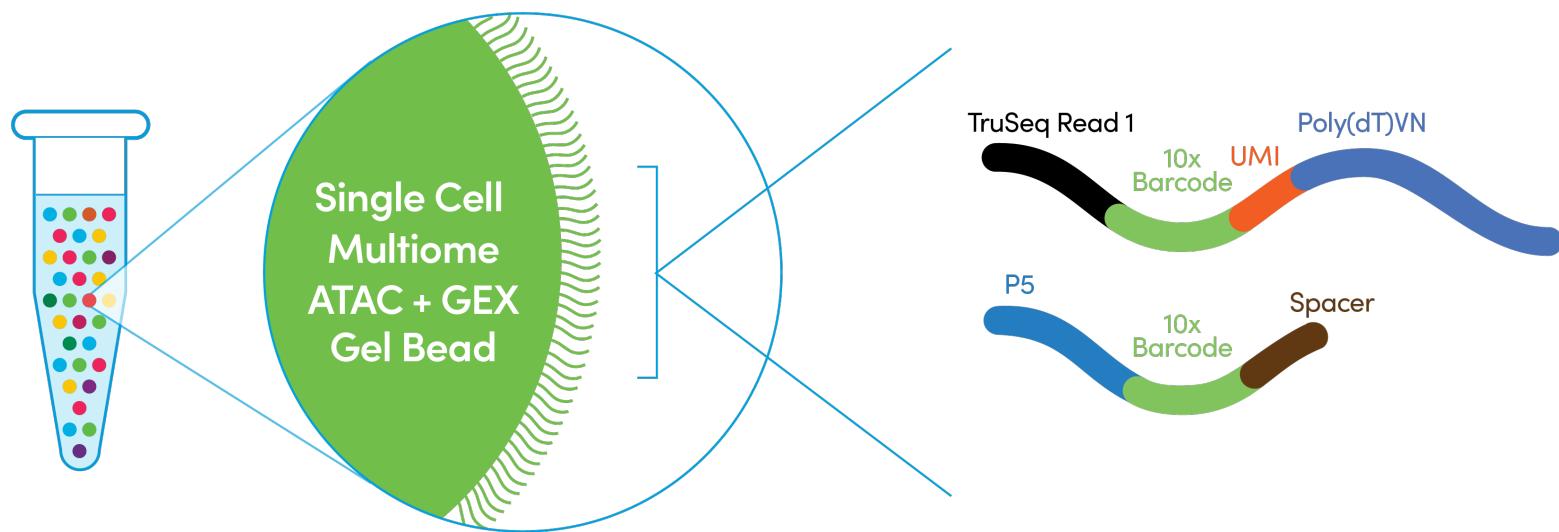
- Joint profiling of chromatin accessibility, DNA methylation and transcription in single cells (single-cell nucleosome, methylation and transcription sequencing)



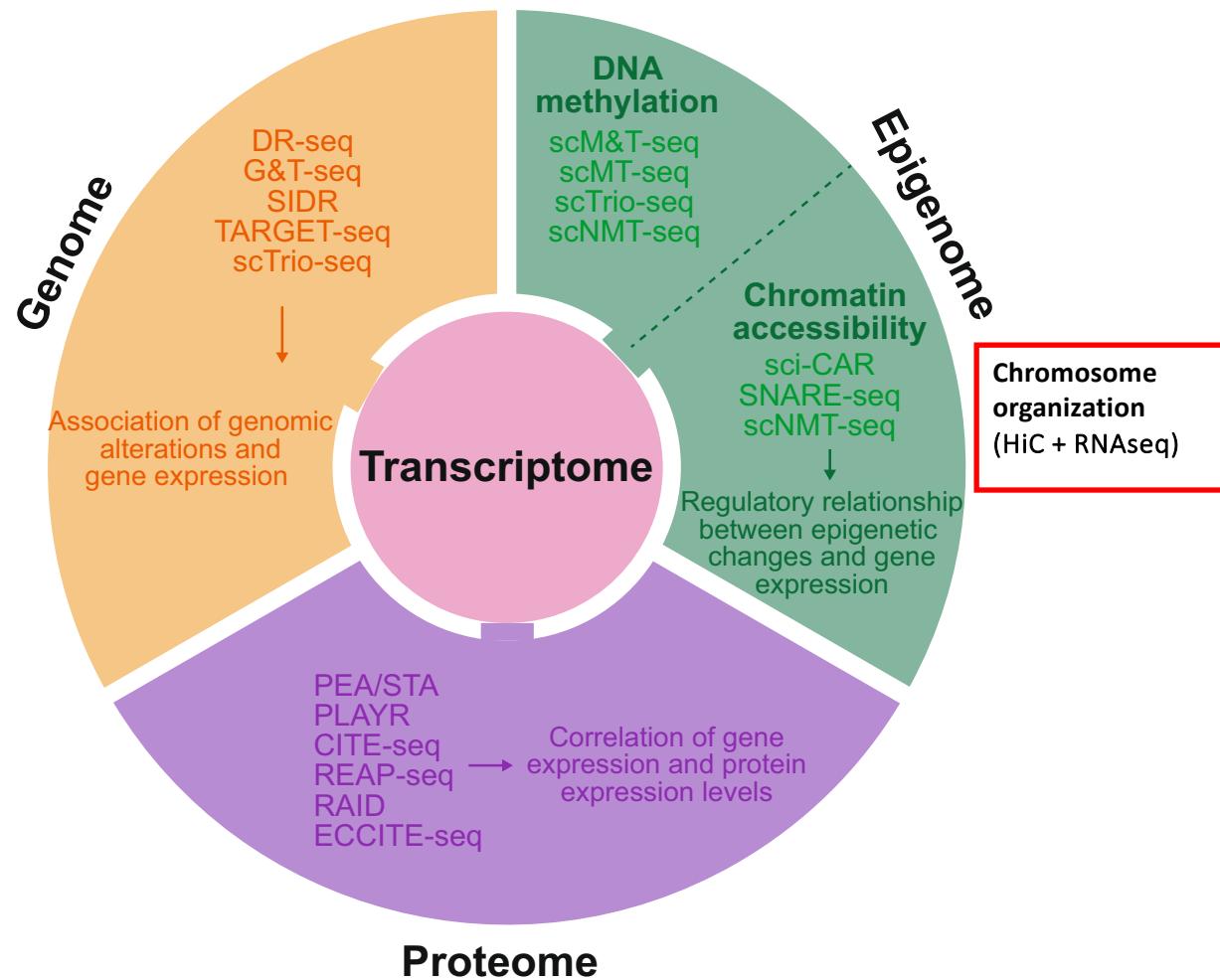
Nature Comm., 2018, 9, 781

Exp Mol Med, 2020, 52, 1428–1442

10X Single Cell Multiome (scATAC + scRNA)

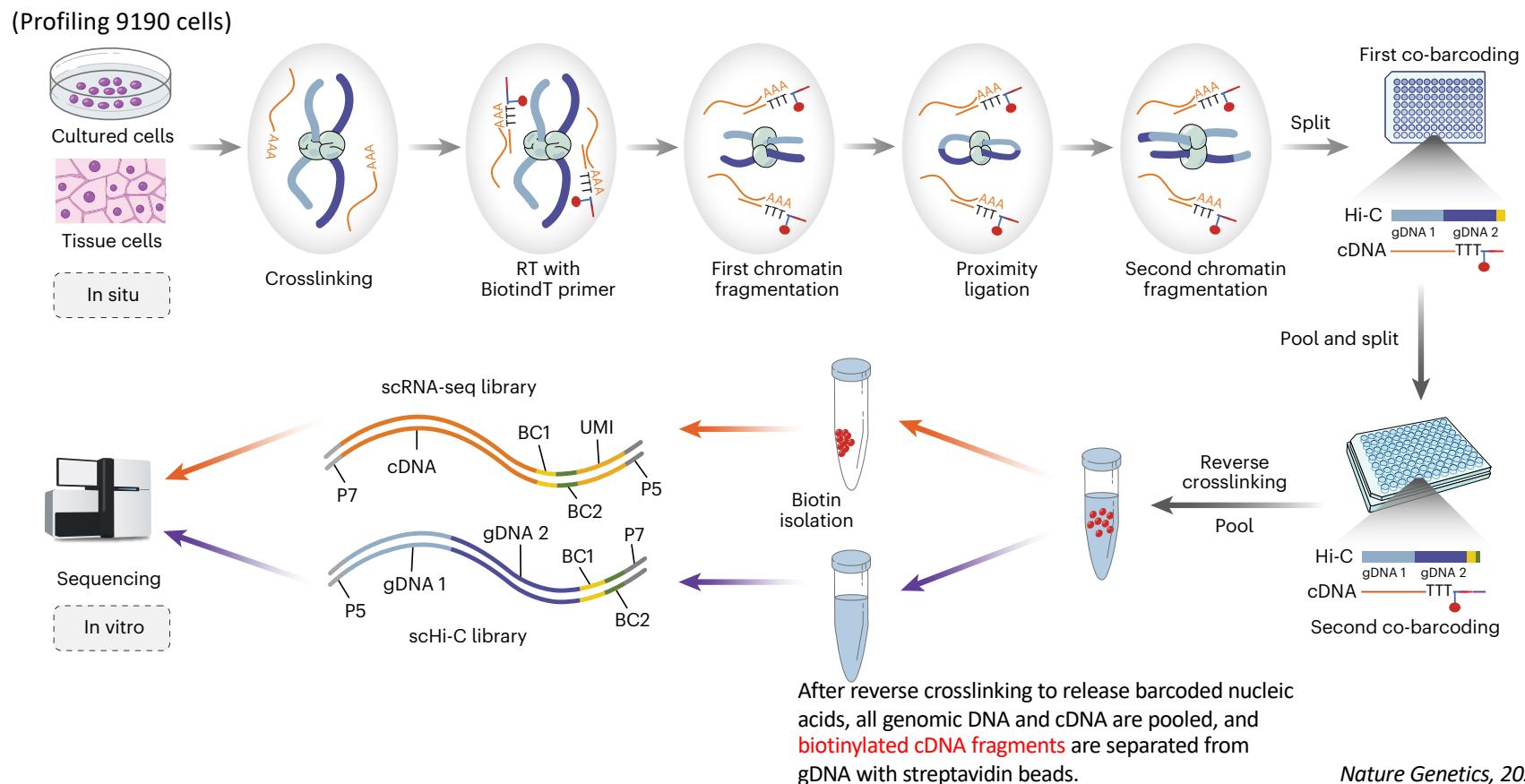


Multi-omics profiling of single cells



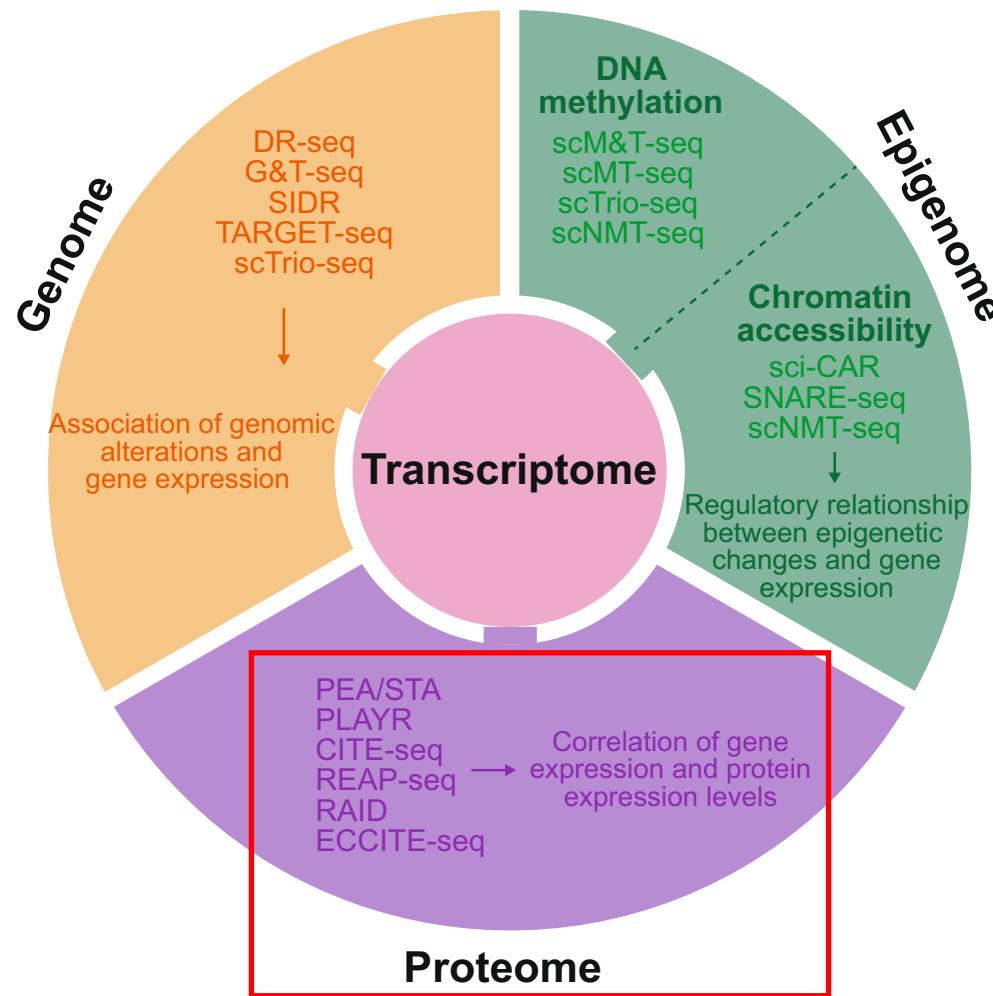
GAGE-seq

- Genome architecture and gene expression by sequencing a scalable, robust single-cell co-assay measuring **3D genome structure** and **transcriptome** simultaneously **within the same cell**

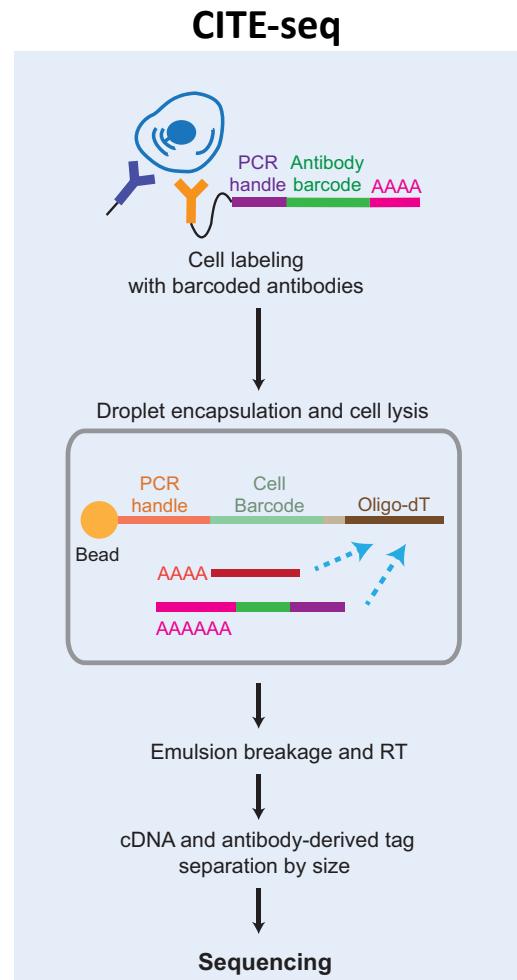


Nature Genetics, 2024, 56, 1701-1711

Multi-omics profiling of single cells



Single cell transcriptome and proteome

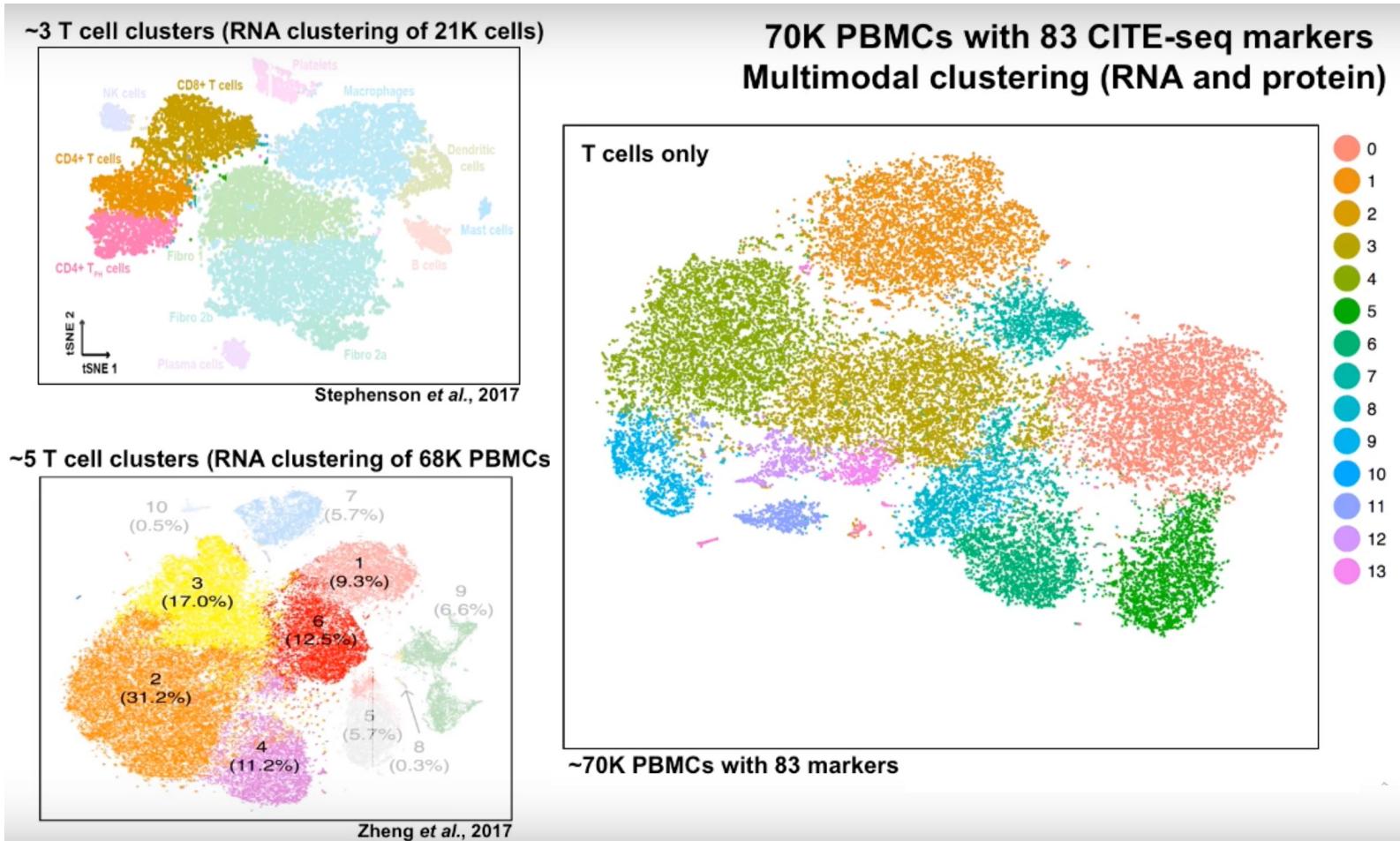


Nature Methods, 2017, 14, 865–868

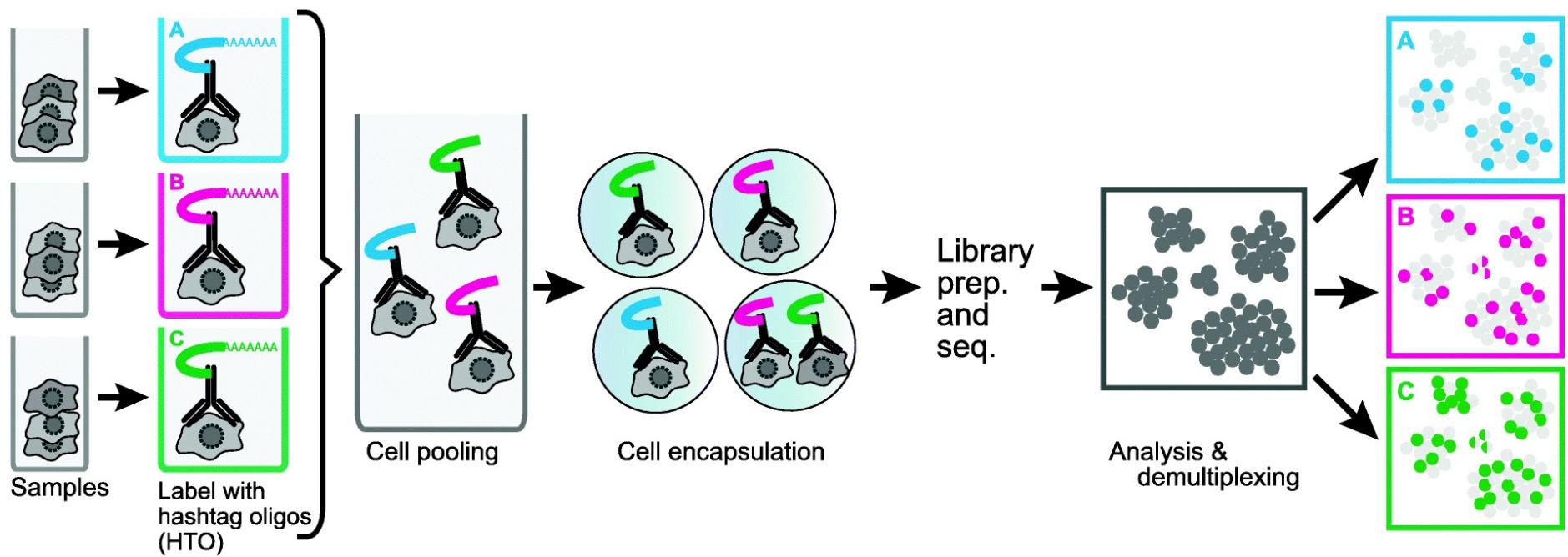
Exp Mol Med, 2020, 52, 1428–1442

Multimodal clustering resolves more T cell clusters

13 T cell clusters with distinct protein and RNA markers

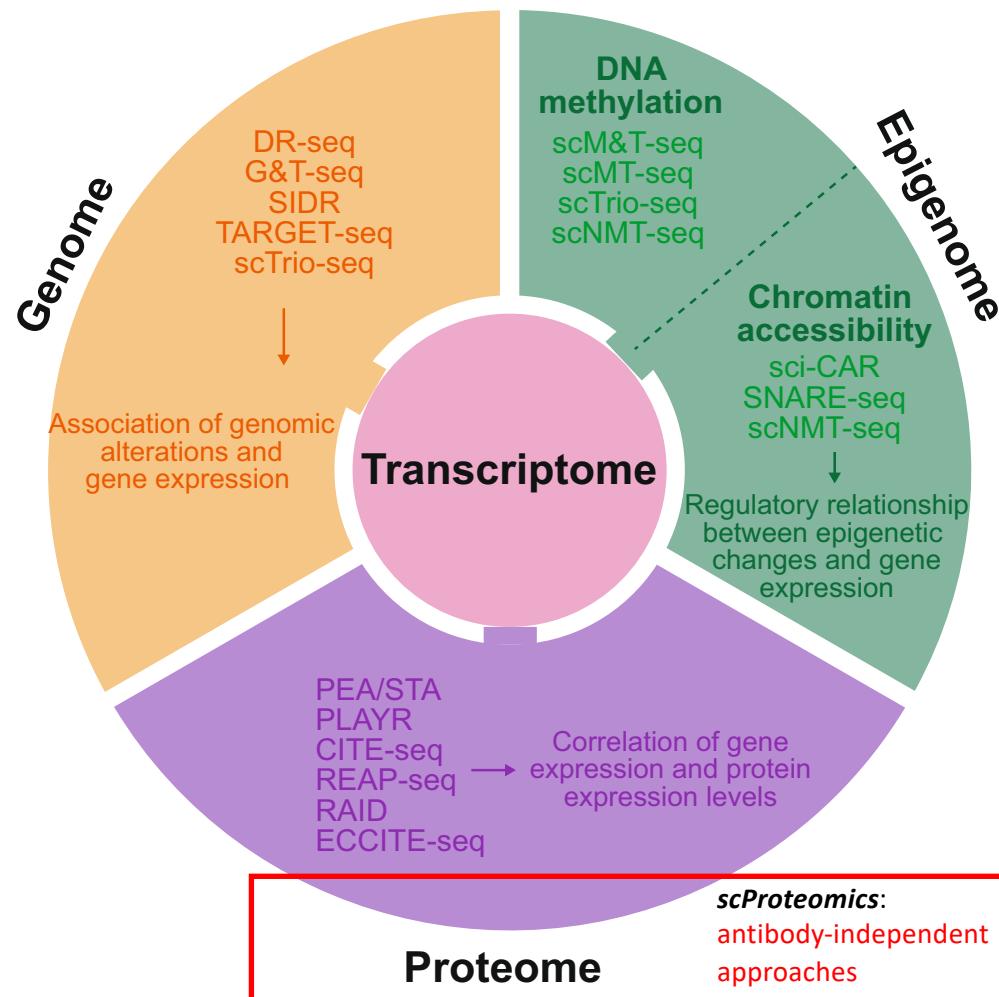


Cell Hashing: apply CITE-seq to multiplex multiple samples



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

Multi-omics profiling of single cells



Single cell whole transcriptome & proteome analysis

RESEARCH

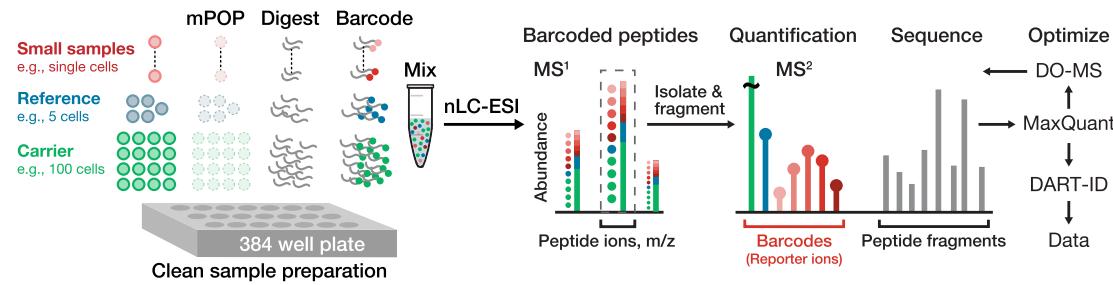
Open Access



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

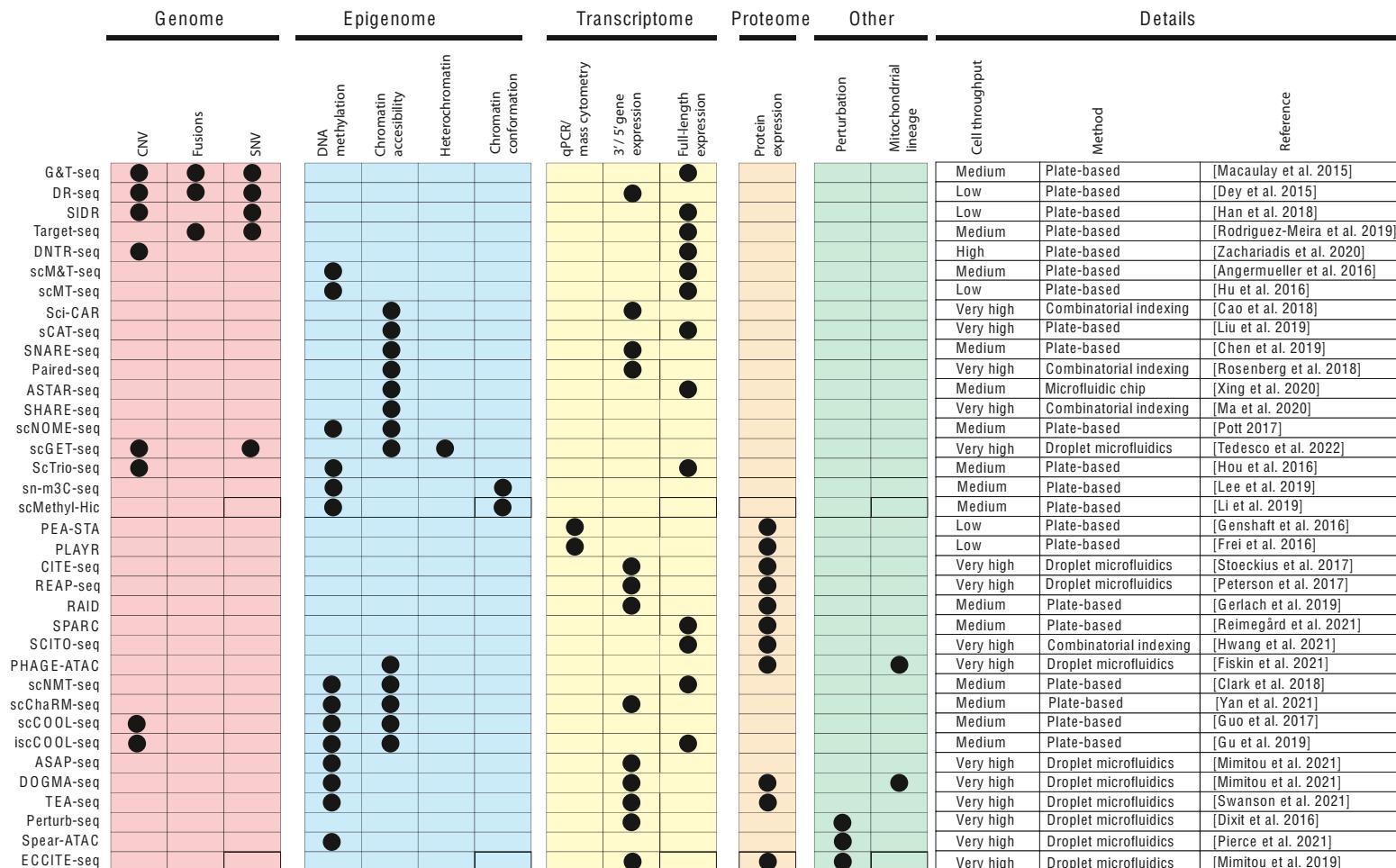
Integrated
analysis

scRNAseq

Nikolai Slavov group
Genome Biology, 2021, 22, 50

10X GENOMICS

Overview of single-cell multi-omics methods



Conclusion

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

