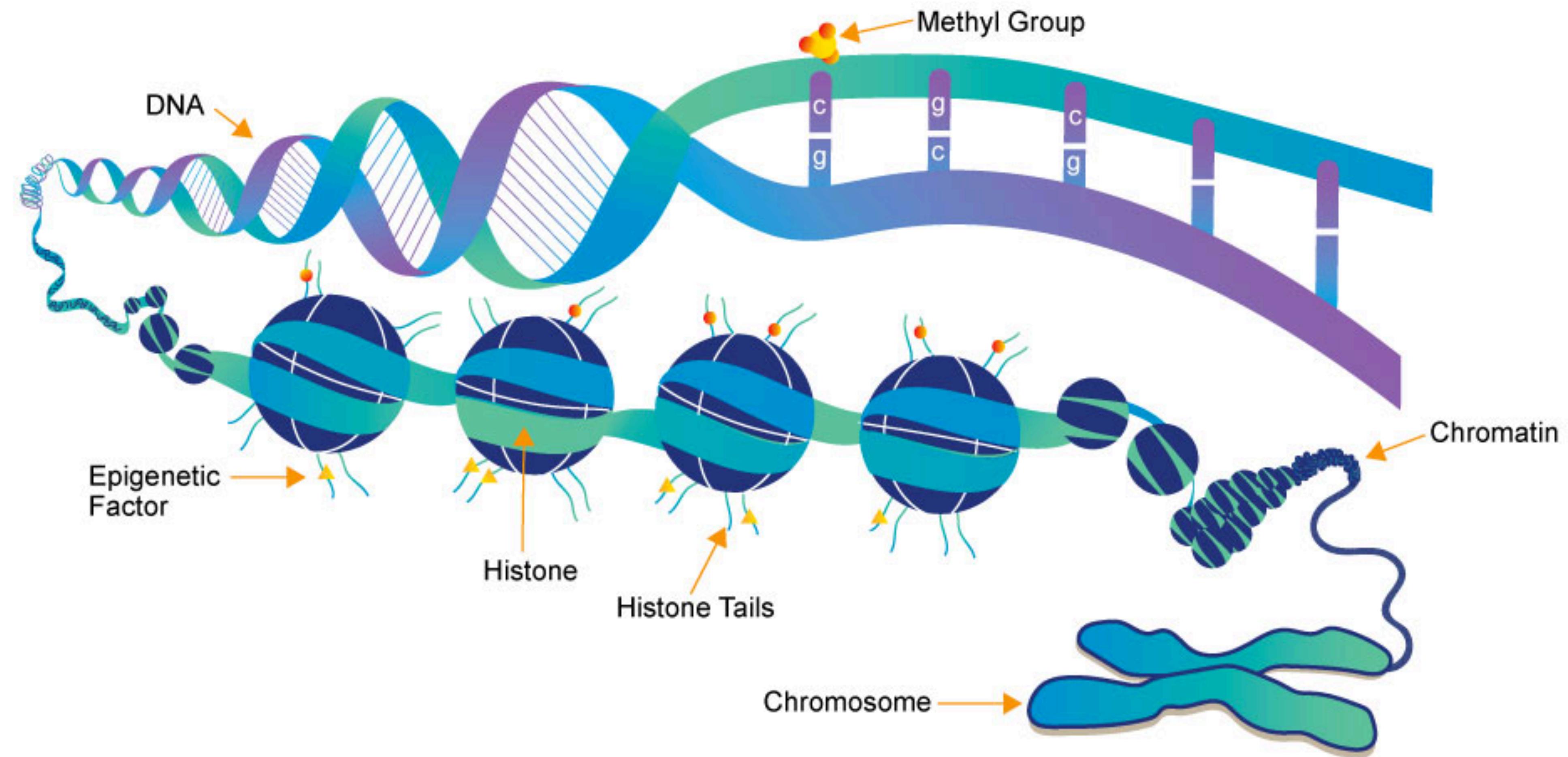


Integrated epigenomic analysis with single-cell CUT&Tag

Bingjie Zhang

Single Cell Genomics Day, 2021

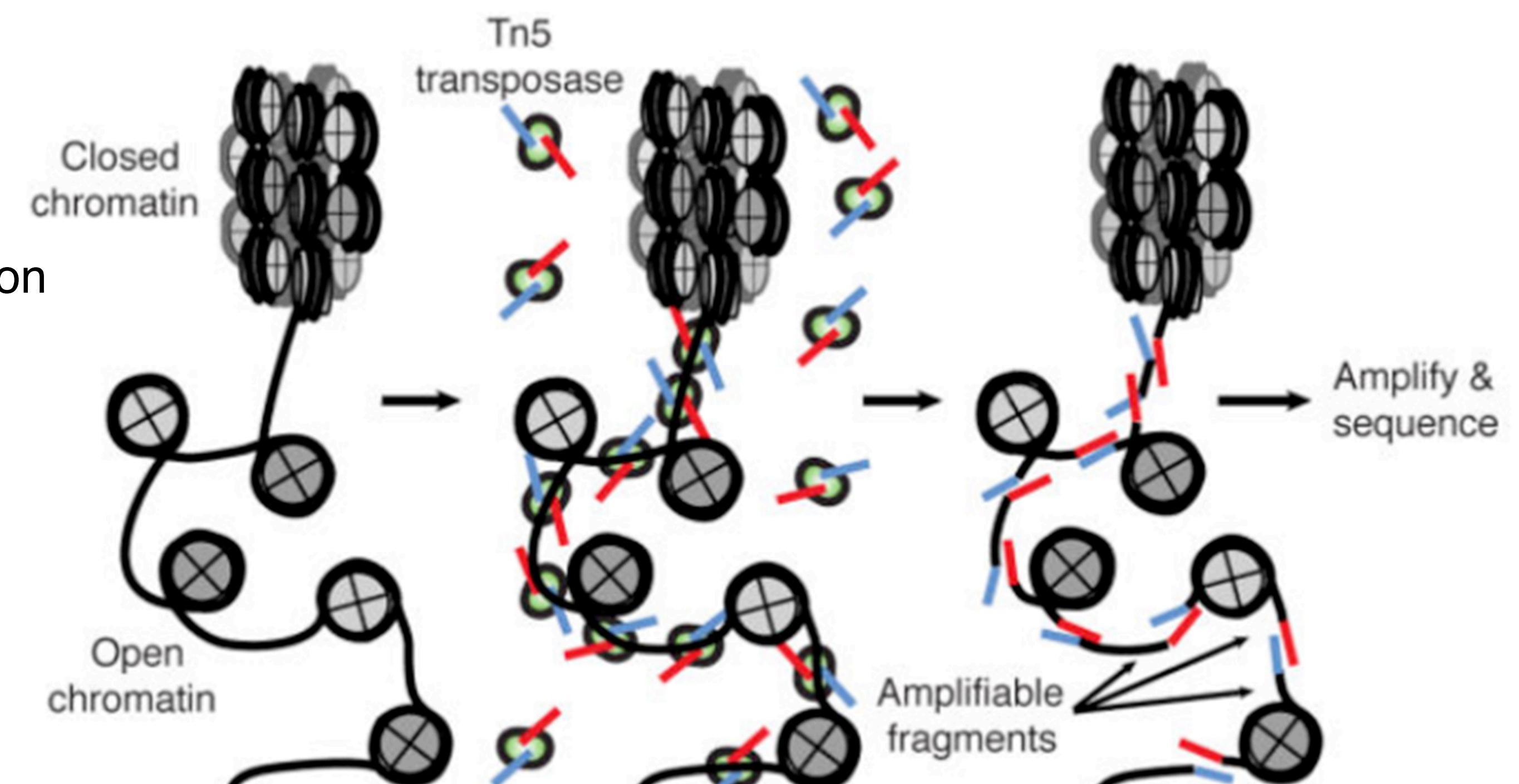
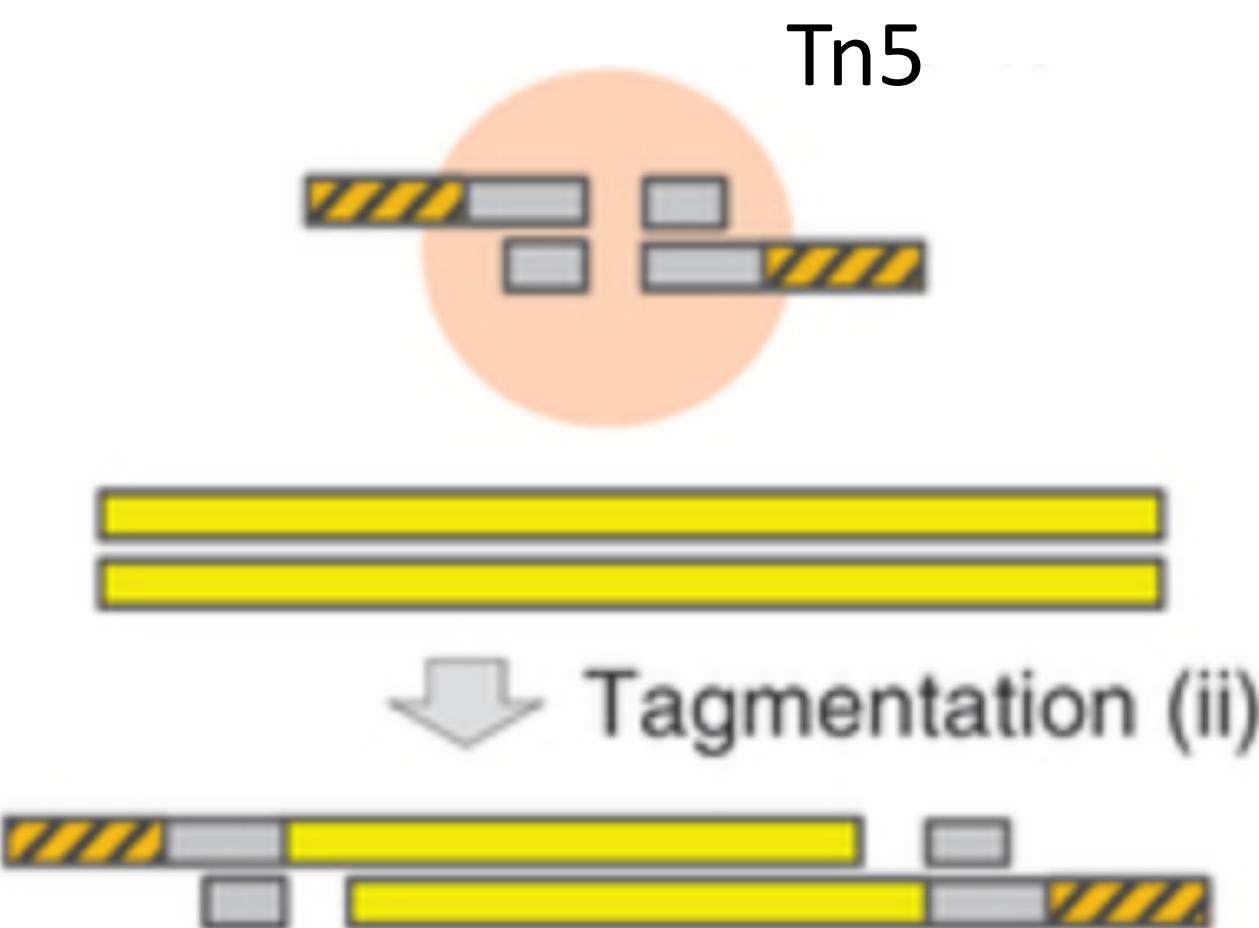
Gene regulation = Genetics + Epigenetics



ATAC-seq: Assay for Transposase Accessible Chromatin

Transposase Tn5

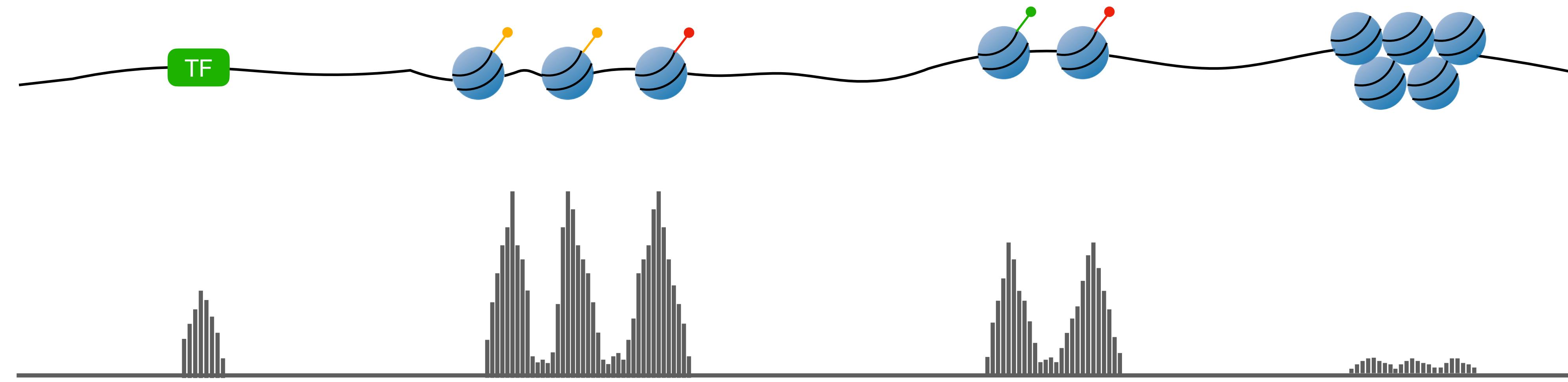
- Catalytic reaction requires magnesium
- Simultaneously cut and ligate adapters
- Stay bound to its DNA substrate after transposition



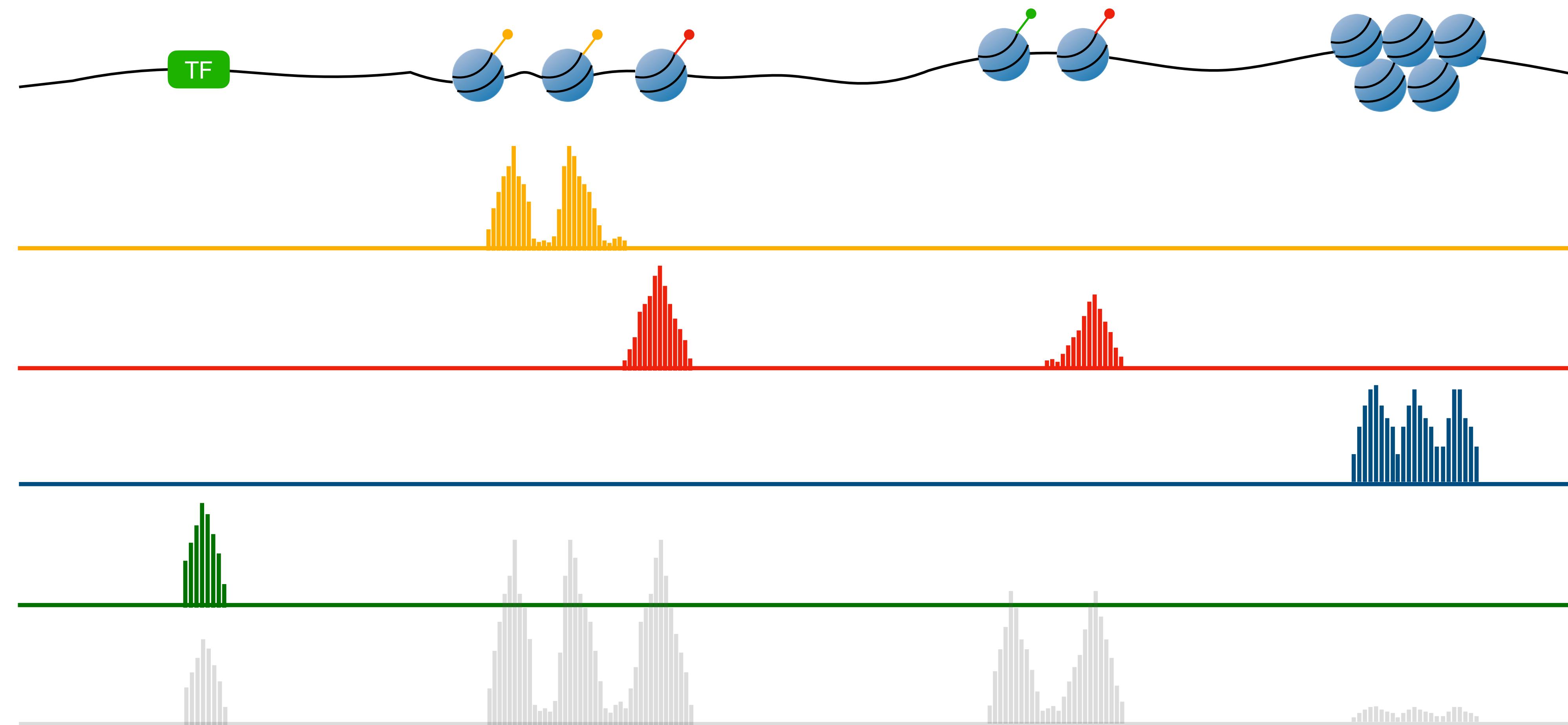
Jason D Buenrostro *Nat Methods*. 2013

Limitations of ATAC-seq

- ATAC is the compilation of all the Tn5 targeted regions
- Hard to distinguish between the real active and the poised promoters/enancers.

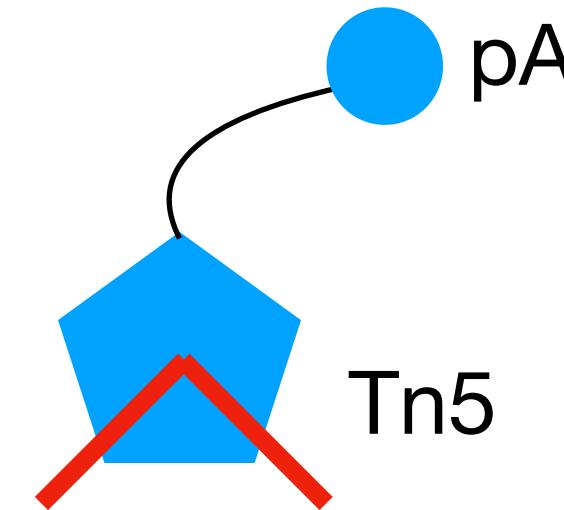


Limitations of ATAC-seq

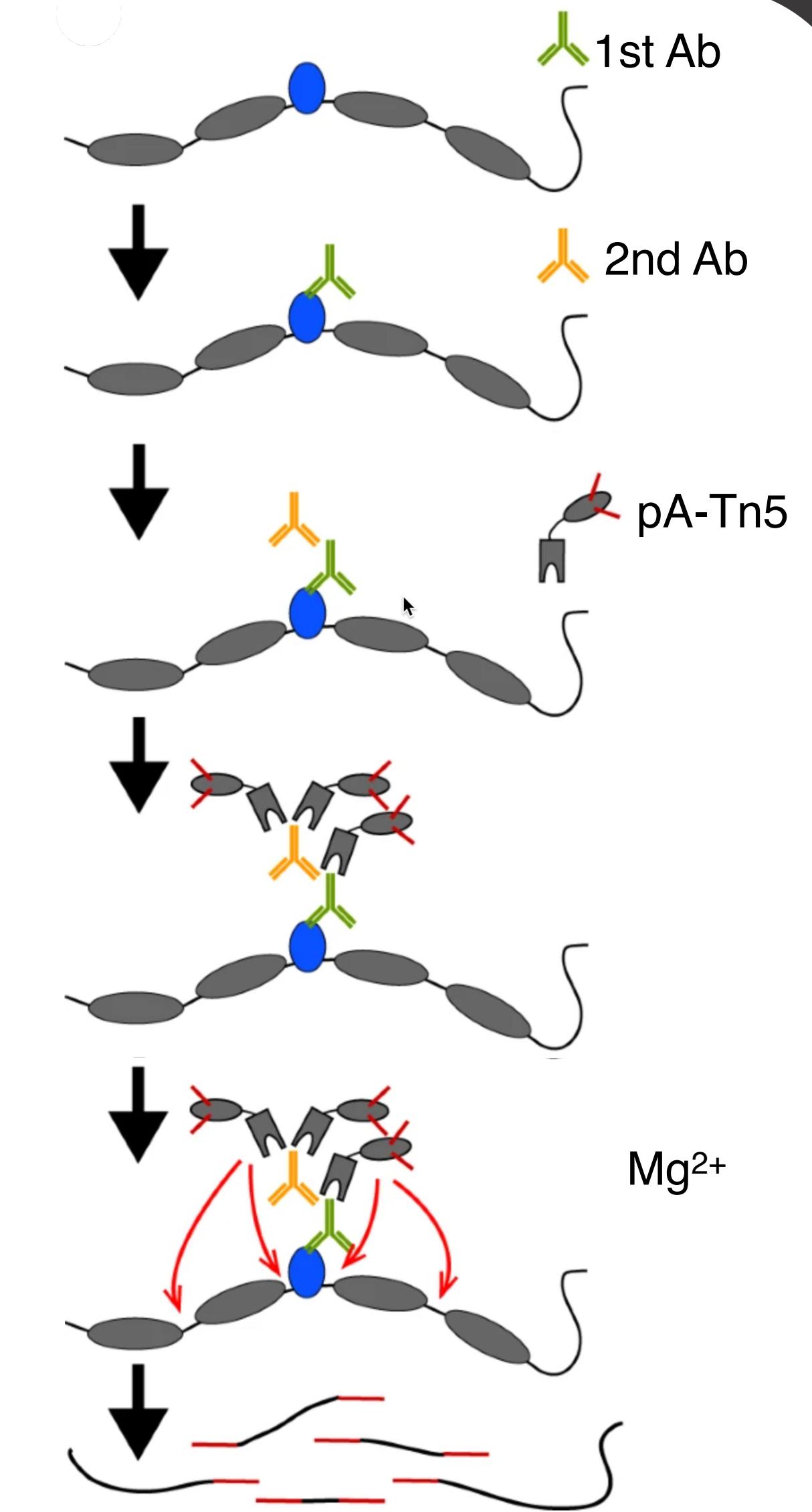
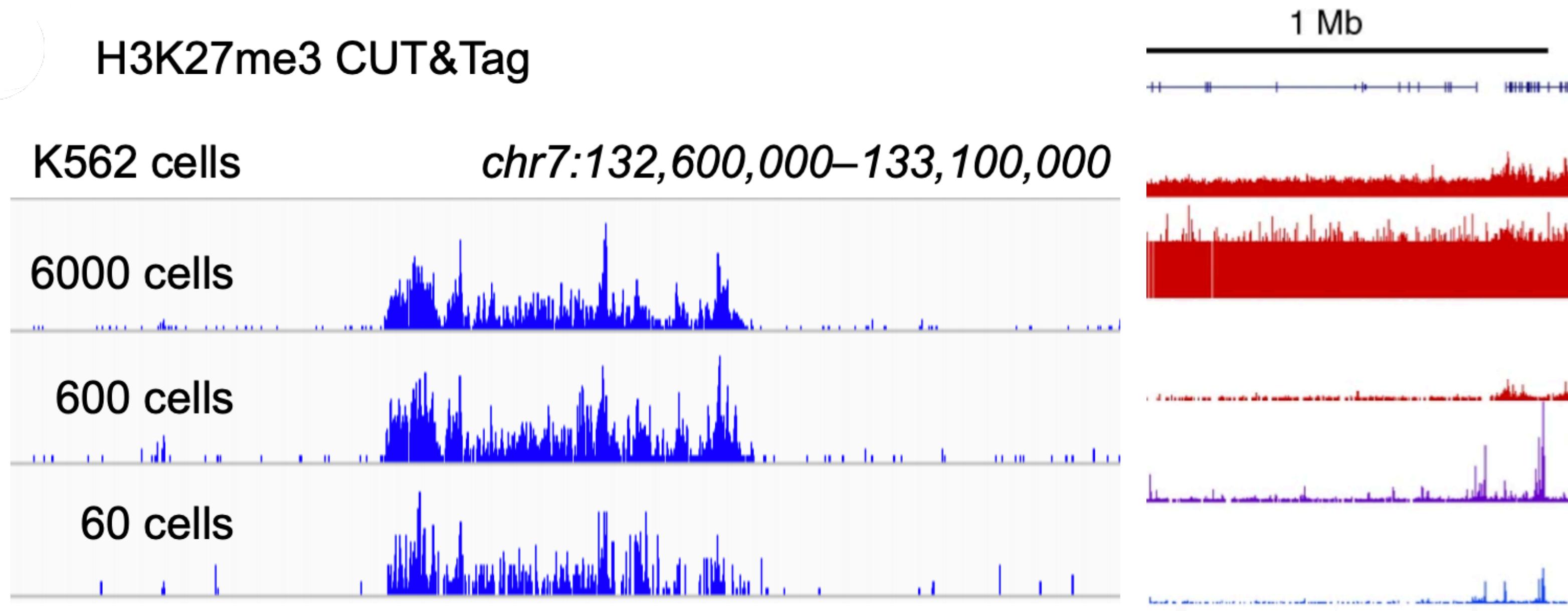


CUT&Tag: Cleavage Under Targets & Tagmentation

Protein A/Tn5 (pA-Tn5) fusion protein loaded with adaptors



H3K27me3 CUT&Tag



Kaya-Okur et al. *Nat Commun.* 2019

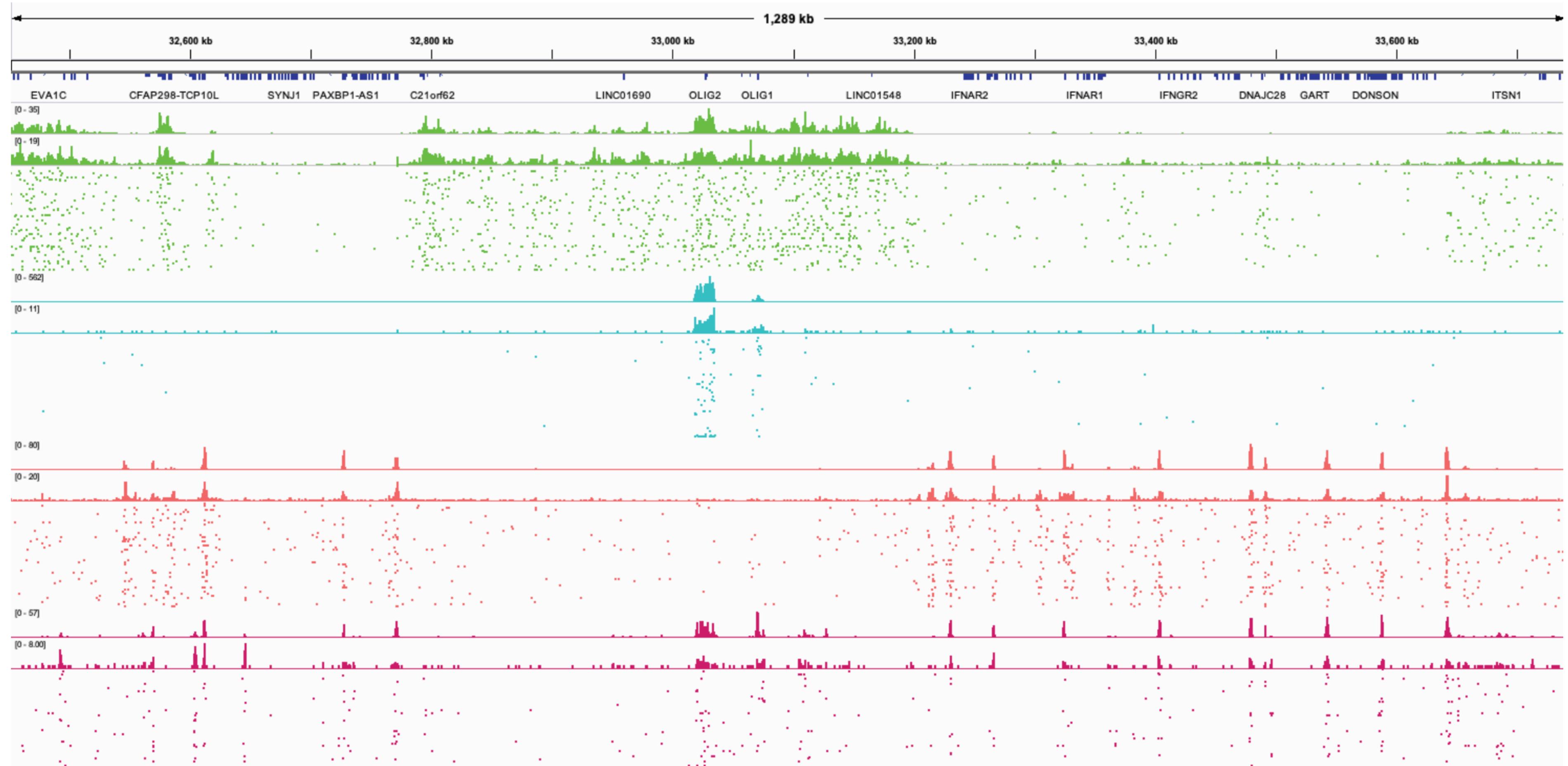
CUT&Tag can be performed at single-cell resolution

K27me3 K562
Bulk Aggregate
Single Cells

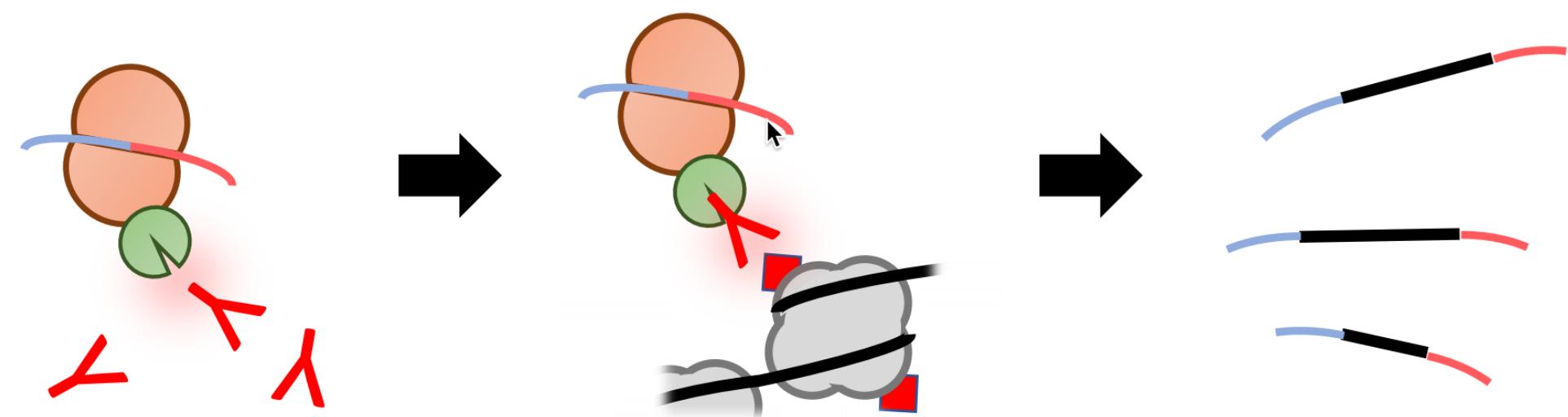
K27me3 hESC

K4me2 K562

K4me2 hESC



CUT&Tag can be performed at single-cell resolution

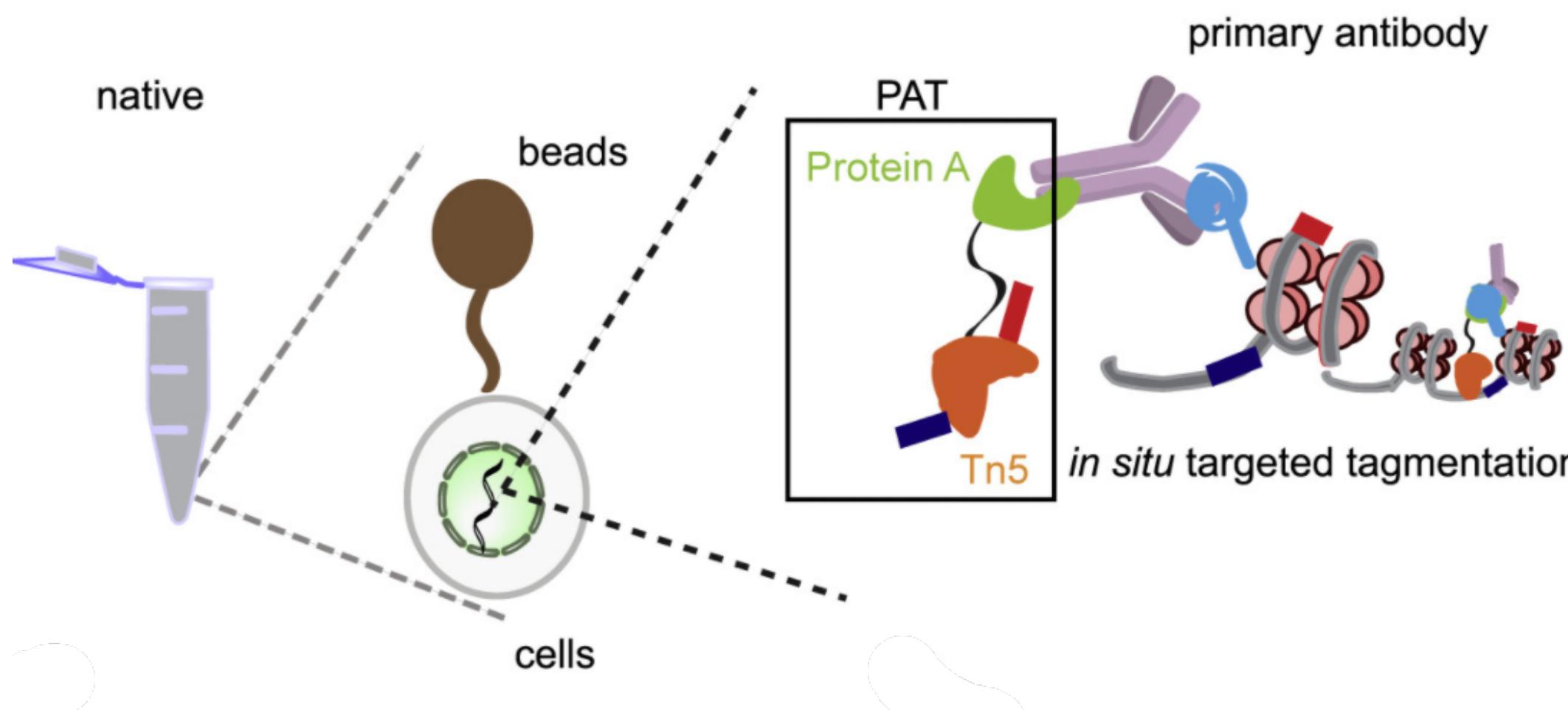


nature communications

Article | Open Access | Published: 20 August 2019

Mapping histone modifications in low cell number and single cells using antibody-guided chromatin tagmentation (ACT-seq)

Benjamin Carter, Wai Lim Ku, Jee Youn Kang, Gangqing Hu, Jonathan Perrie, Qingsong Tang & Keji Zhao



Molecular Cell

Volume 76, Issue 1, 3 October 2019, Pages 206-216.e7

Technology

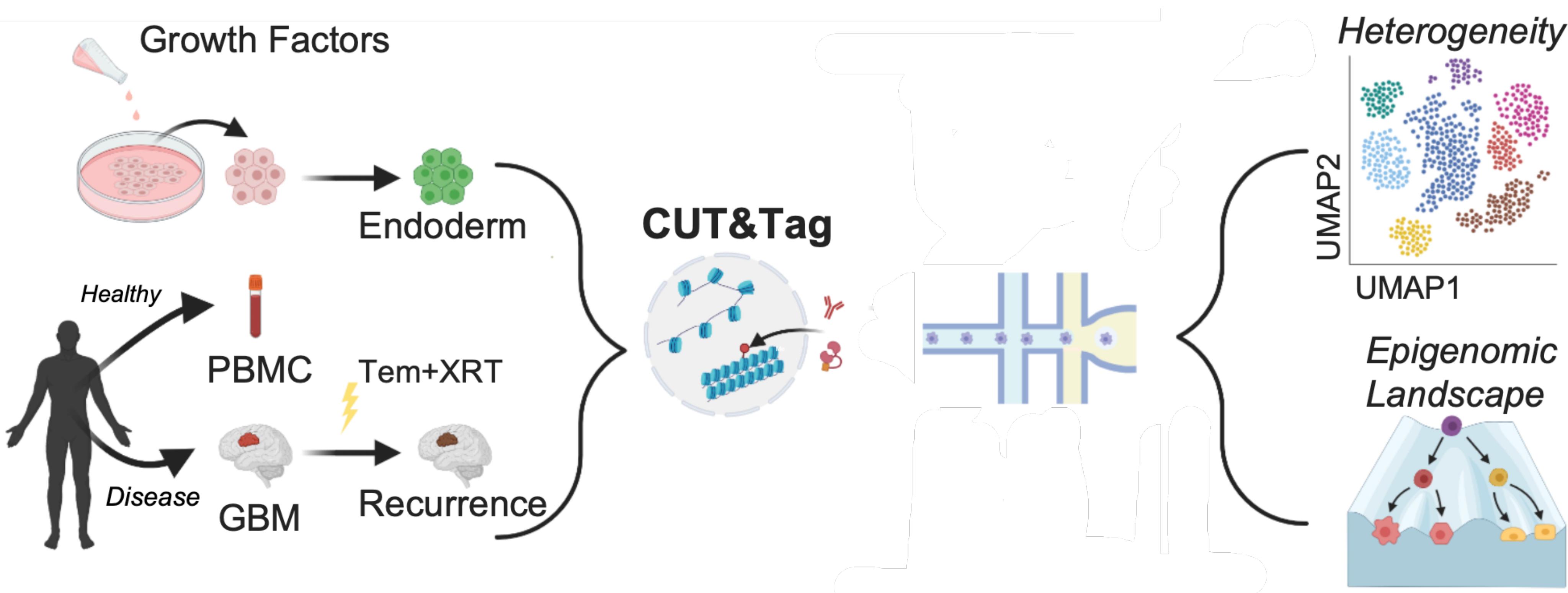
CoBATCH for High-Throughput Single-Cell Epigenomic Profiling

Qianhao Wang ^{1, 2}, Haiqing Xiong ^{1, 2}, Shanshan Ai ^{1, 2}, Xianhong Yu ^{1, 2}, Yaxi Liu ^{1, 2}, Jiejie Zhang ¹, Aibin He ^{1, 3}

Empowered by 10X Chromium

Single-cell analysis of chromatin silencing programs in development and tumor progression

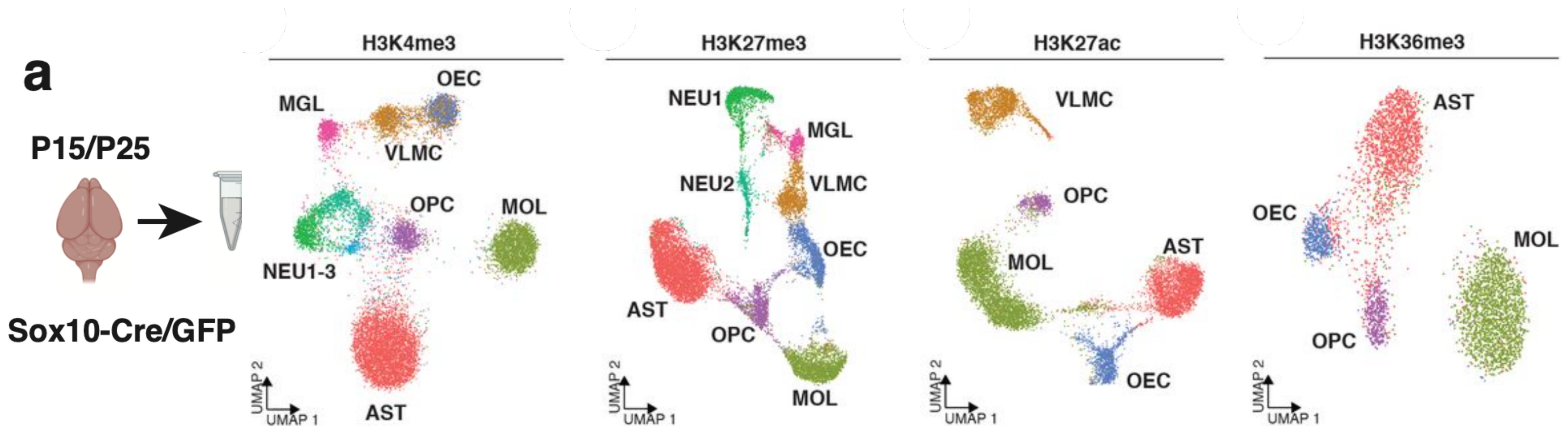
Steven J. Wu,  Scott N. Furlan, Anca B. Mihalas, Hatice S. Kaya-Okur,  Abdullah H. Feroze, Samuel N. Emerson,  Ye Zheng, Kalee Carson, Patrick J. Cimino, C. Dirk Keene,  Jay F. Sarthy,  Raphael Gottardo,  Kami Ahmad,  Steven Henikoff,  Anoop P. Patel



Empowered by 10X Chromium

Single-cell profiling of histone modifications in the mouse brain

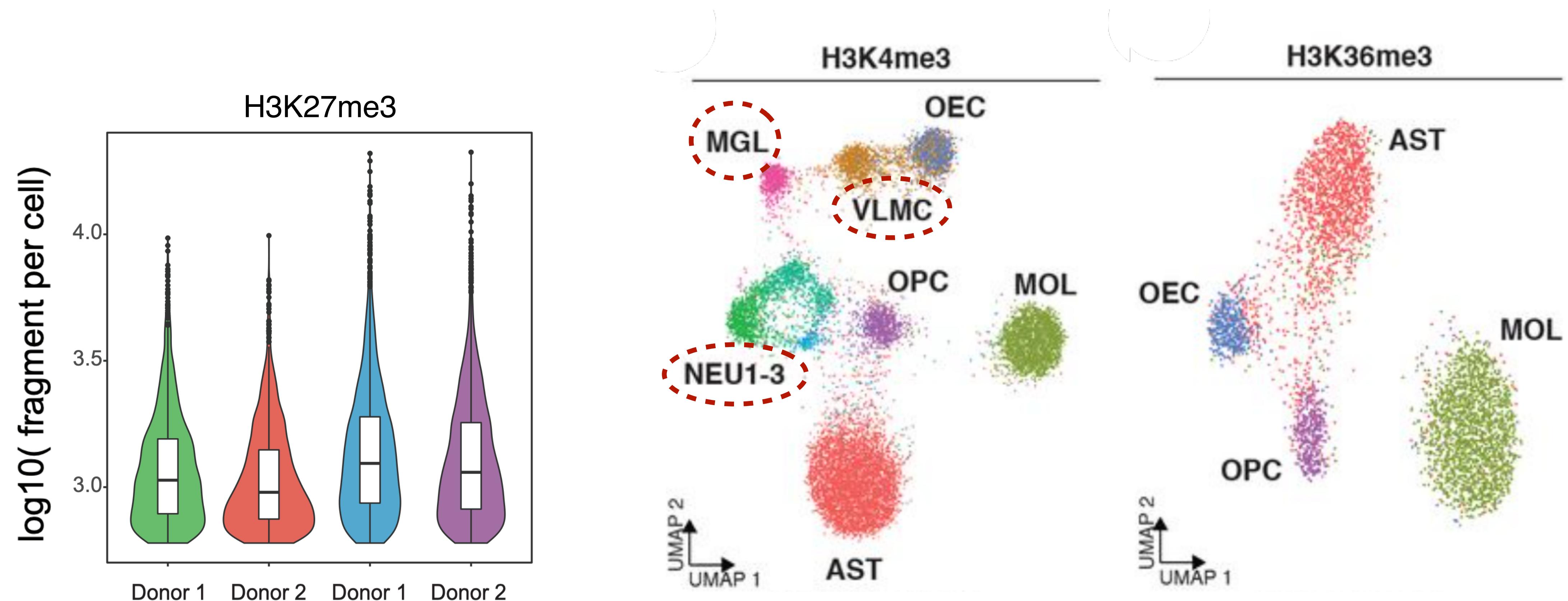
✉ Marek Bartosovic, Mukund Kabbe, ✉ Gonçalo Castelo-Branco



- Too many centrifuge steps.
- Nuclei are easy to clump in high salt buffer (1% BSA in specific buffers).

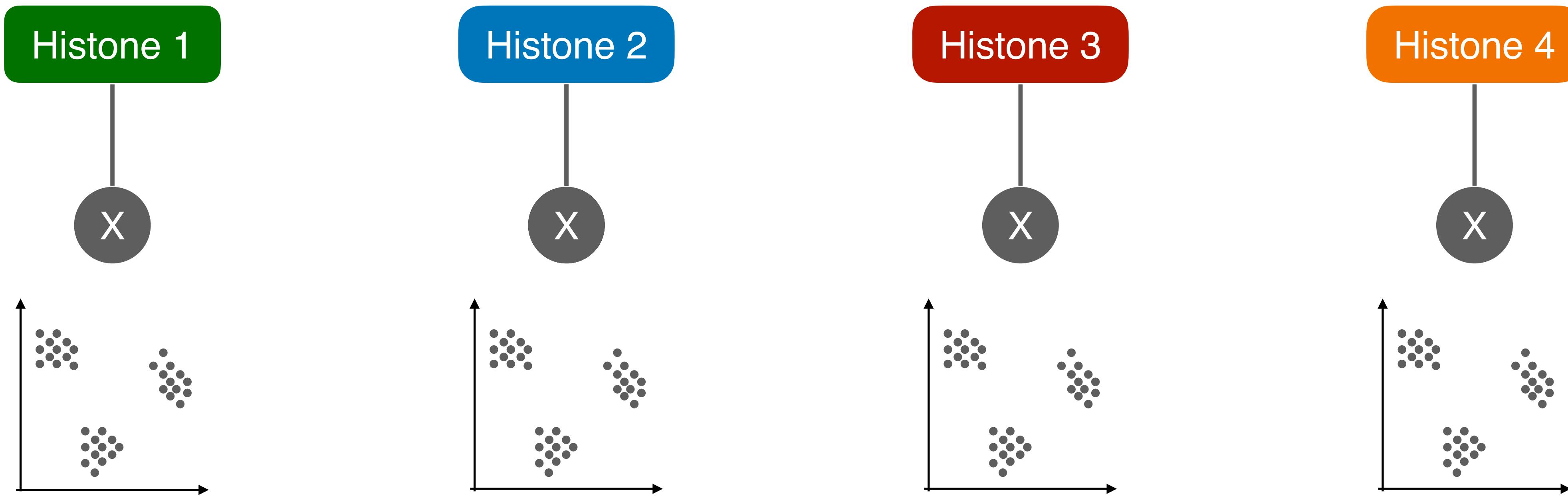
Limitations of scCUT&Tag

- Data is not ideally suited for clustering / discovery.
- Challenging to integrate datasets from different marks.



Wu et al, *bioRxiv* 2020

A solution in theory



Histone 1

Histone 2

Histone 3

Histone 4



Histone 1

Histone 2

Histone 3

Histone 4



Histone 1

Histone 2

Histone 3

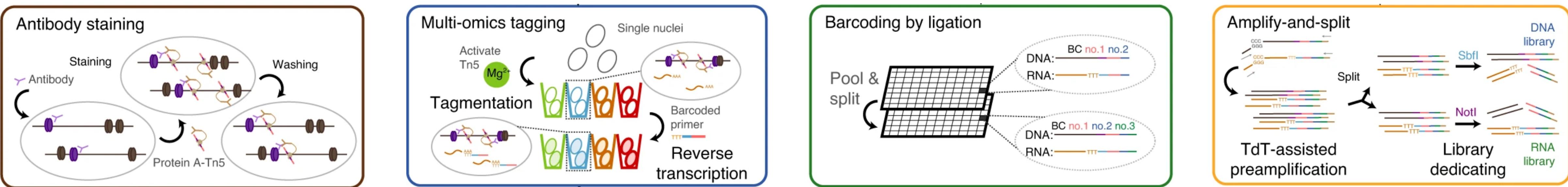
Histone 4

Joint scCUT&Tag/RNA co-assay: Paired-Tag

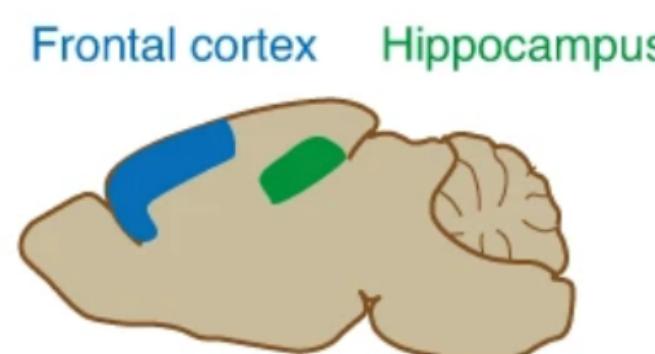
nature methods

Joint profiling of histone modifications and transcriptome in single cells from mouse brain

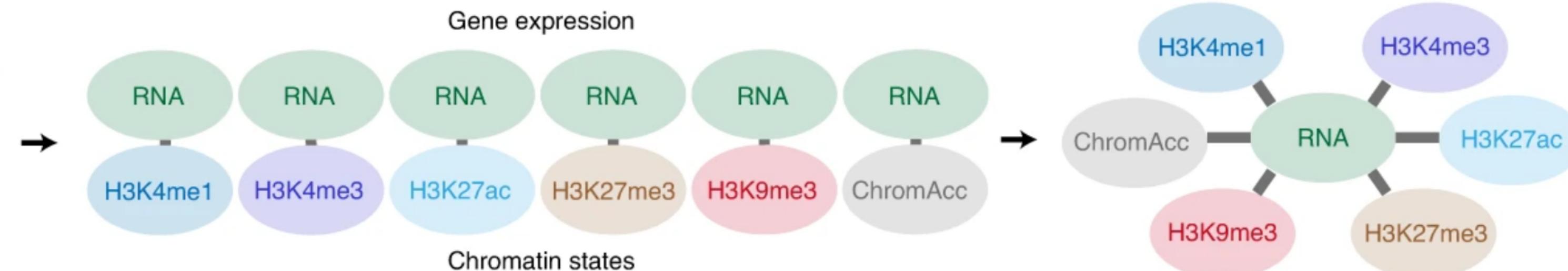
Chenxu Zhu, Yanxiao Zhang, Yang Eric Li, Jacinta Lucero, M. Margarita Behrens & Bing Ren 



Mouse brain regions



Single-cell multi-omics profiling

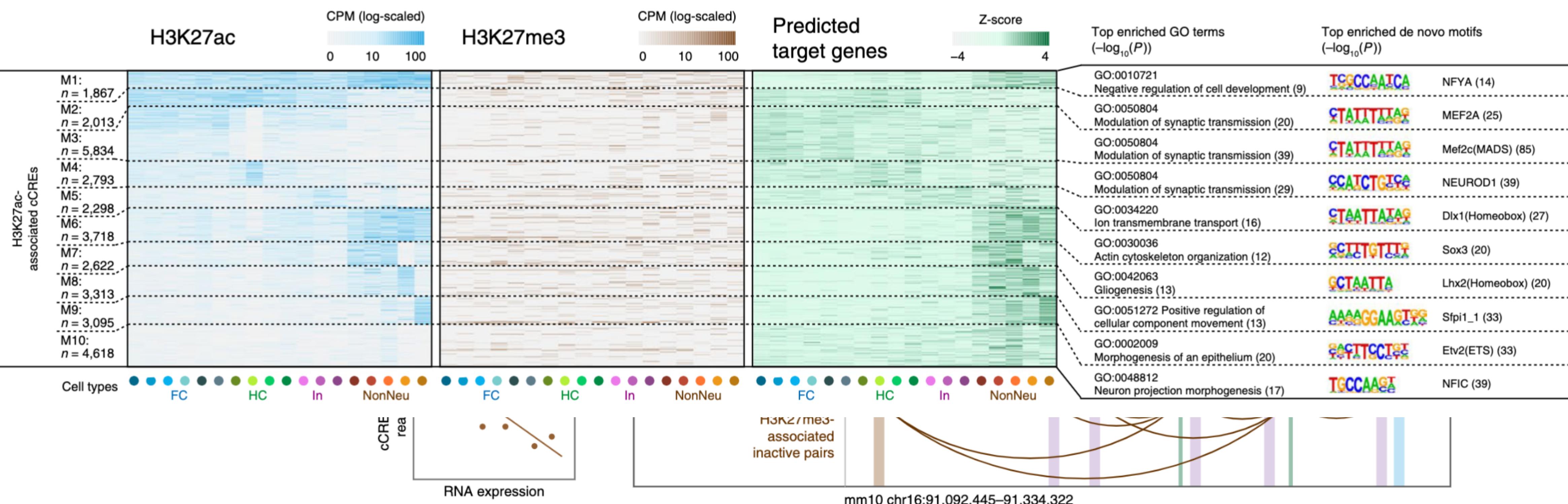


Chenxu Zhu et al, *Nature Methods*. 2021

Integrative analysis of chromatin state and gene expression

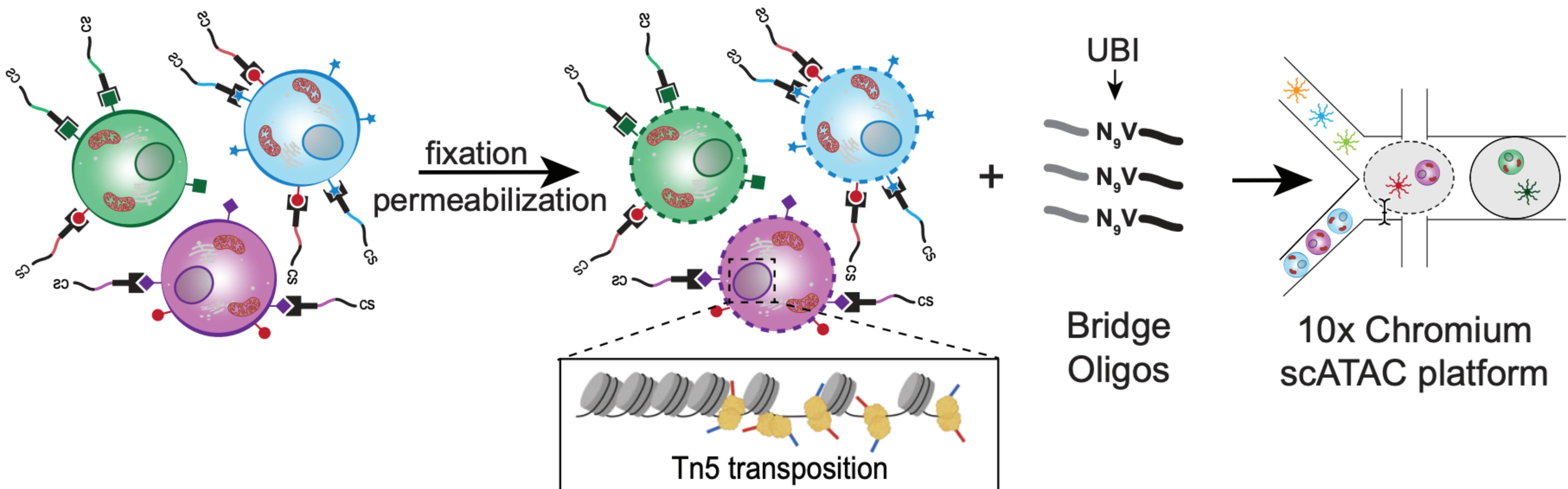
Integrative analysis of chromatin state and gene expression connects distal cCREs to putative target genes

CREs: *Cis*-regulatory elements

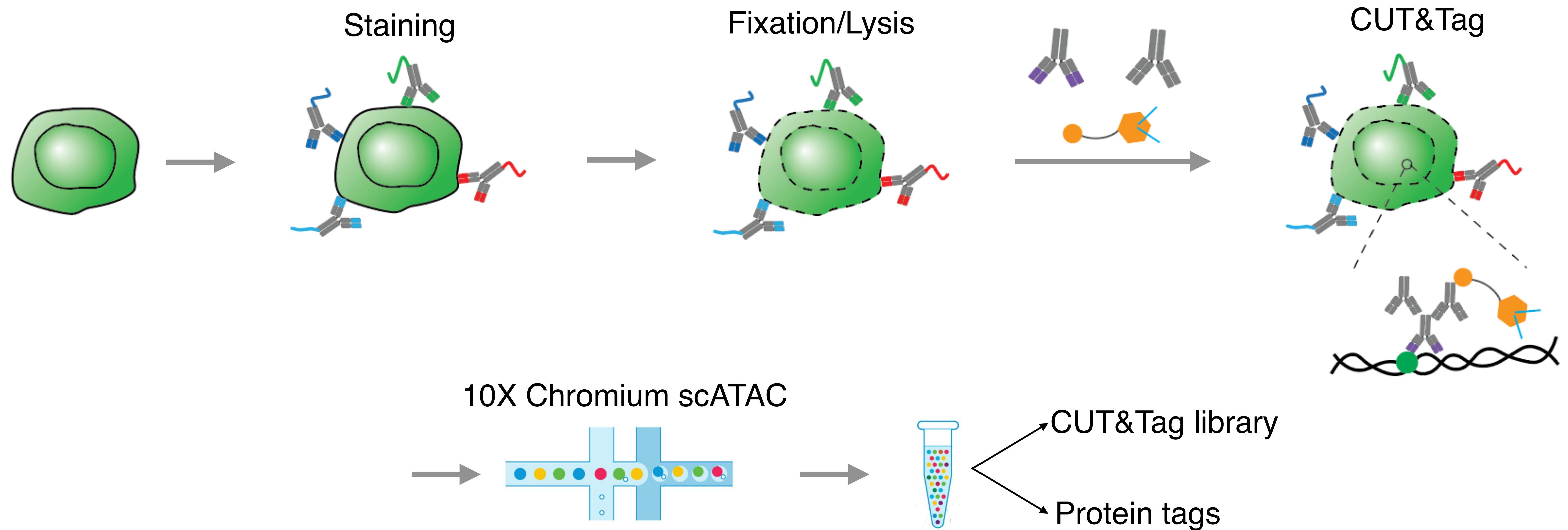


Alternative approach: joint profiling with surface proteins

ASAP-seq: ATAC with Select Antigen Profiling by sequencing

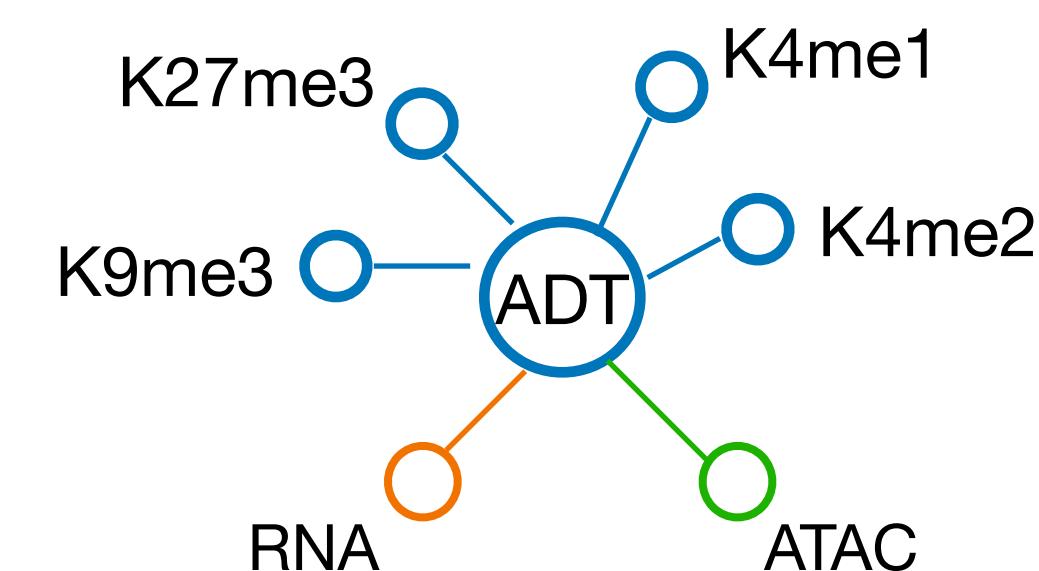
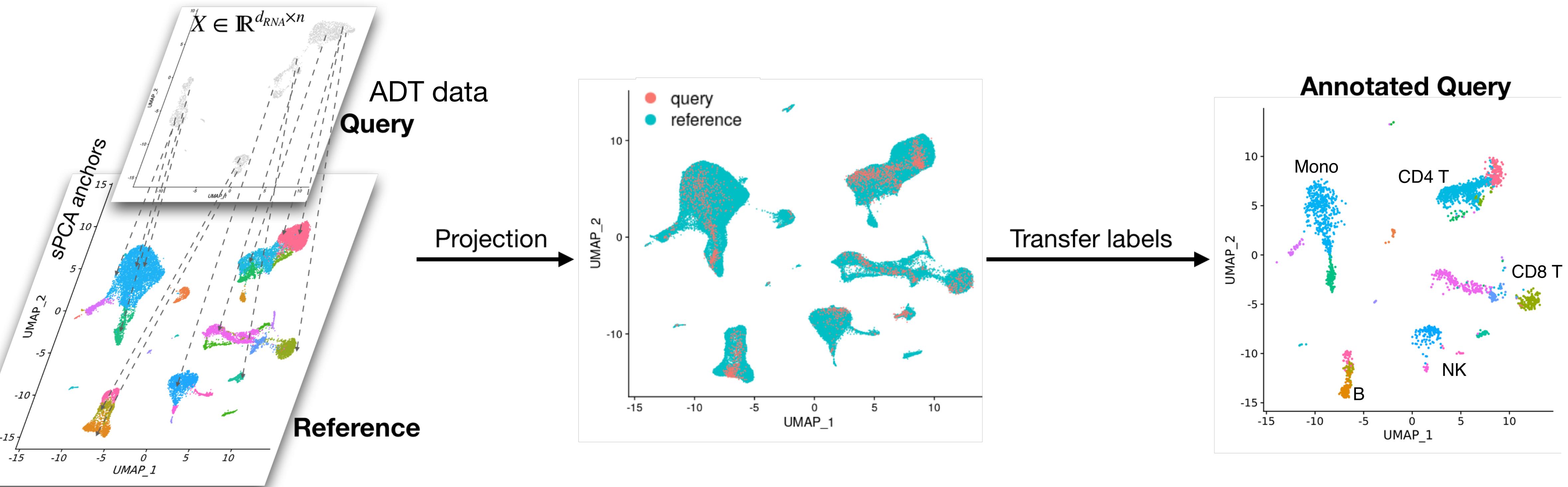


scCUT&Tag-Pro: scCUT&Tag/surface proteins co-assay



Acknowledgment to Eleni Mimitou and Peter Smibert

Reference mapping in Seurat V4

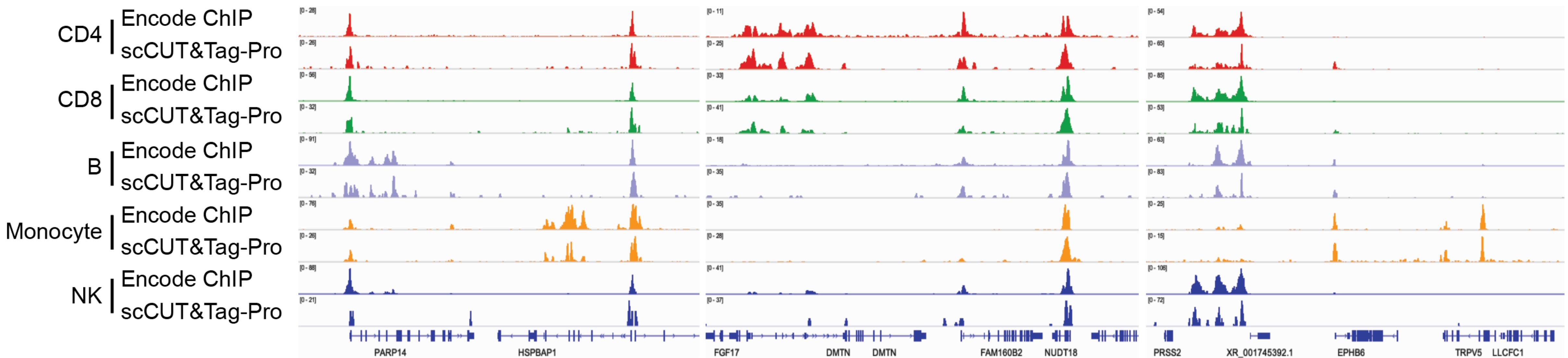


<http://azimuth.satijalab.org/app/azimuth>

Figure by: Yuhao
Yuhao *bioRxiv* 2020.10.12

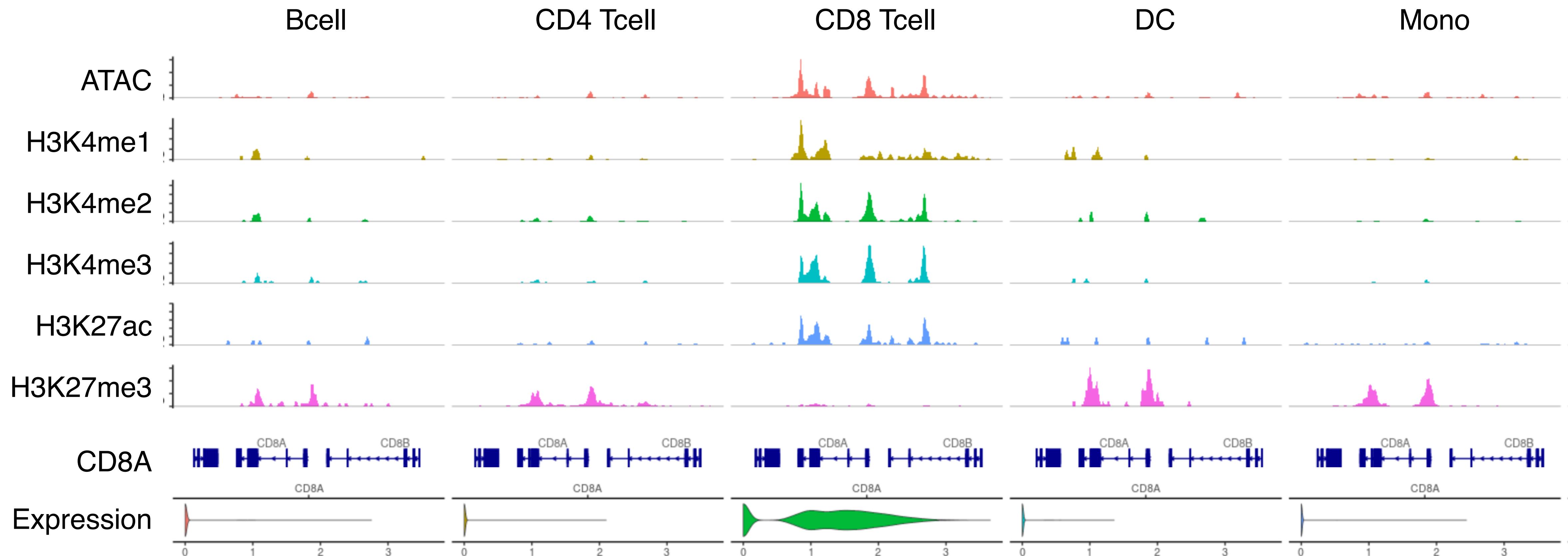
scCUT&Tag-Pro of PBMC

PBMC H3K27ac



Unpublished data

Multimodal data integration

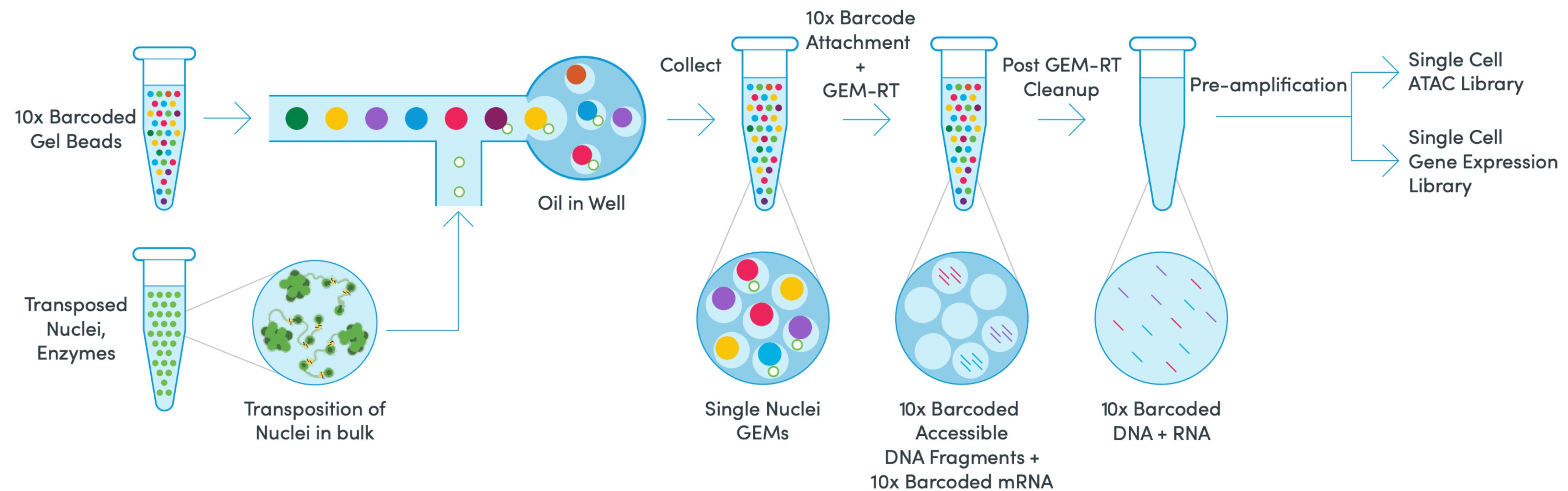


Integration and visualisation: Signac

Acknowledgment to Avi Srivastava
Unpublished data

Future perspectives

Adapt to 10X Multiome Kit

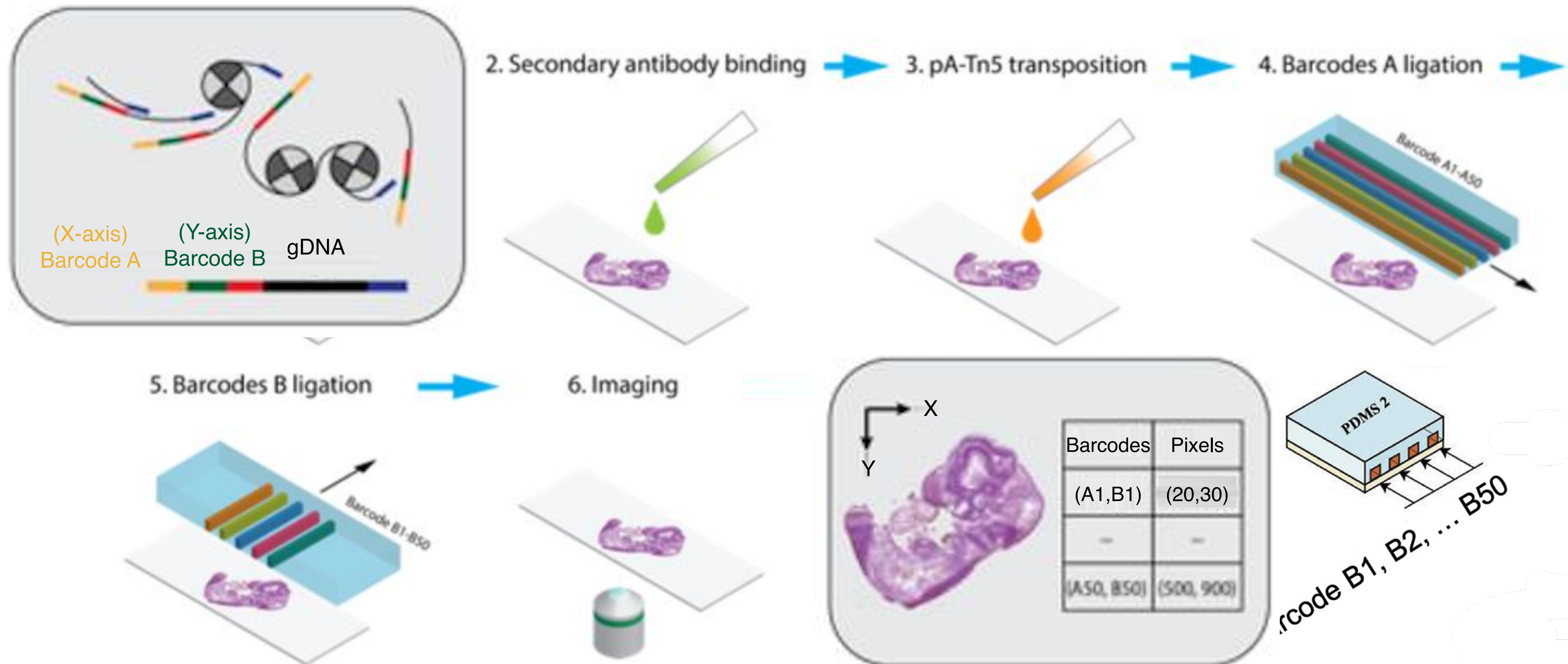


Longer incubation time, multiple washes and fixation condition may result in RNA data with lower quality

Future perspectives

Spatial Epigenome Sequencing at Tissue Scale and Cellular Level

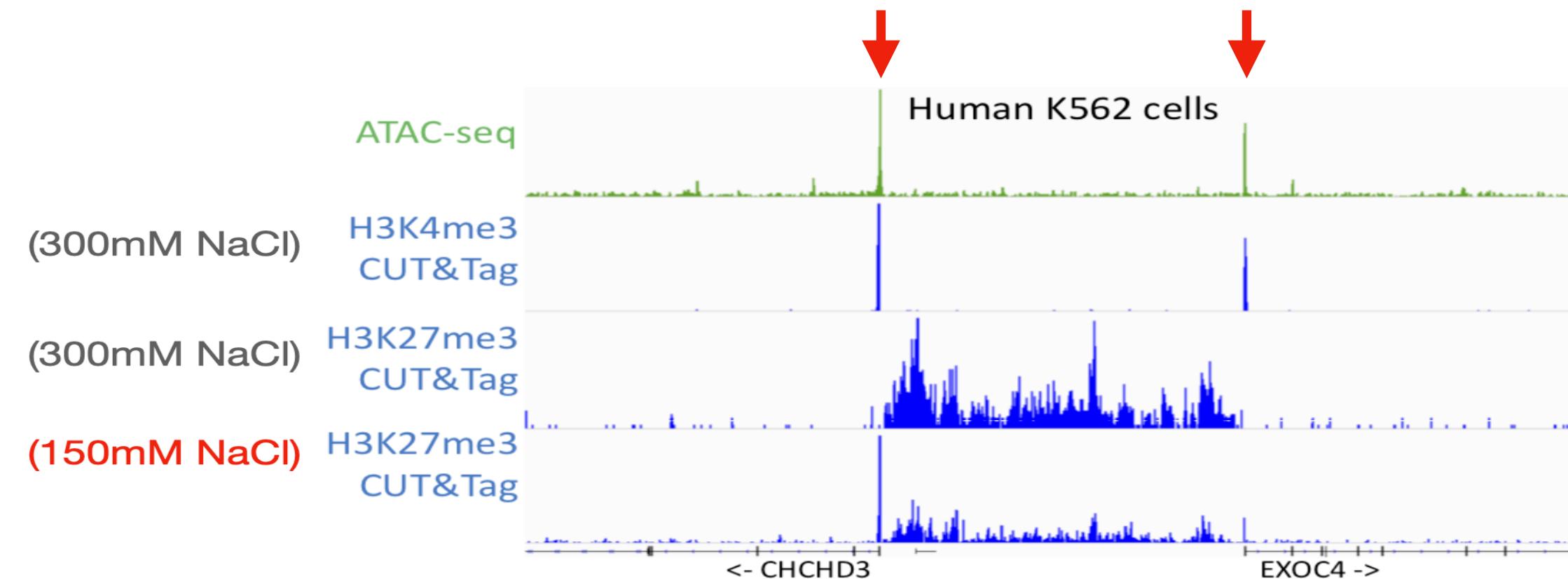
Yanxiang Deng, Di Zhang, Yang Liu, Graham Su, Archibald Enninful, Zhiliang Bai, Rong Fan



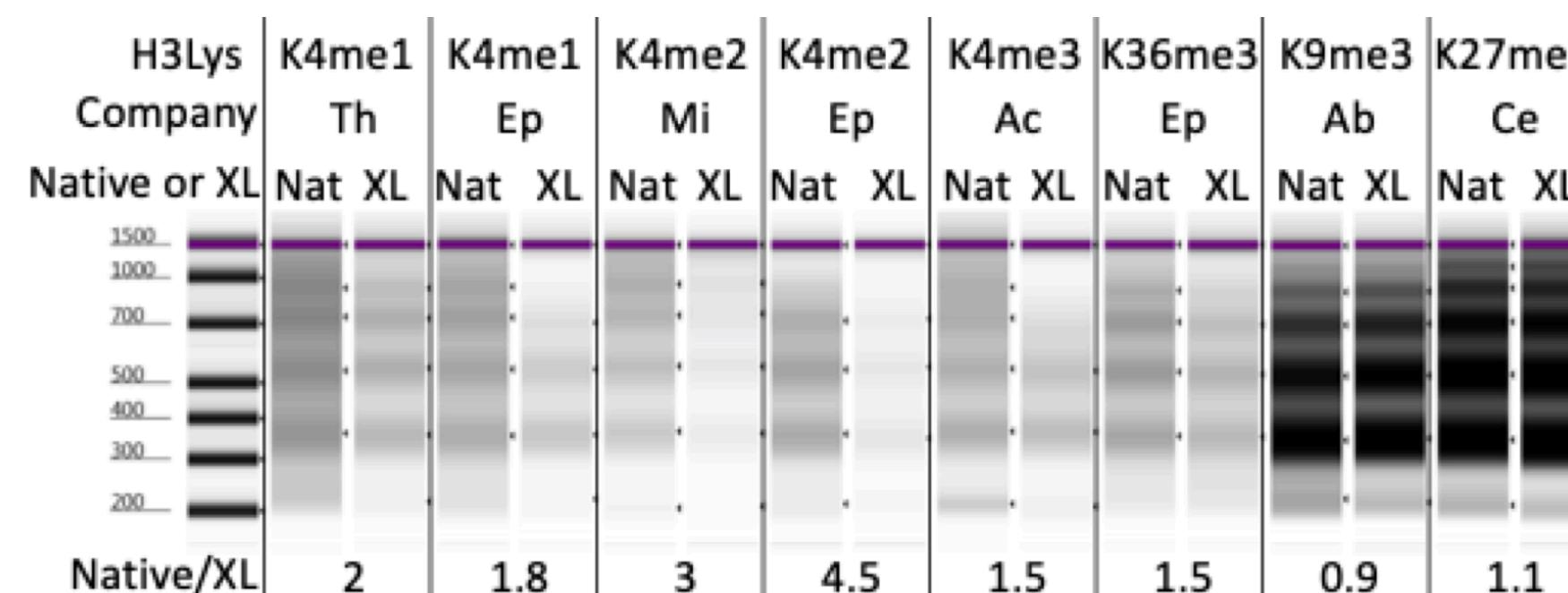
Yanxiang Deng et al, *bioRxiv* 2021

Things to consider

- 300 mM NaCl is required to suppress artifactual binding of pA-Tn5 to accessible DNA



- Reduced library yields with lightly cross-linked nuclei with some epitopes and antibodies.



Suitable for TFs ?

Reference

<https://www.whatisepigenetics.com/fundamentals/#:~:text=Epigenetics%20is%20the%20study%20of,how%20cells%20read%20the%20genes.>