Scheine (best 4 out of 5), first week of March

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From fluorescence microscopy to sequencing and back

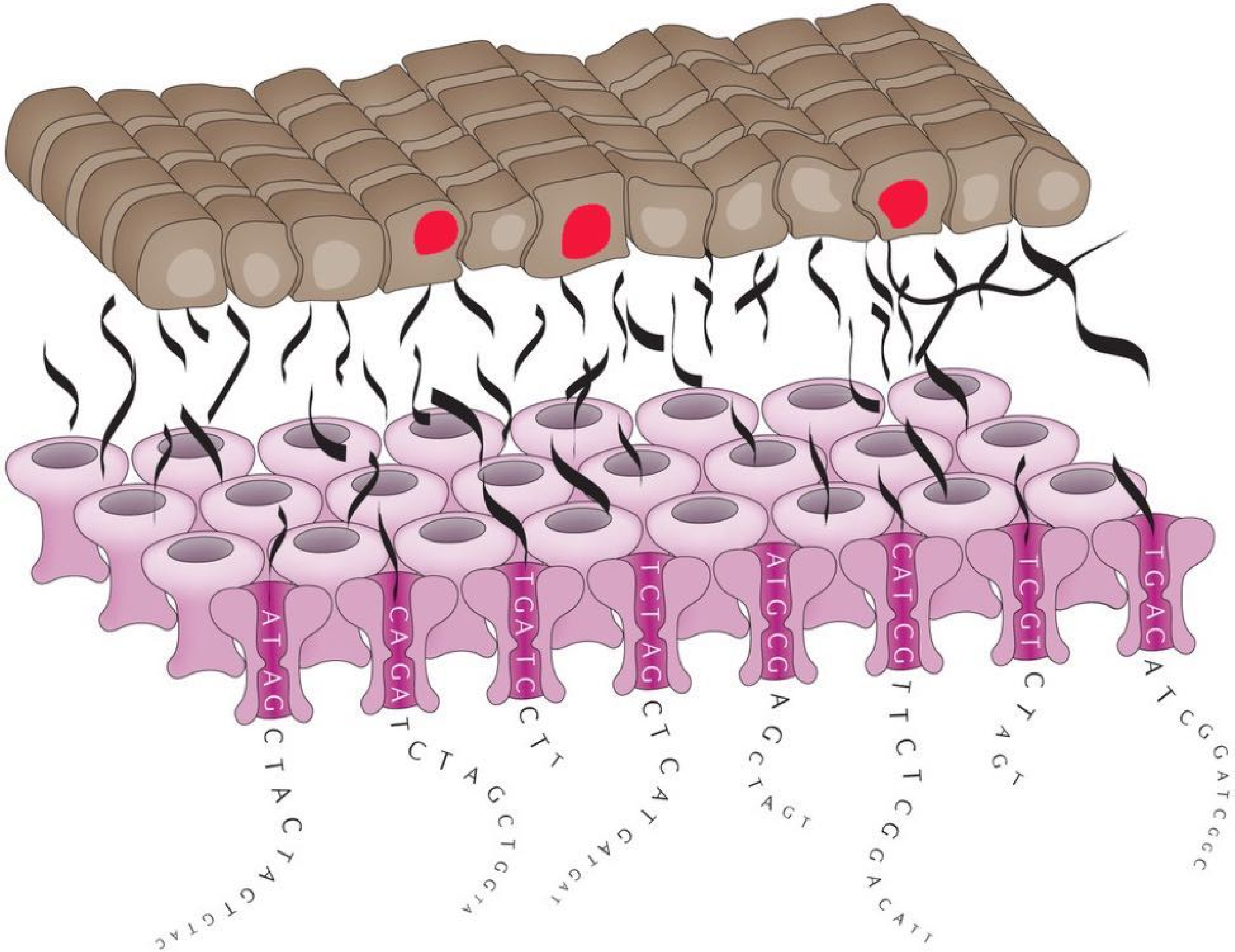
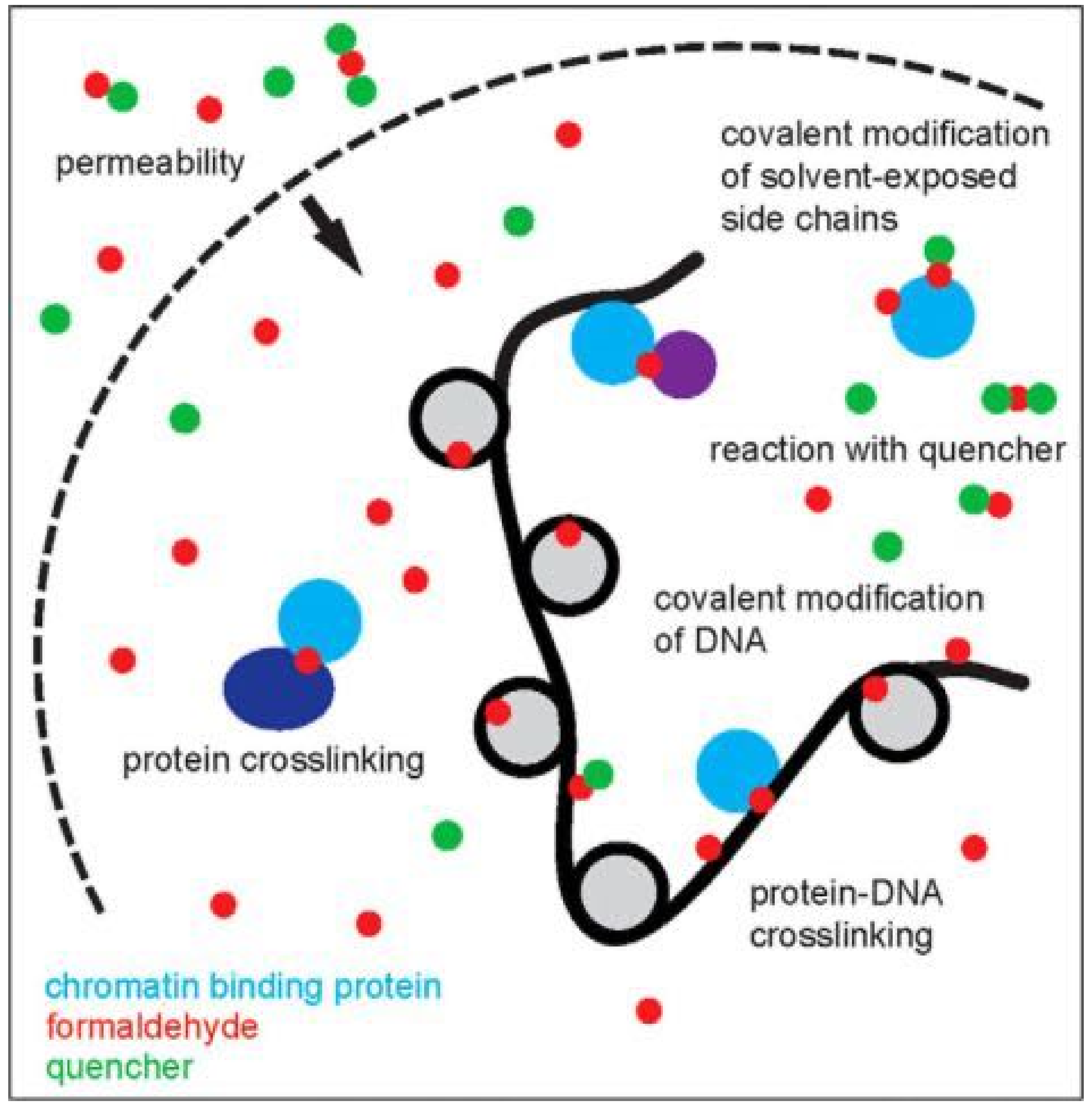
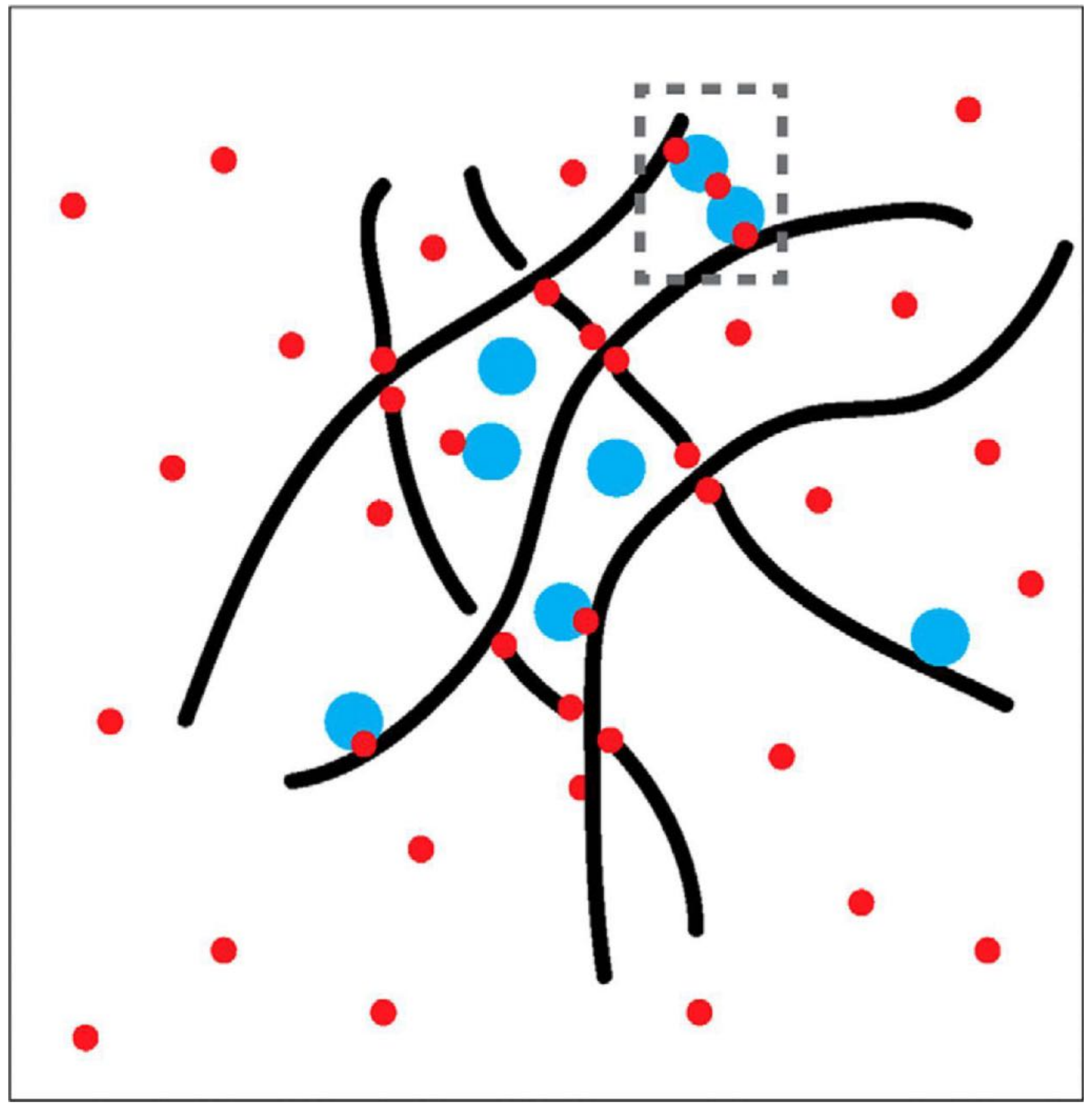
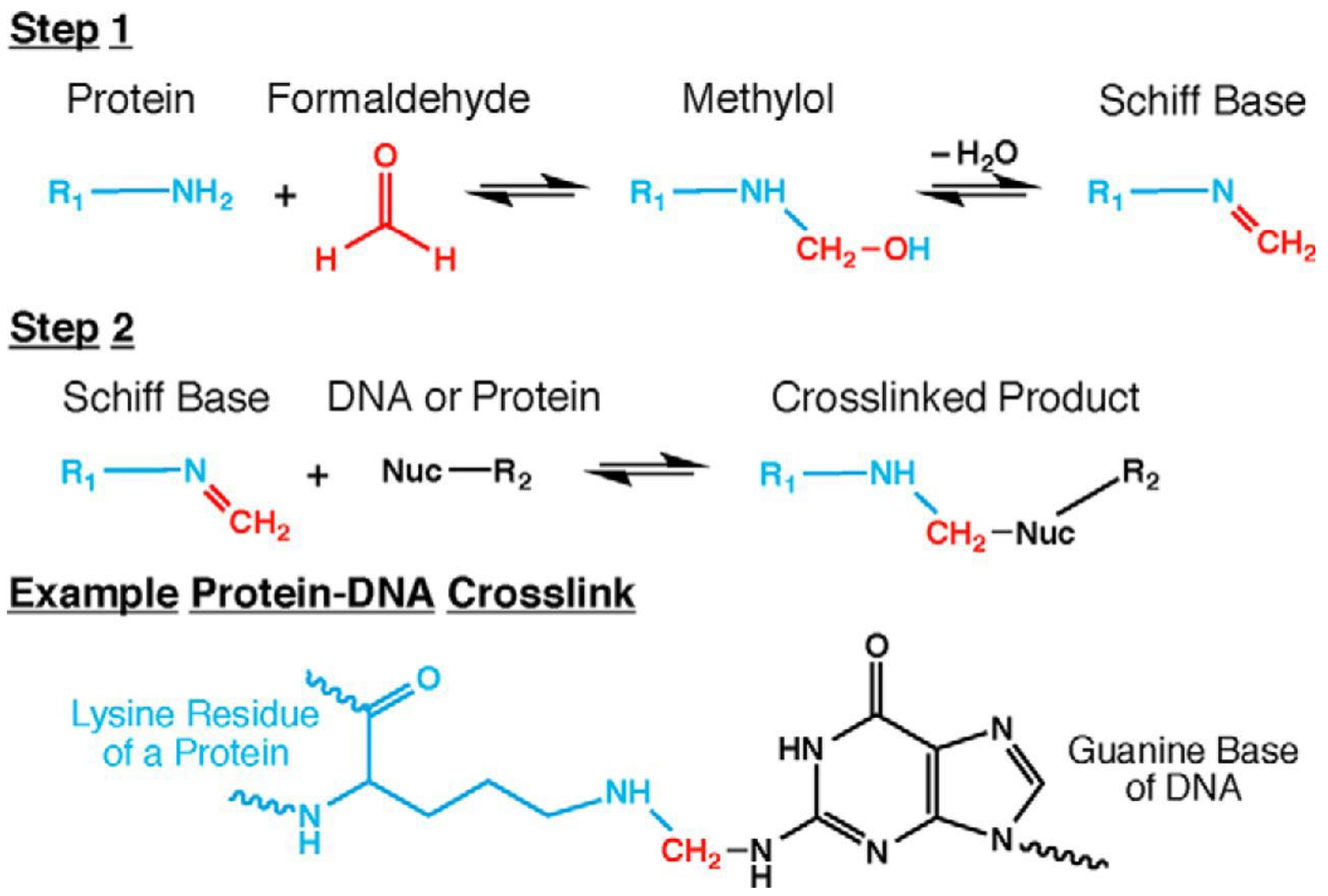


Image from Crosetto,
Nat Rev Genet, 2015

Formaldehyde cross-linking of proteins and DNA

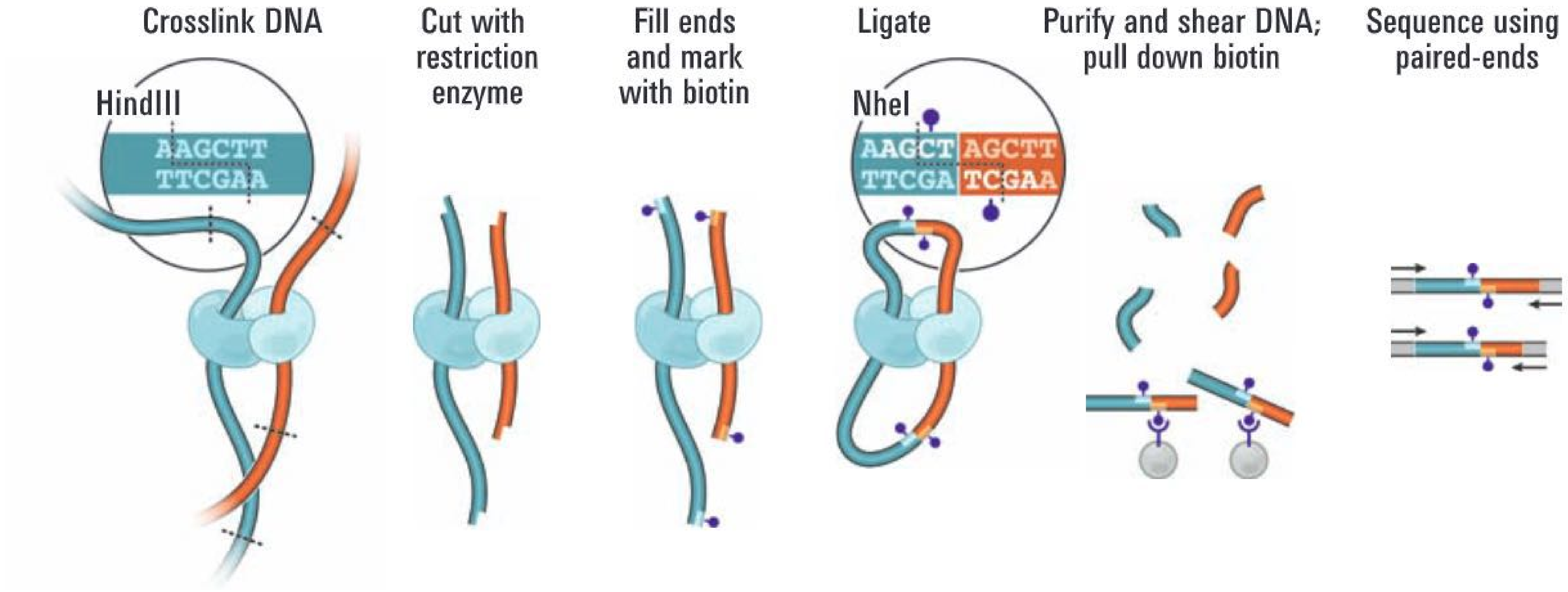


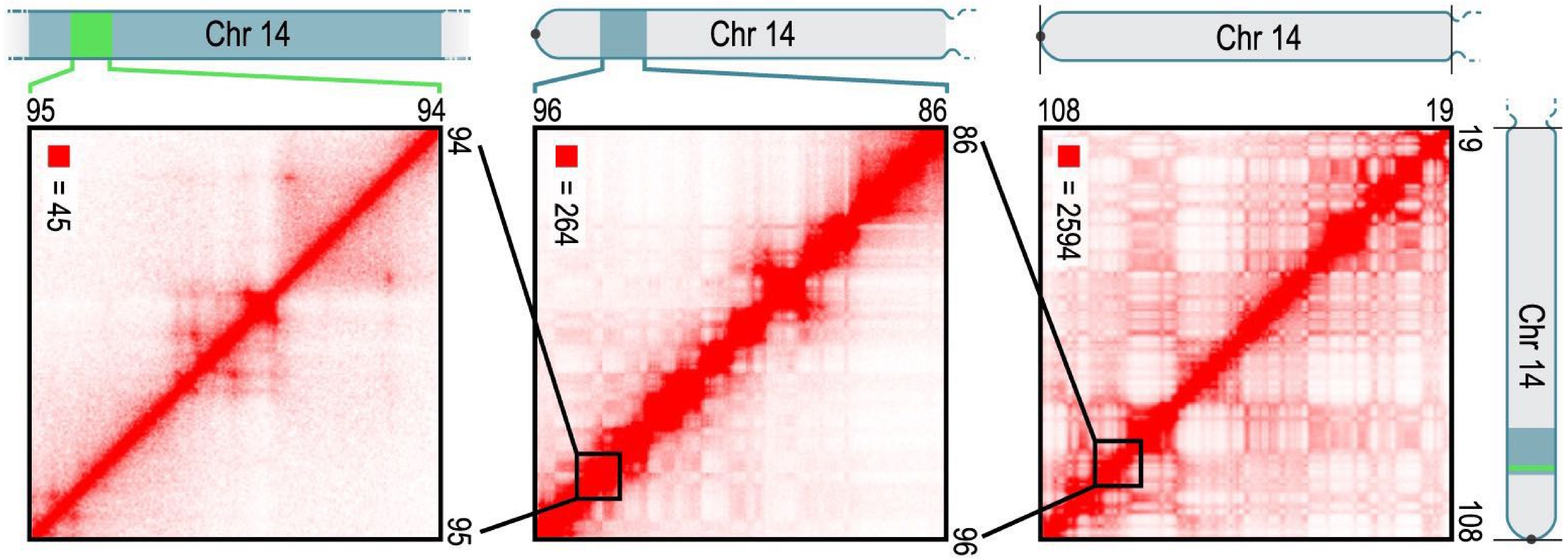


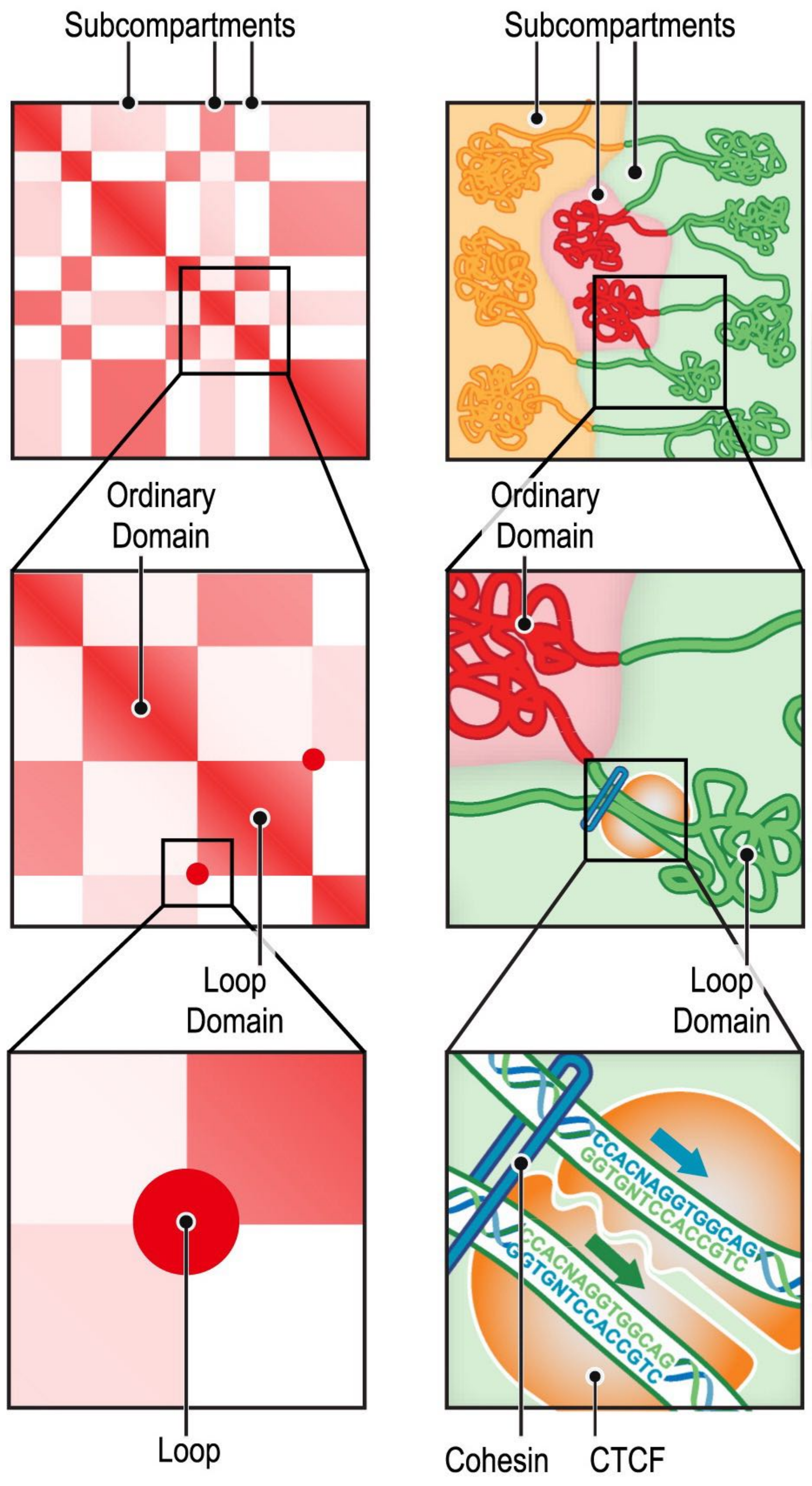


Hoffman, Journal of Biological Chemistry 290, 2015

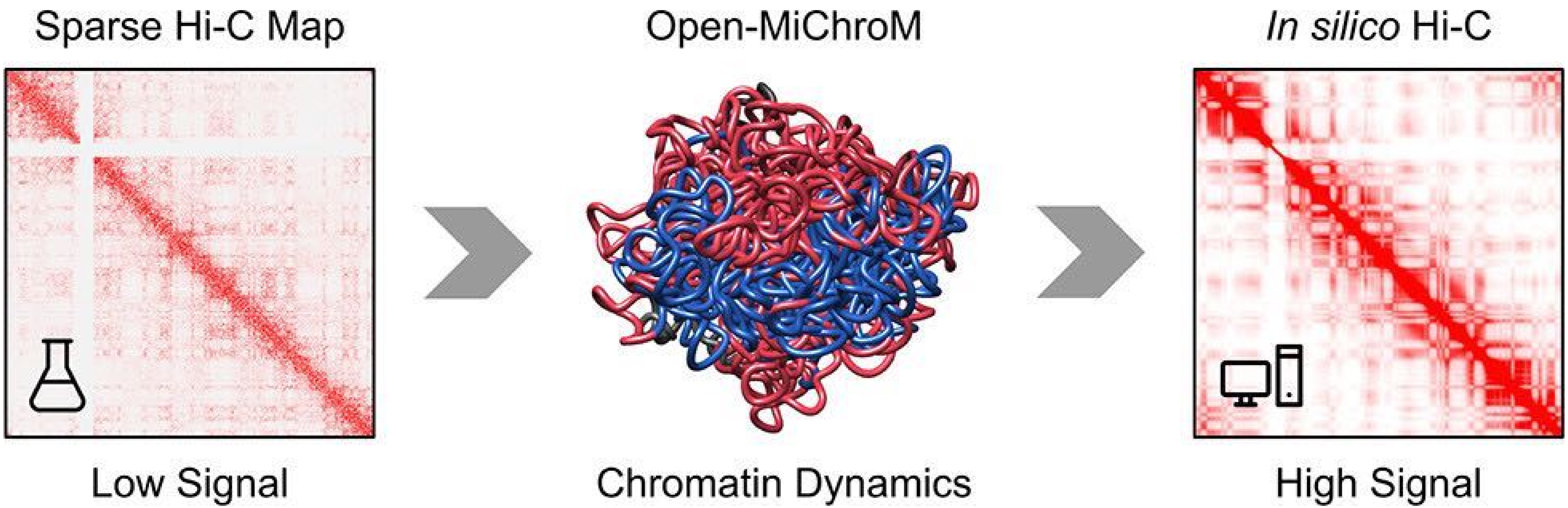
Chromatin **C**onformation **C**apture (3C) analysis of chromatin organization *in situ* by cross-linking and sequencing







Single cell genome folding maps from Hi-C



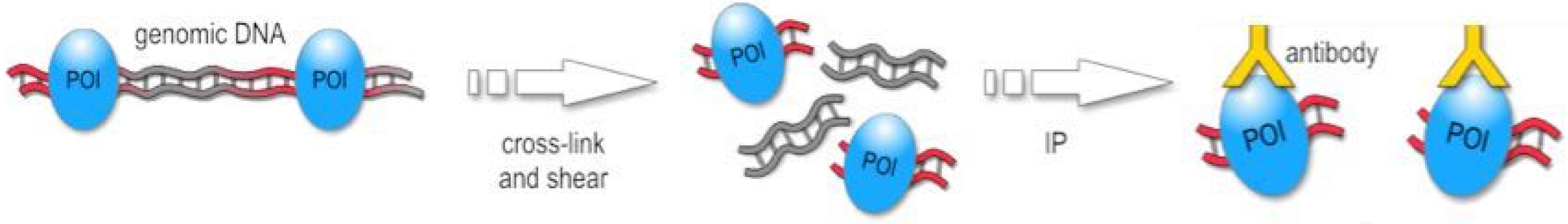
Oliveira, J. Phys. Chem. B 2021, 125, 31, 8757–8767

Quantitative information on chromatin binding from ChIP-seq

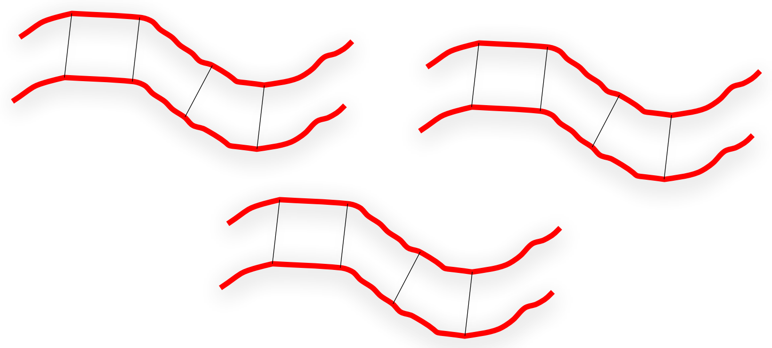
DNA + bound protein

Fragment DNA

Immunoprecipitate



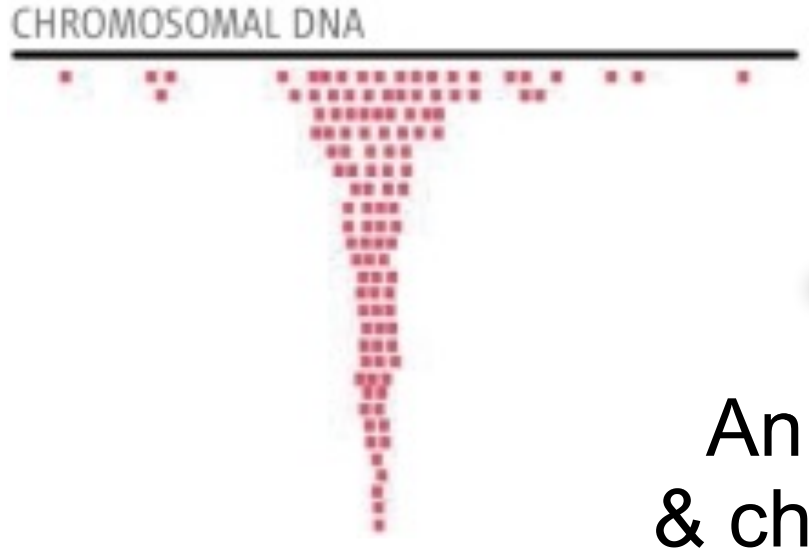
Release DNA



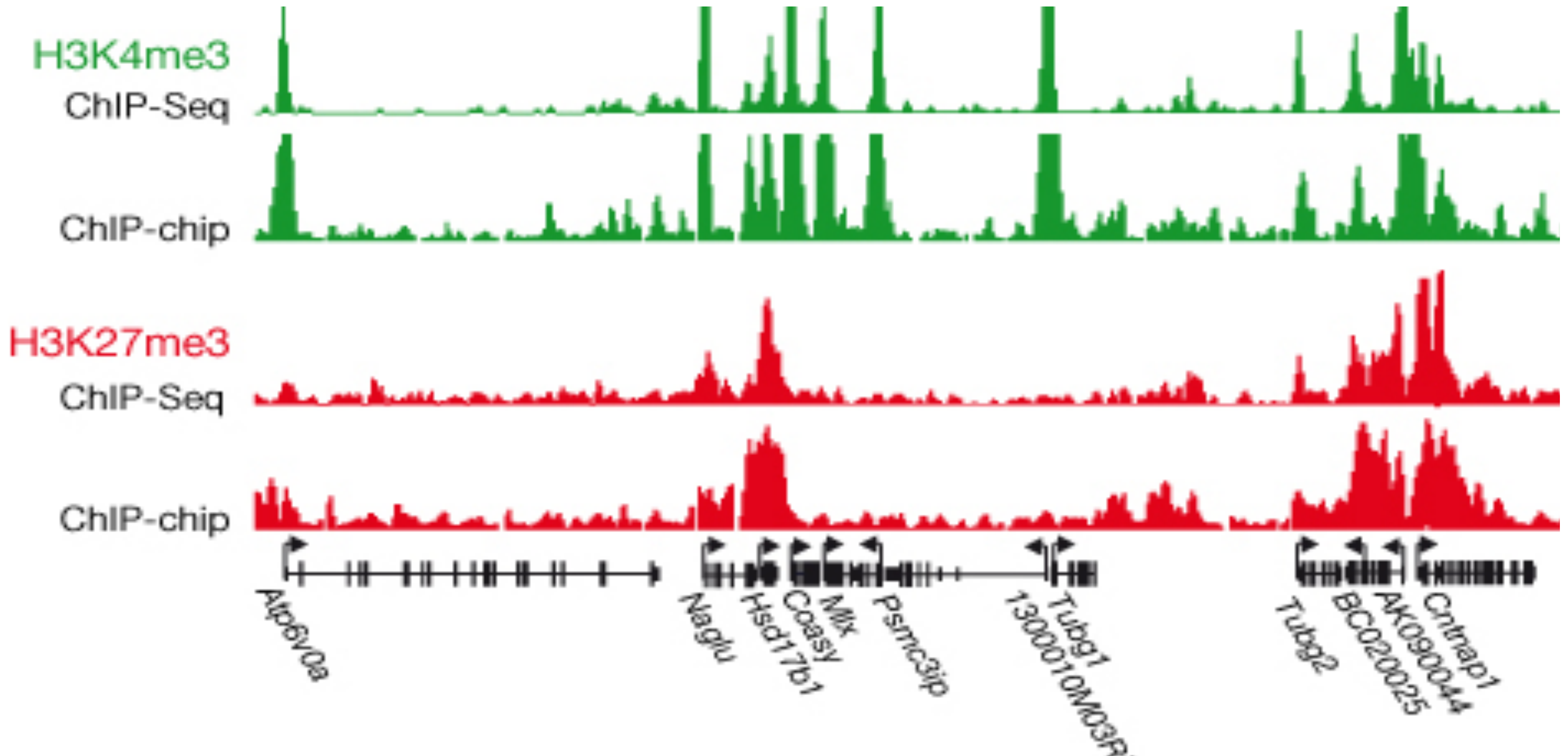
Prepare sequencing library

Sequence

Map sequence tags to genome & identify peaks



Annotate peaks & chromatin states



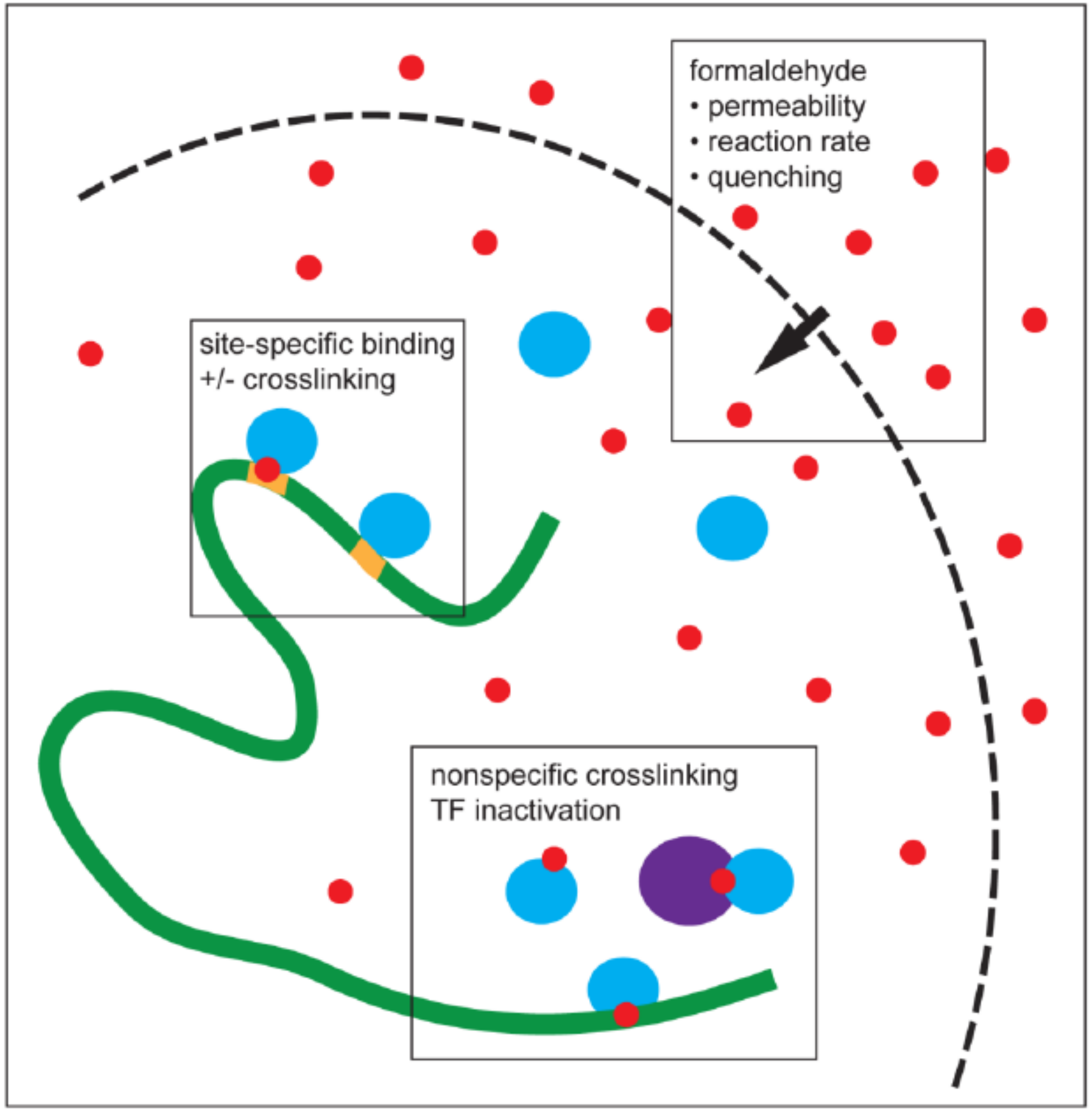
Adapted from slide set by: Stuart M. Brown, Ph.D.,
Center for Health Informatics & Bioinformatics, NYU School of Medicine

Measuring Chromatin Interaction Dynamics on the Second Time Scale at Single-Copy Genes

Kunal Poorey,[1]* Ramya Viswanathan,[1]* Melissa N. Carver,[1] Tatiana S. Karpova,[2]
Shana M. Cirimotich,[1] James G. McNally,[2] Stefan Bekiranov,[1]† David T. Auble[1]†

www.sciencemag.org **SCIENCE** VOL 342 18 OCTOBER 2013

Measure binding kinetics of transcription factors at single binding sites from ChIP-seq analysis by the CLK (cross-linking kinetic) method

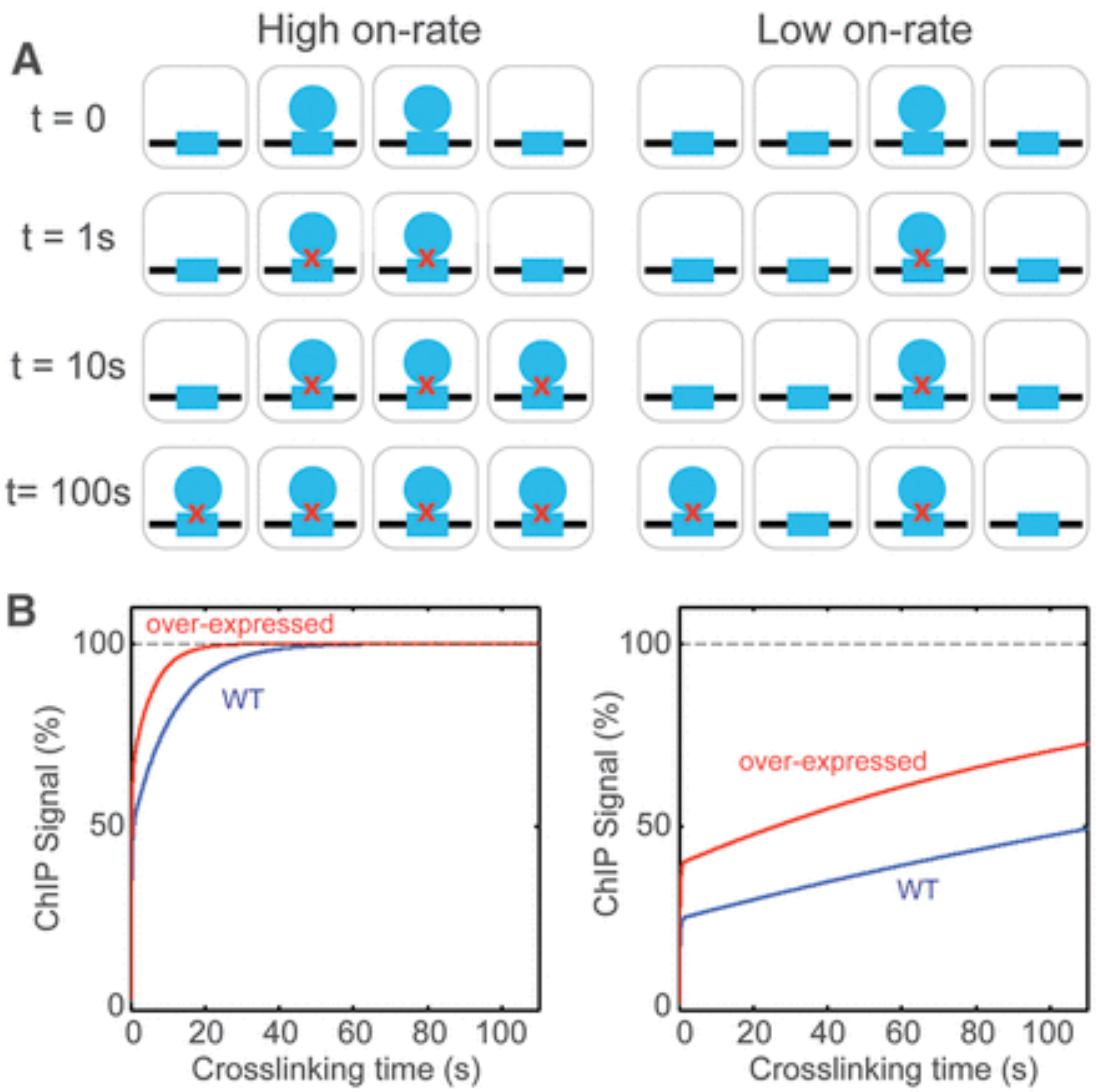


formaldehyde
- permeability
- reaction rate
- quenching

site-specific binding
+/- crosslinking

nonspecific crosslinking
TF inactivation

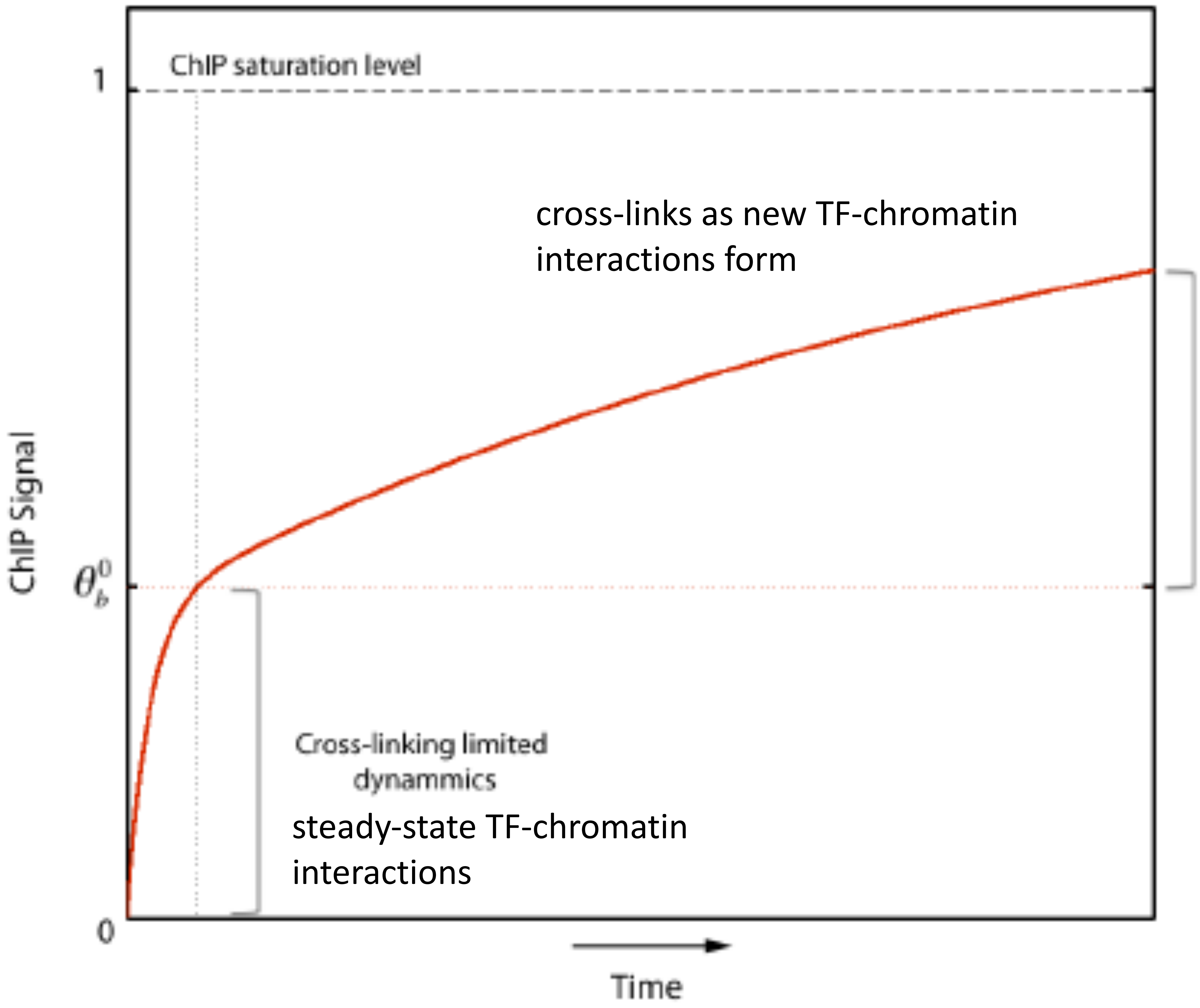
Measuring Chromatin Interaction Dynamics on the Second Time Scale at Single-Copy Genes by ChIP-seq



Assumptions

- TF in excess over binding sites
- TFs are cross-linked to specific and un-specific sites
- unbound TFs are not inactivated by cross-linking
- formaldehyde is not limiting
- ChIP signal is proportional to the number of TF-chromatin interactions

CLK curve



$$\mathit{unbound\ TF}\ \underset{r_{1b}}{\overset{r_{1f}}{\rightleftarrows}}\ \mathit{bound\ TF}\ \xrightarrow{r_2}\ \mathit{crosslinked\ TF}$$

On rate limiting chromain dynamics

short cross-linking time

$$\theta_{xl}(t) \approx \theta_b^0(1-e^{-k_{xl}C_{FH}t})$$

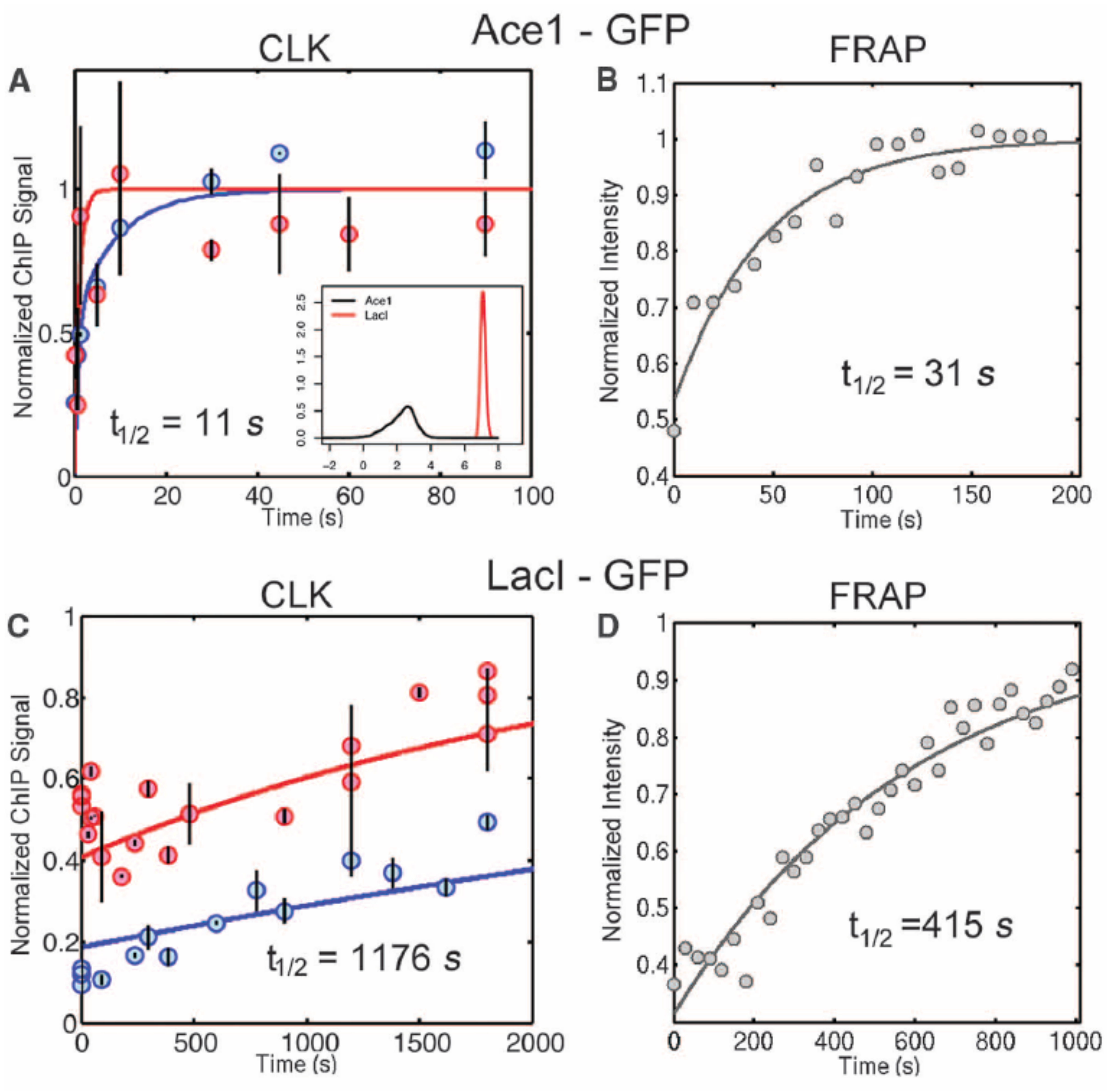
Fraction cross-linked

Fraction bound at t = 0

$$\theta_{xl}(t) \approx 1-(1-\theta_b^0)e^{-k_a C_{TF} t}.$$

TF concentration

Comparison of FRAP and CLK



ACE1 at the
CUP1 gene array

LacI at the
*lac*O array

From fluorescence microscopy to sequencing and back

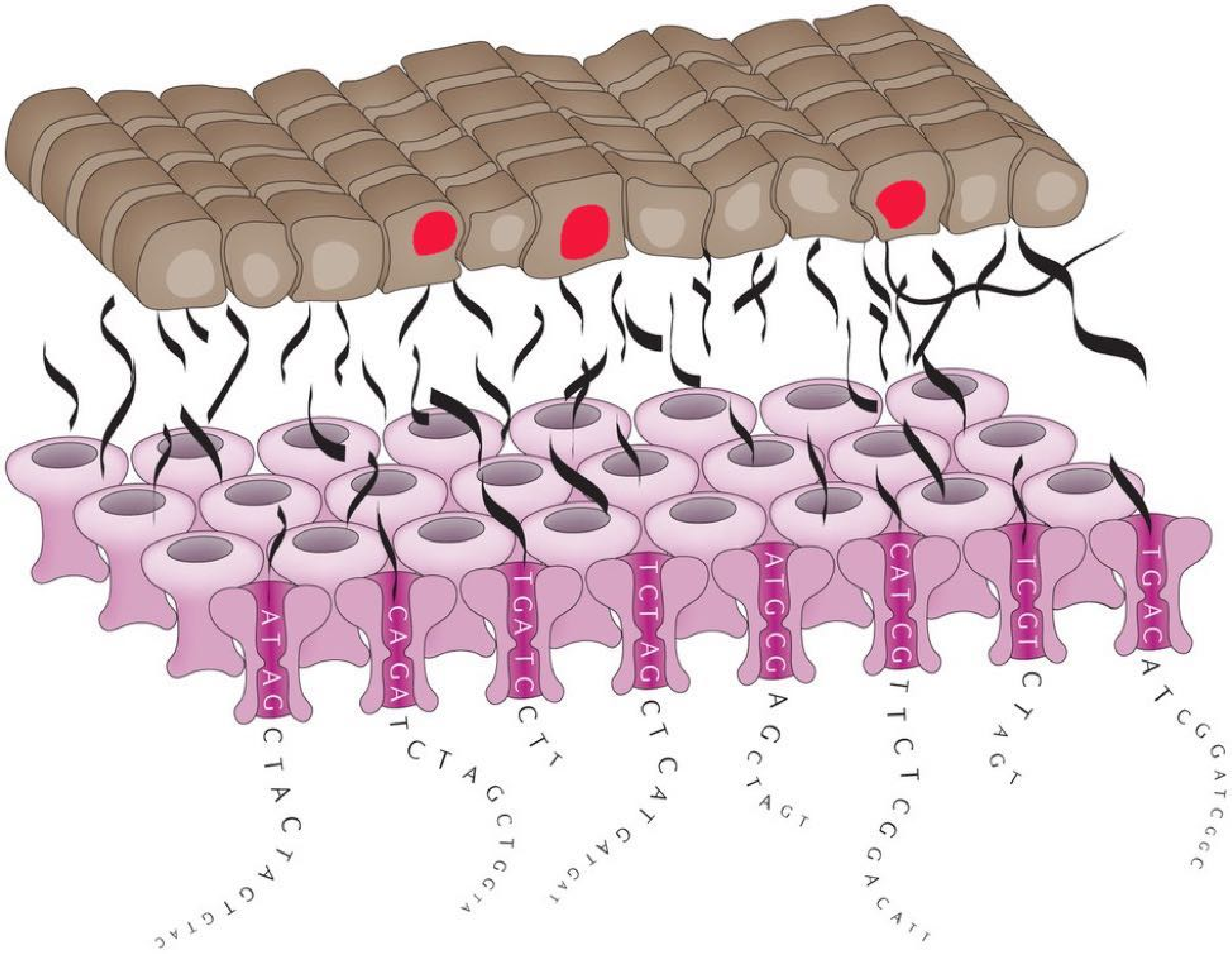
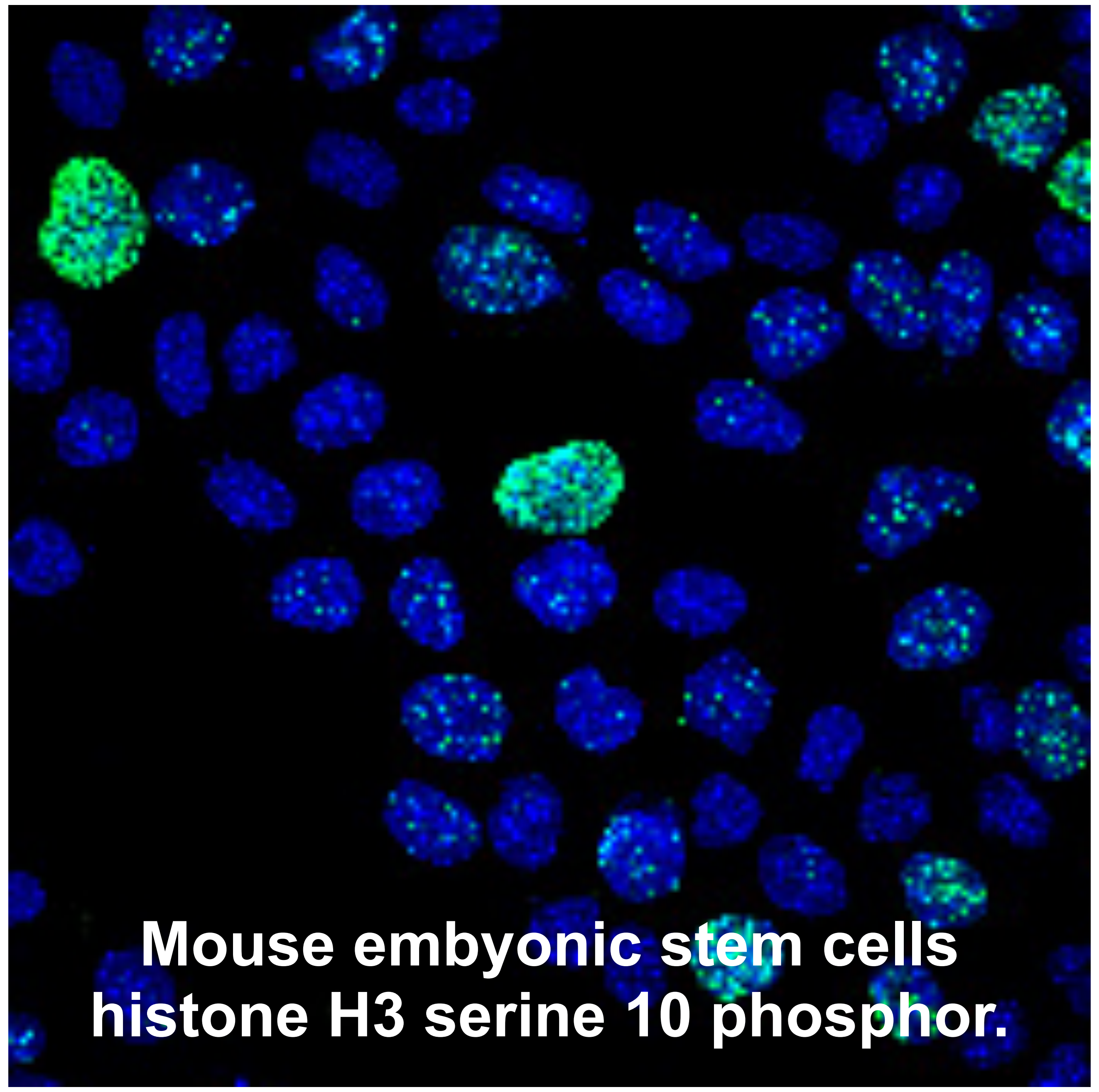


Image from Crosetto,
Nat Rev Genet, 2015

How to integrate microscopy with sequencing?

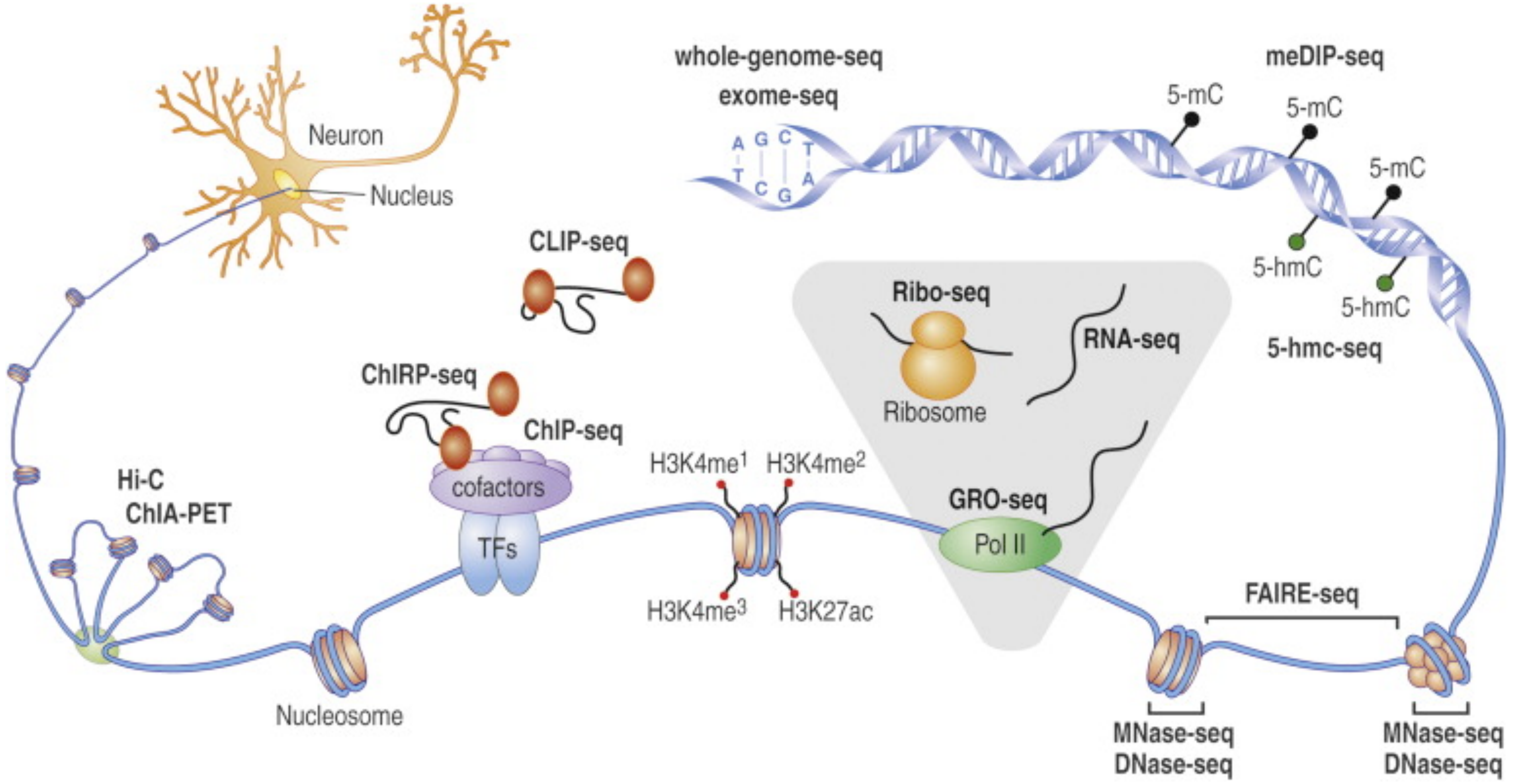
Fluorescence microscopy



Mouse embyonic stem cells histone H3 serine 10 phosphor.

- Single (living) cells
- No molecular resolution
- Lacks genome reference

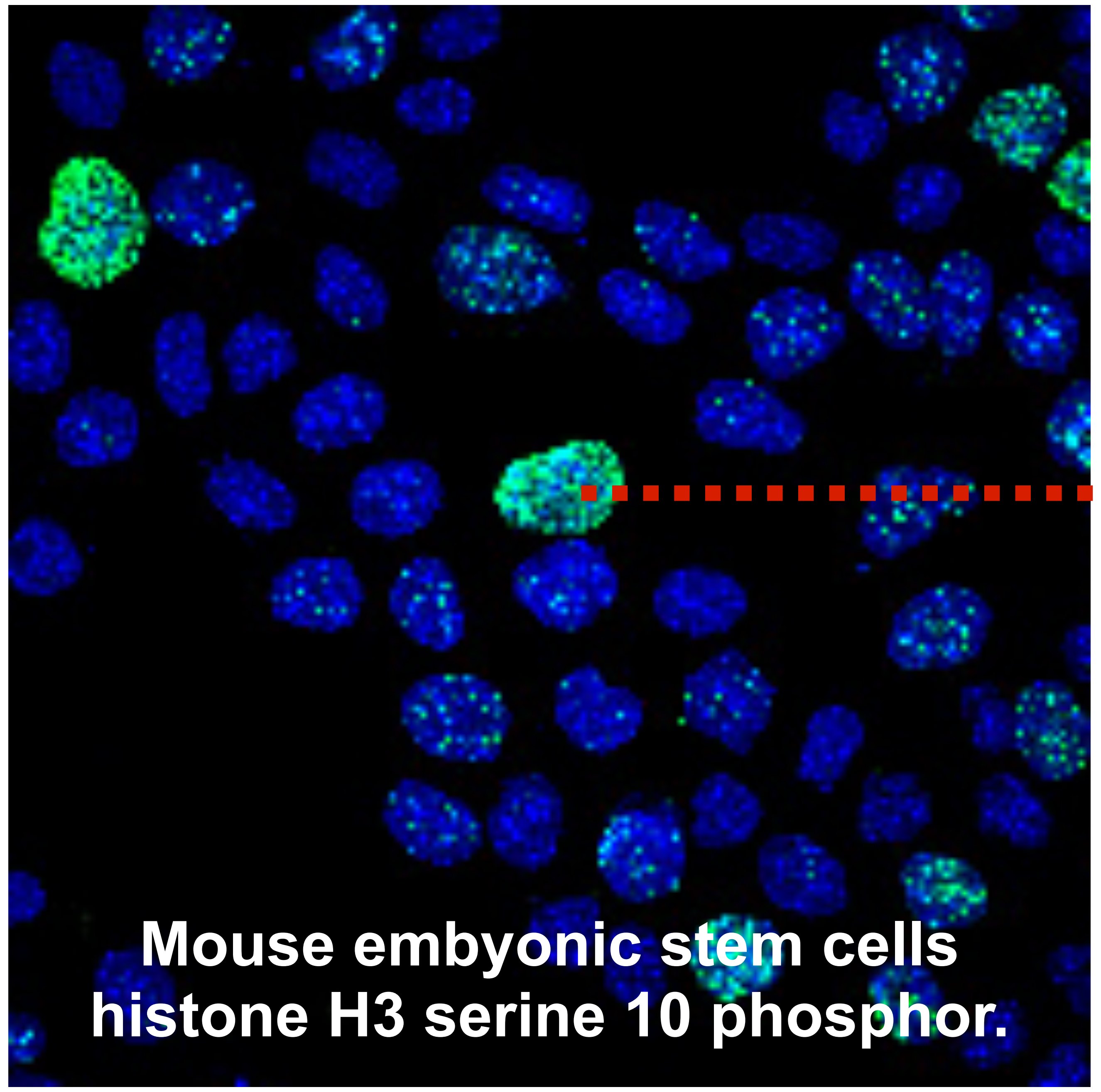
Sequencing



- 10^4-10^7 dead cells
- Base pair resolution (0.34 nm)
- Genome sequence reference

Microscopy meets sequencing

Fluorescence microscopy



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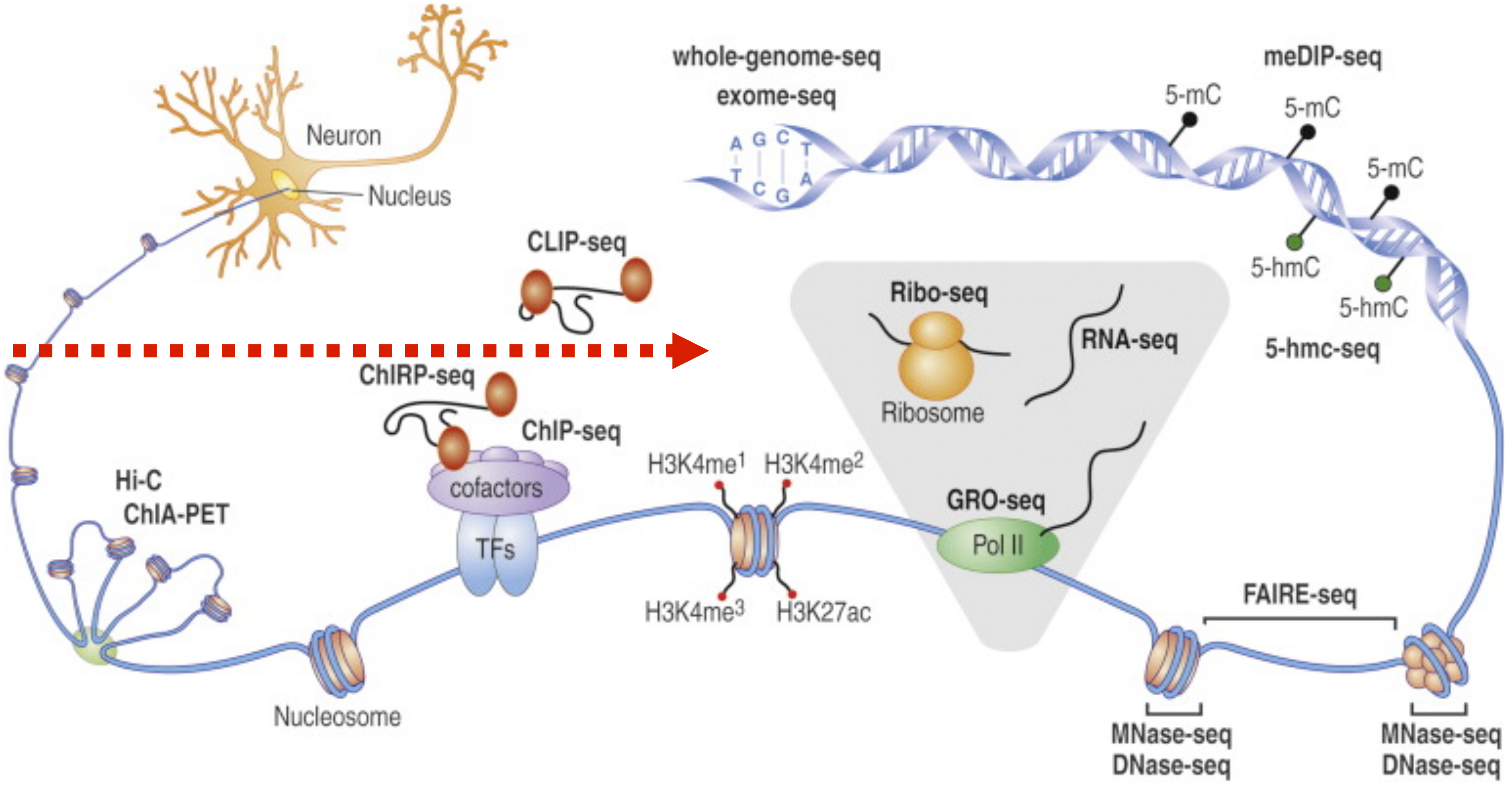
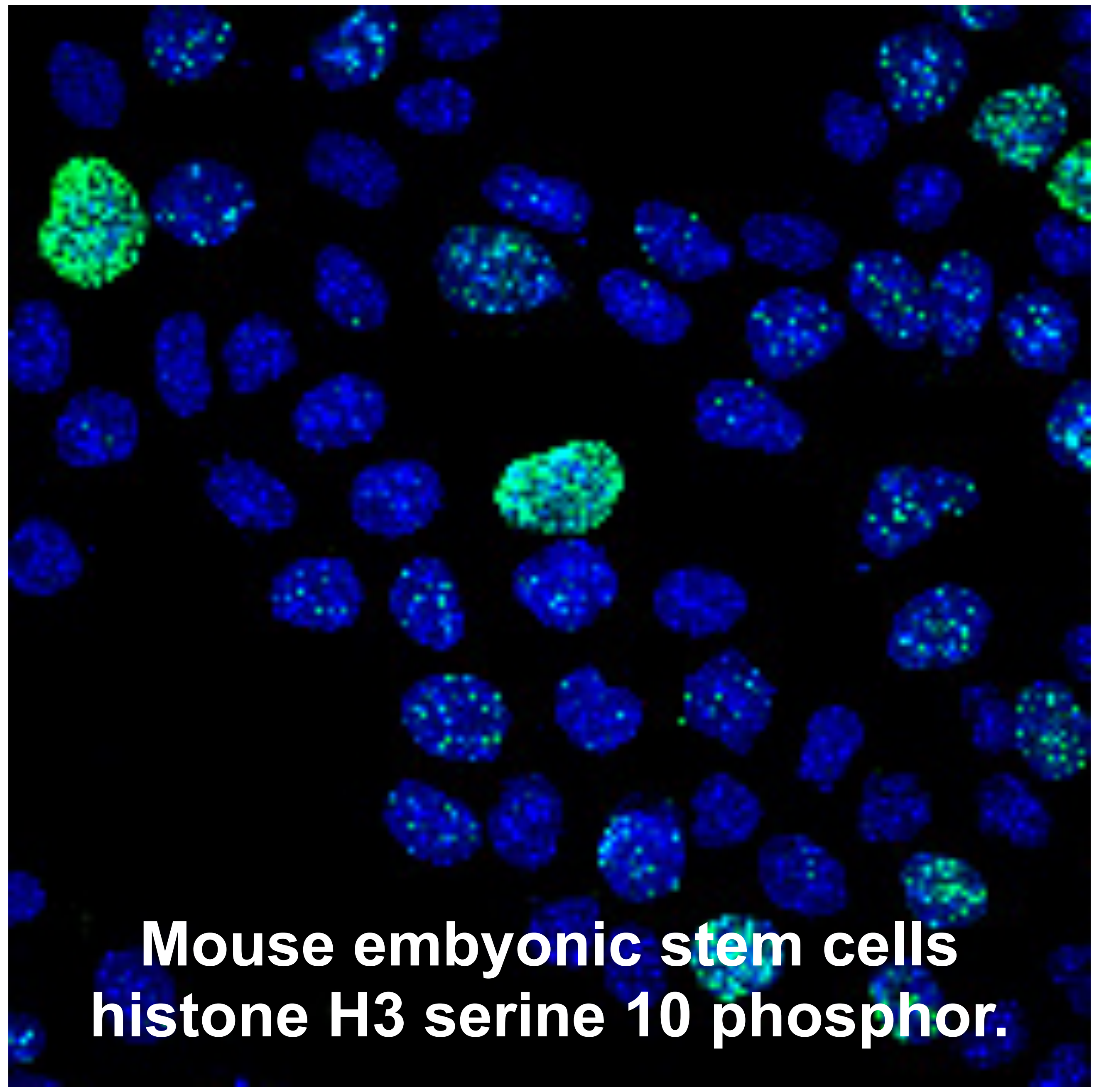


- **Single cells**
- Base pair resolution (0.34 nm)
- Genome sequence reference

Microscopy meets sequencing

Fluorescence microscopy



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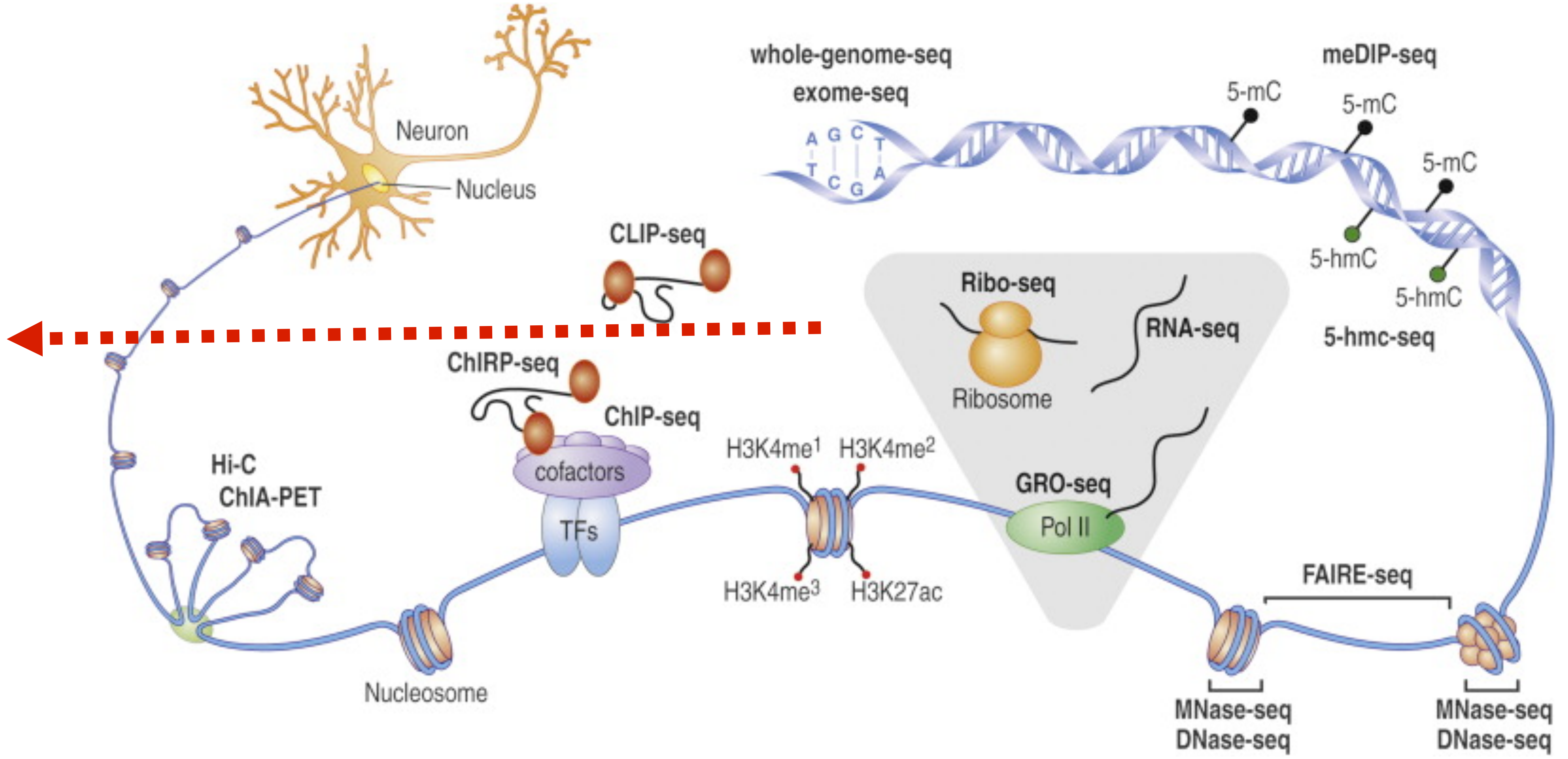
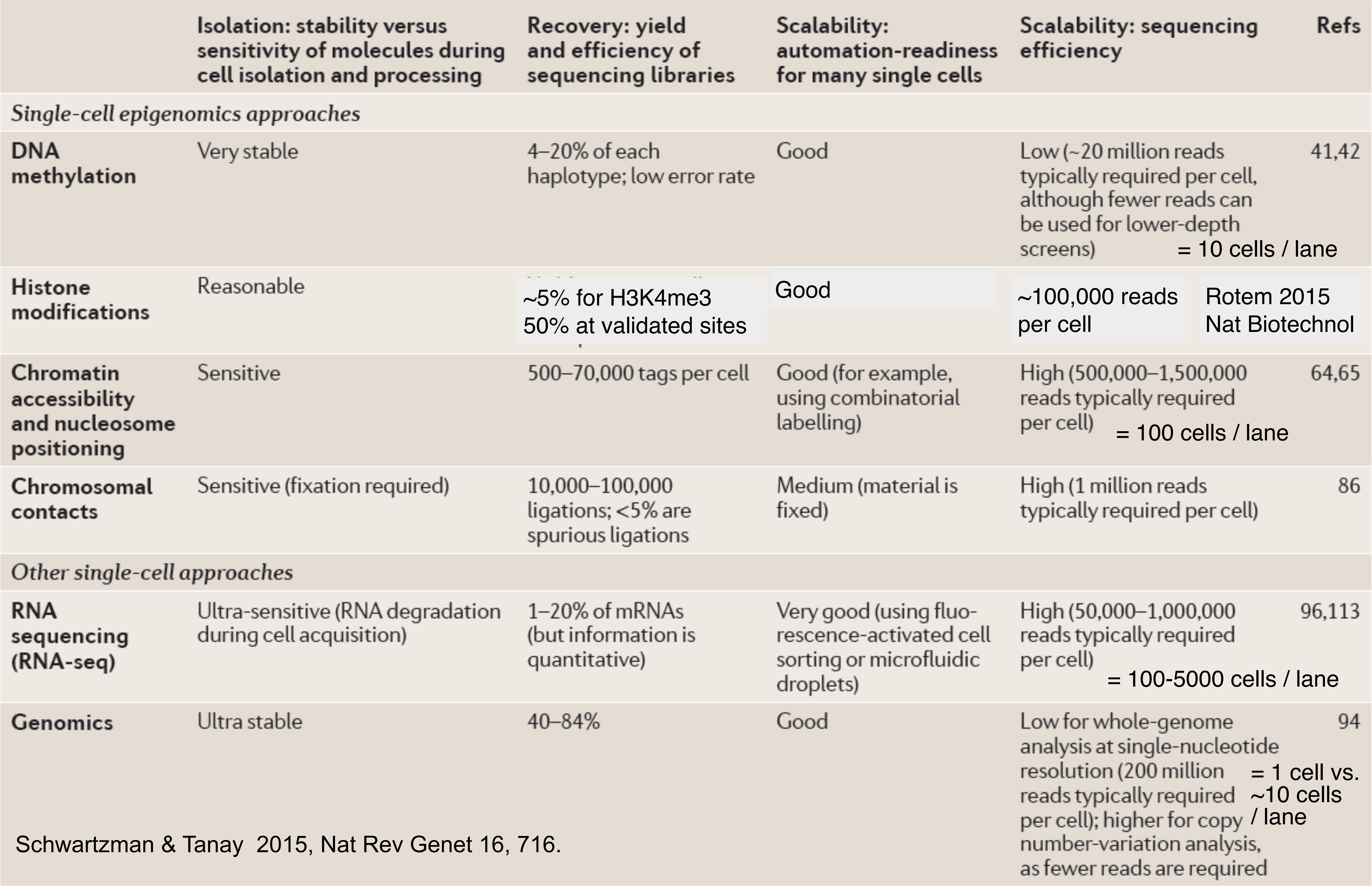


- **Single cells**
- Base pair resolution (0.34 nm)
- Genome sequence reference

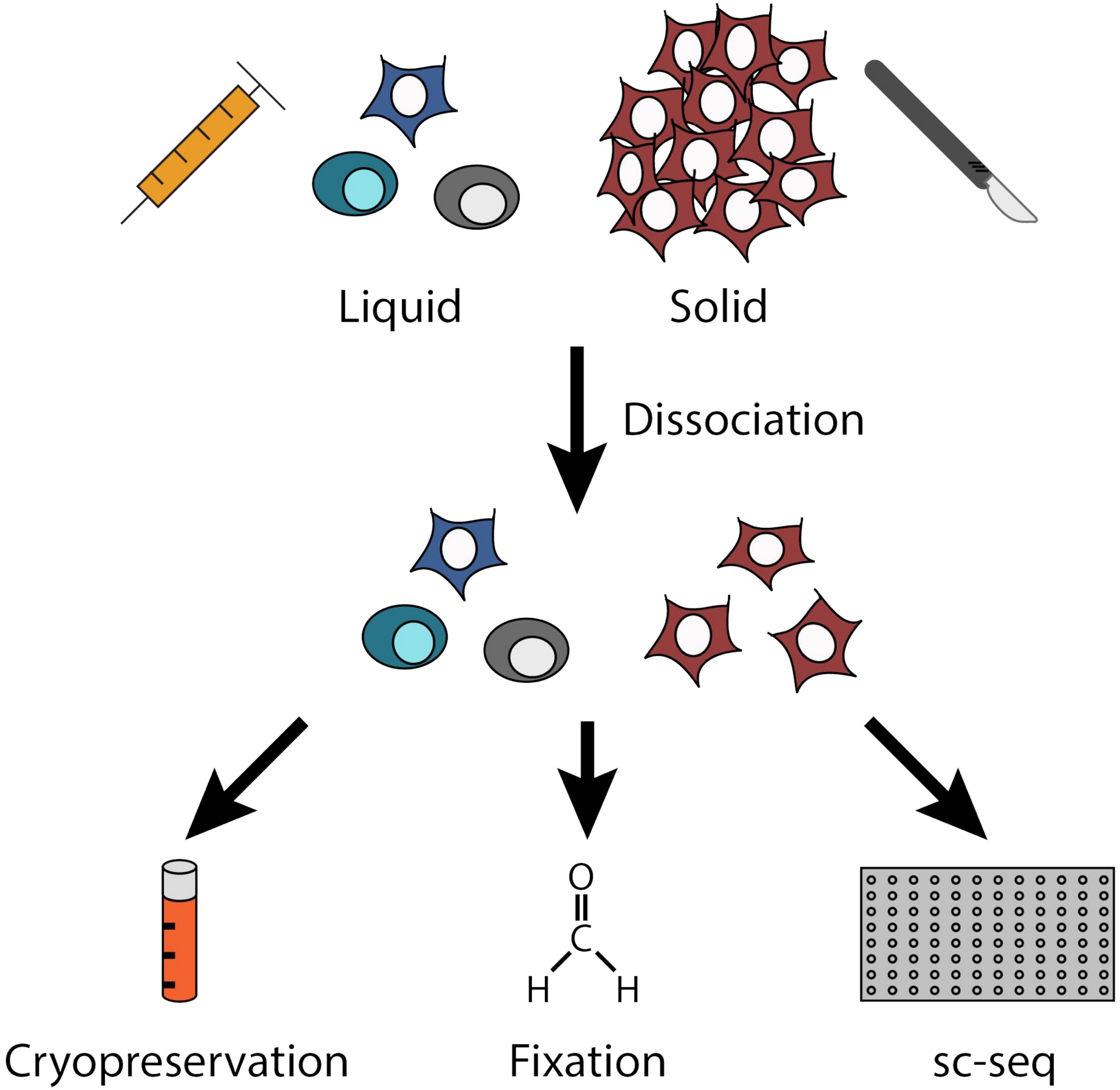
Single-cell sequencing based readouts



	Isolation: stability versus sensitivity of molecules during cell isolation and processing	Recovery: yield and efficiency of sequencing libraries	Scalability: automation-readiness for many single cells	Scalability: sequencing efficiency	Refs
Single-cell epigenomics approaches					
DNA methylation	Very stable	4–20% of each haplotype; low error rate	Good	Low (~20 million reads typically required per cell, although fewer reads can be used for lower-depth screens) = 10 cells / lane	41,42
Histone modifications	Reasonable	~5% for H3K4me3 50% at validated sites	Good	~100,000 reads per cell	Rotem 2015 Nat Biotechnol
Chromatin accessibility and nucleosome positioning	Sensitive	500–70,000 tags per cell	Good (for example, using combinatorial labelling)	High (500,000–1,500,000 reads typically required per cell) = 100 cells / lane	64,65
Chromosomal contacts	Sensitive (fixation required)	10,000–100,000 ligations; <5% are spurious ligations	Medium (material is fixed)	High (1 million reads typically required per cell)	86
Other single-cell approaches					
RNA sequencing (RNA-seq)	Ultra-sensitive (RNA degradation during cell acquisition)	1–20% of mRNAs (but information is quantitative)	Very good (using fluorescence-activated cell sorting or microfluidic droplets)	High (50,000–1,000,000 reads typically required per cell) = 100-5000 cells / lane	96,113
Genomics	Ultra stable	40–84%	Good	Low for whole-genome analysis at single-nucleotide resolution (200 million reads typically required per cell); higher for copy number-variation analysis, as fewer reads are required = 1 cell vs. ~10 cells / lane	94

Schwartzman & Tanay 2015, Nat Rev Genet 16, 716.

Sample processing for single cell sequencing



Liquid

Solid

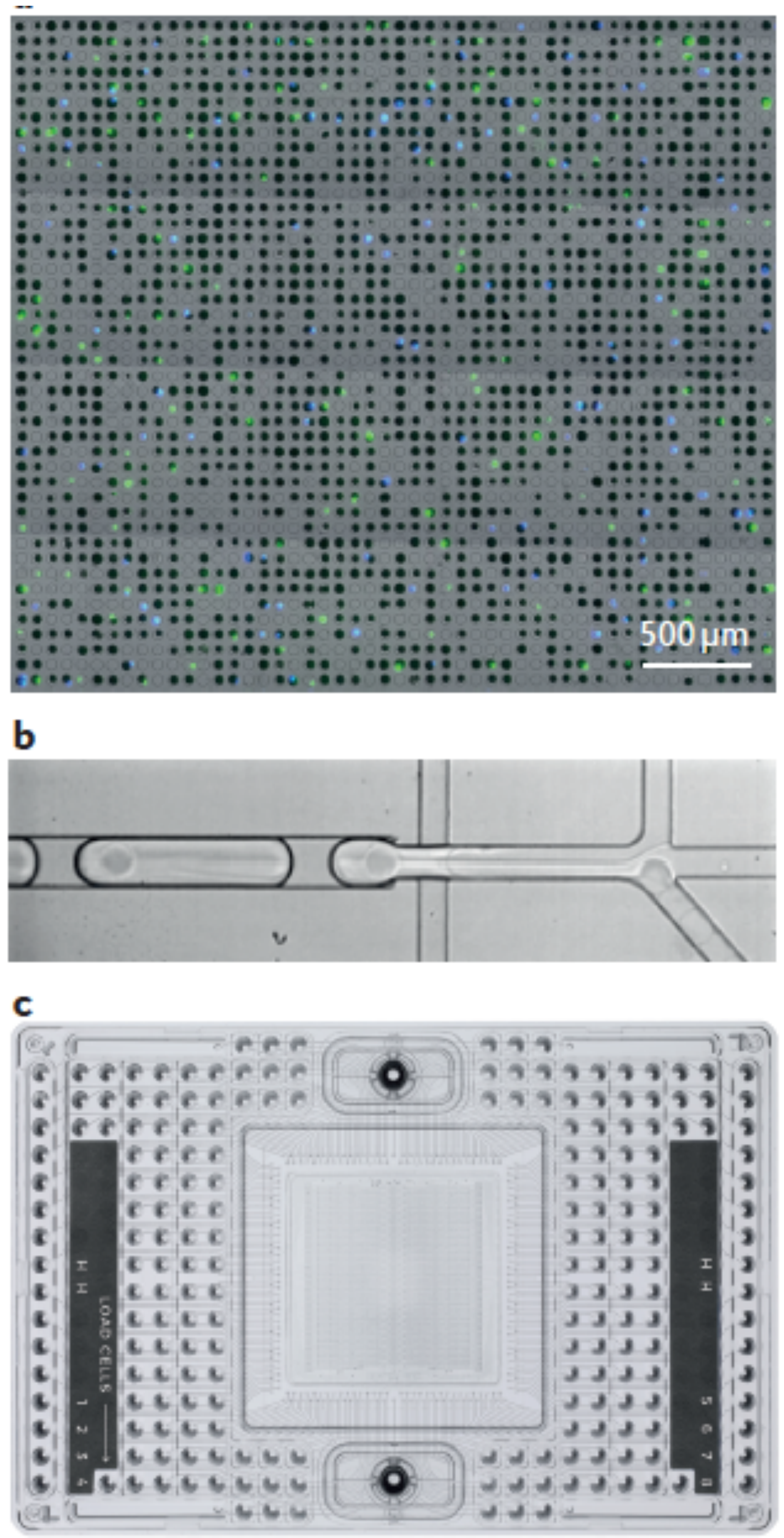
Dissociation

Cryopreservation

Fixation

sc-seq

Capturing single cells



Microwells

FACS based

commercial systems (dispenser)

Droplets

emulsion pumps

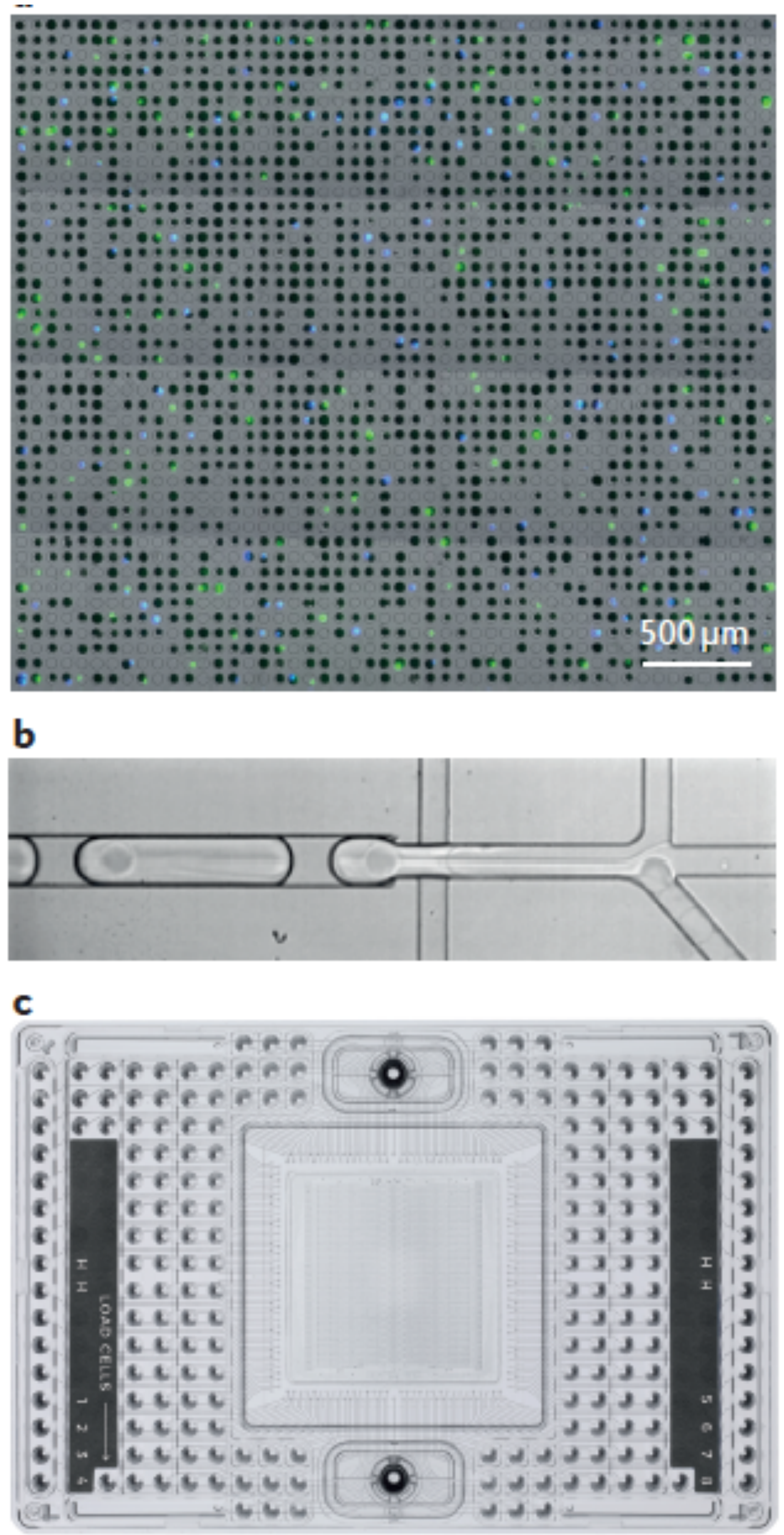
commercial systems (drop-seq)

Microfluidics

cell capture

commercial systems (nano traps)

SINGLE CELL SEQUENCING - CAPTURING



Microwells

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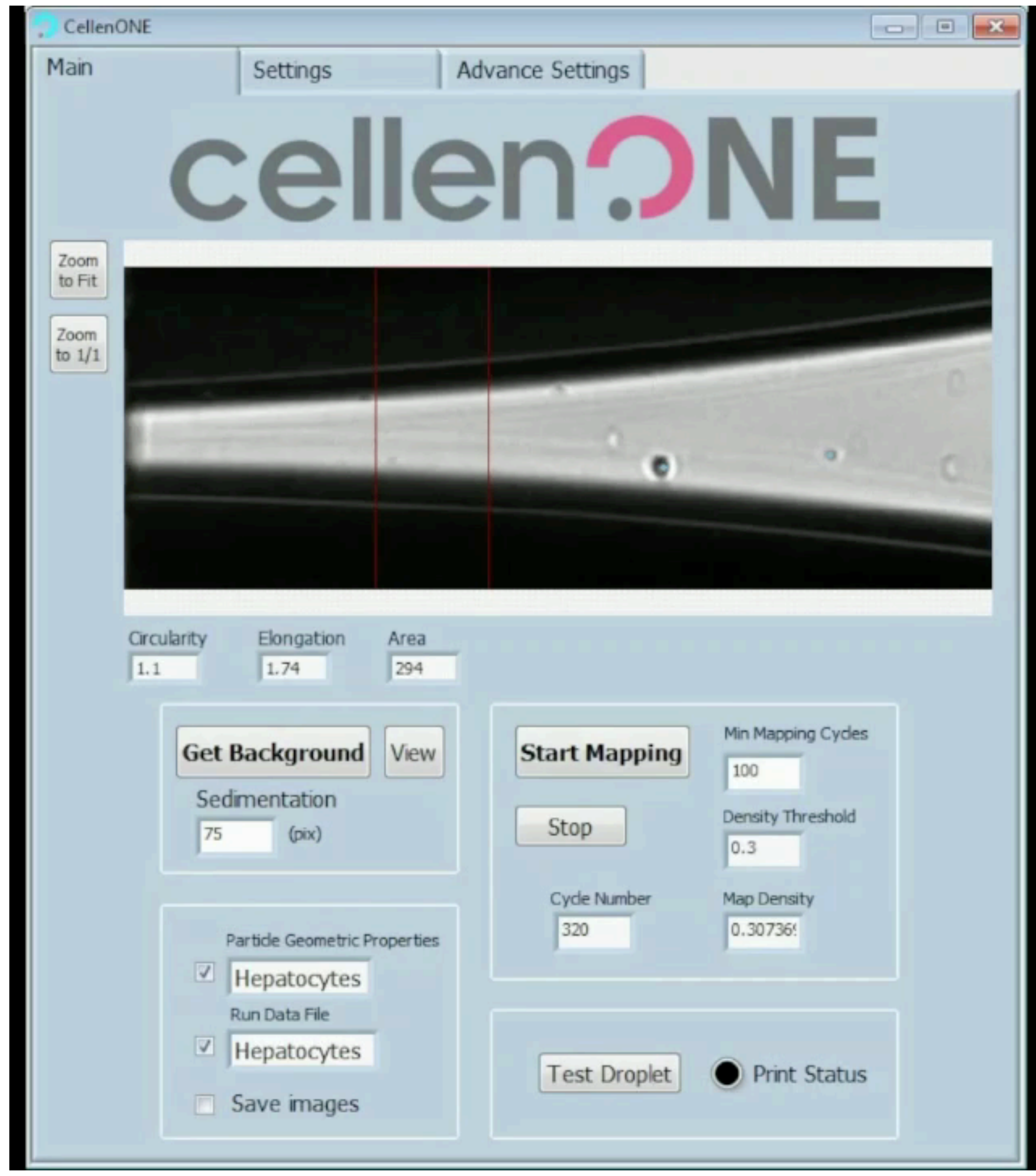


Circularity 1.1

Elongation 1.74

Area 294

Sedimentation 75 (pix)

Min Mapping Cycles 100

Density Threshold 0.3

Cycle Number 320

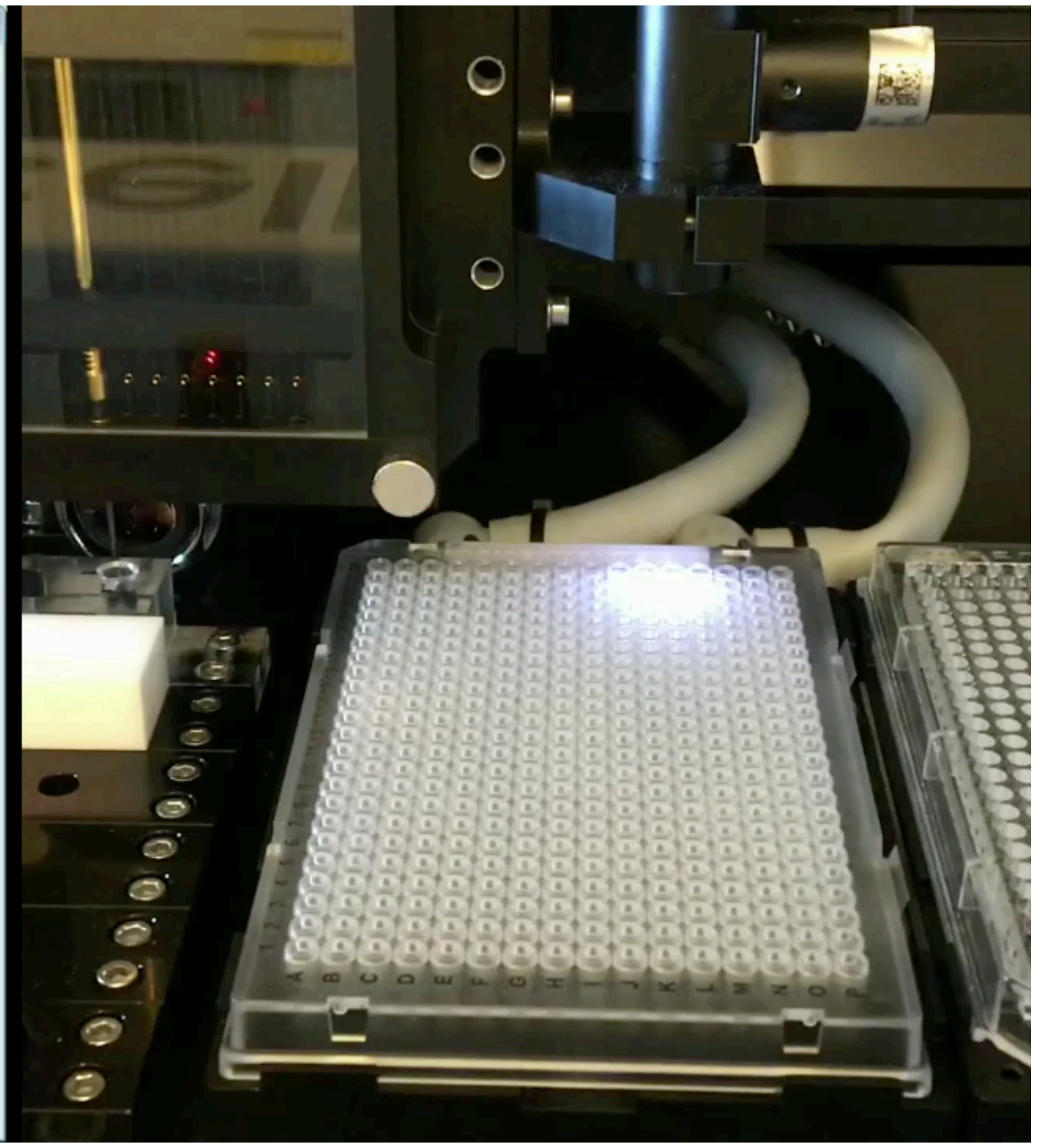
Map Density 0.30736

Particle Geometric Properties ☑ Hepatocytes

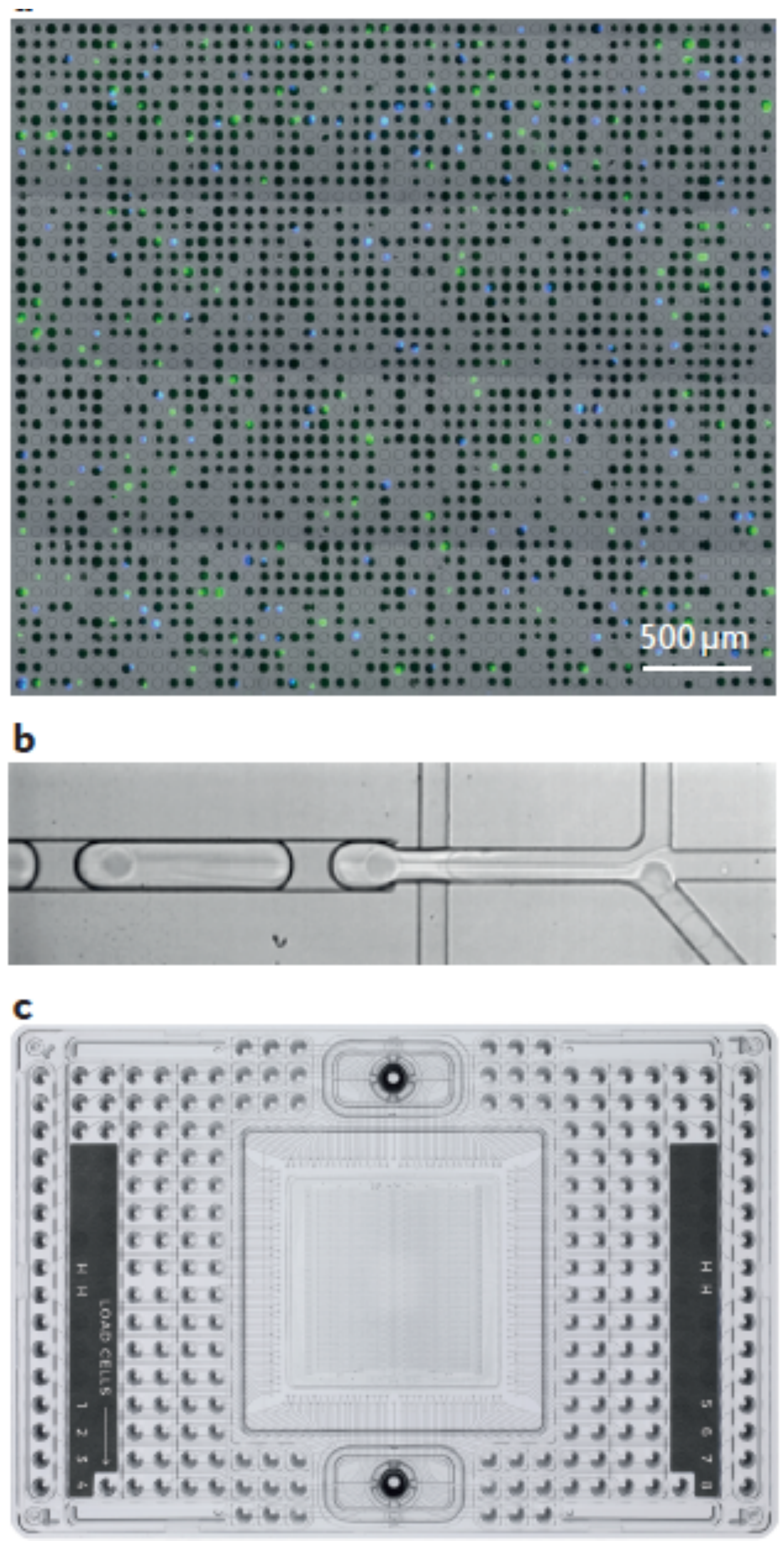
Run Data File ☑ Hepatocytes

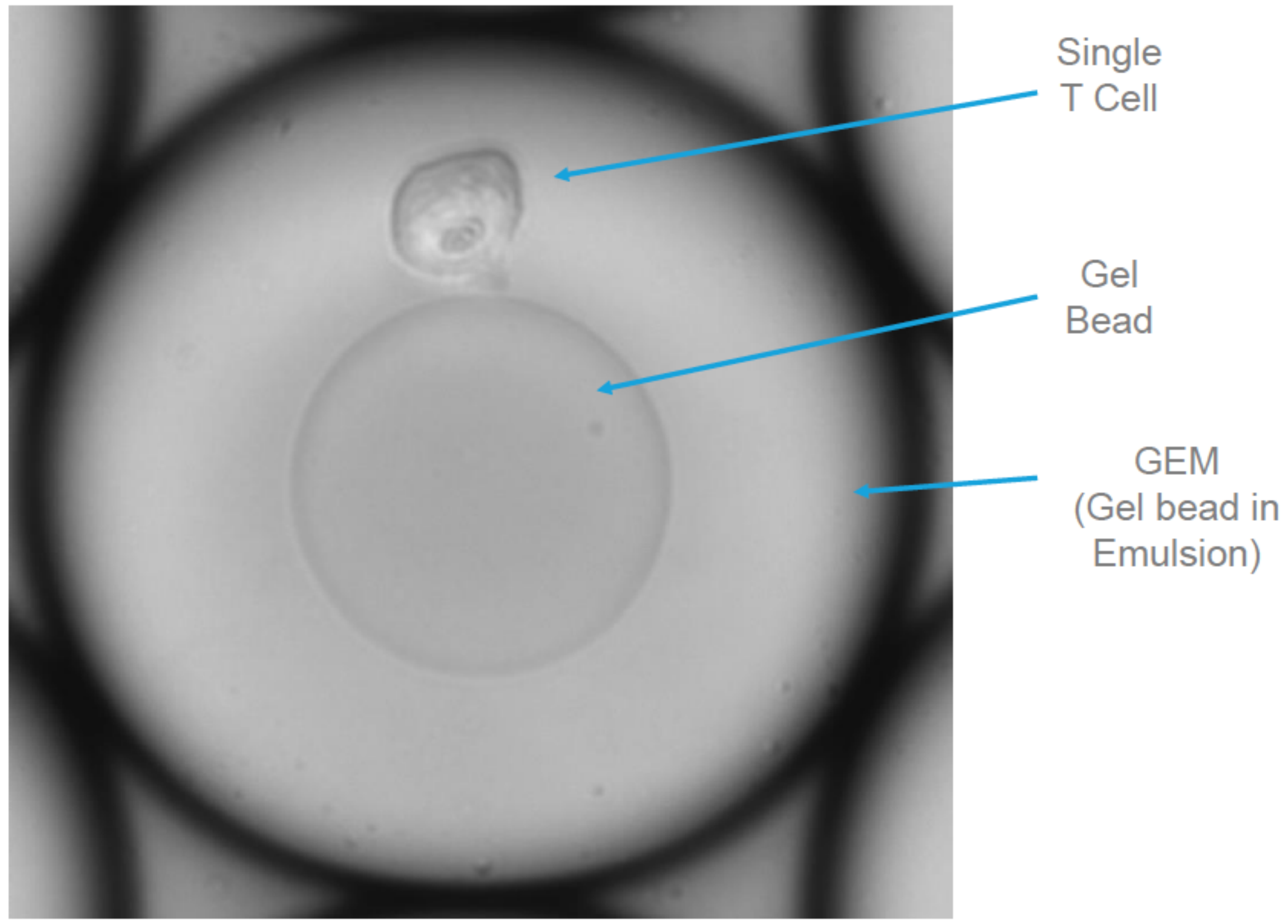
☐ Save images

● Print Status



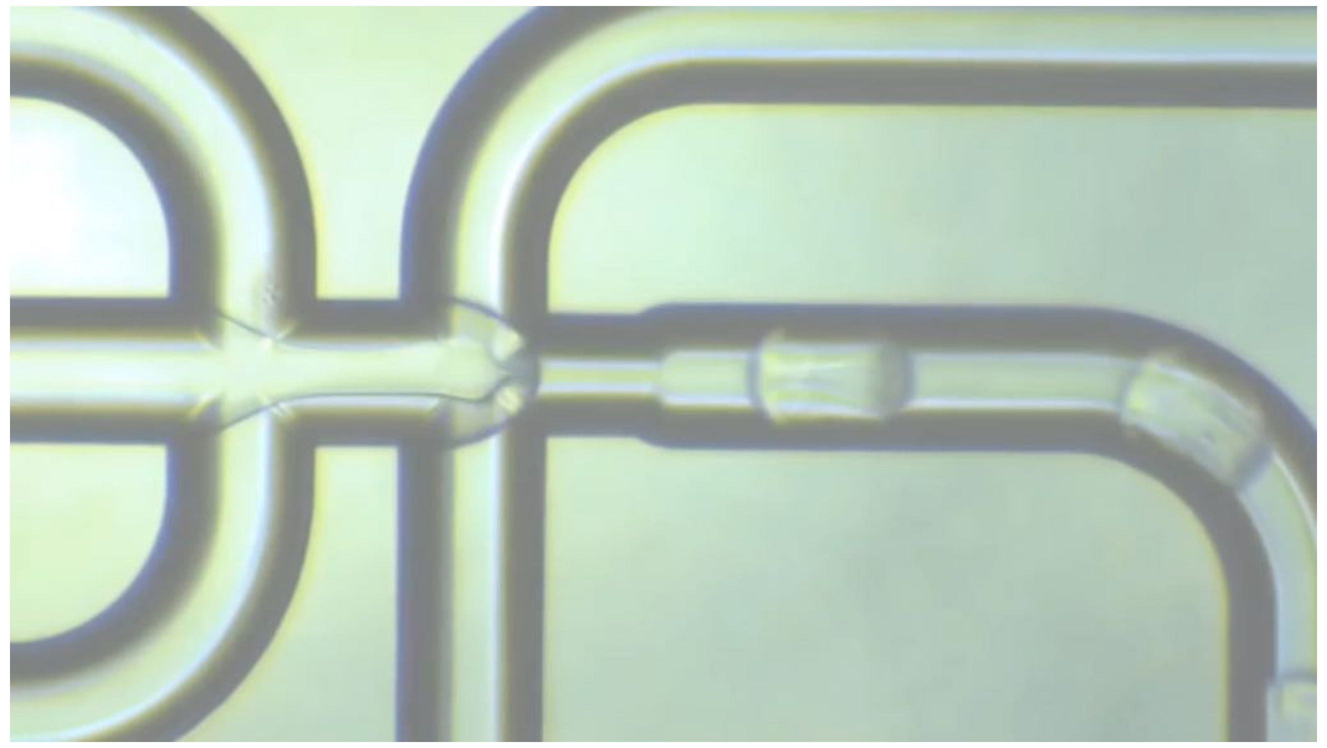
SINGLE CELL SEQUENCING - CAPTURING



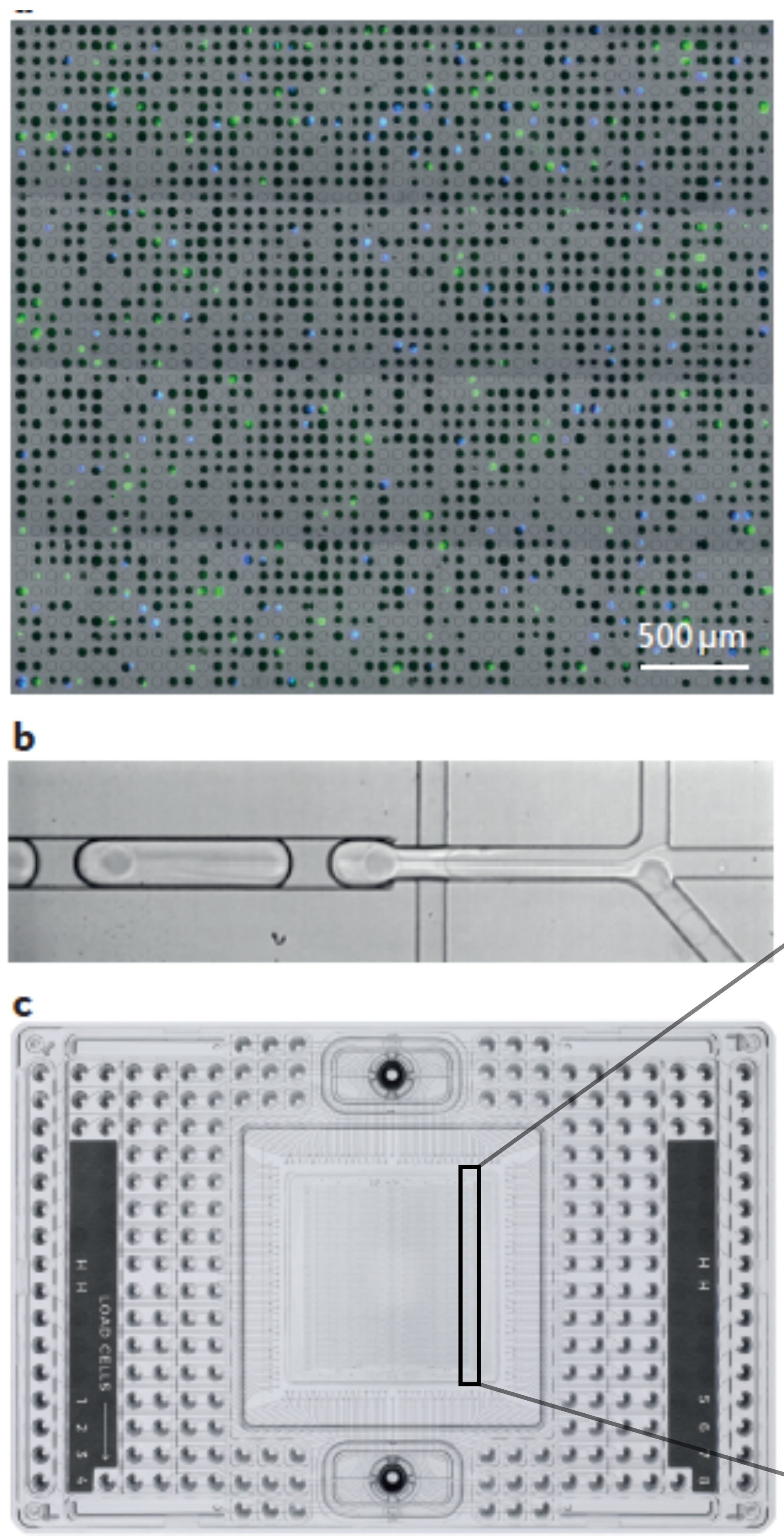


Droplets

emulsion pumps
commercial systems (drop-seq)



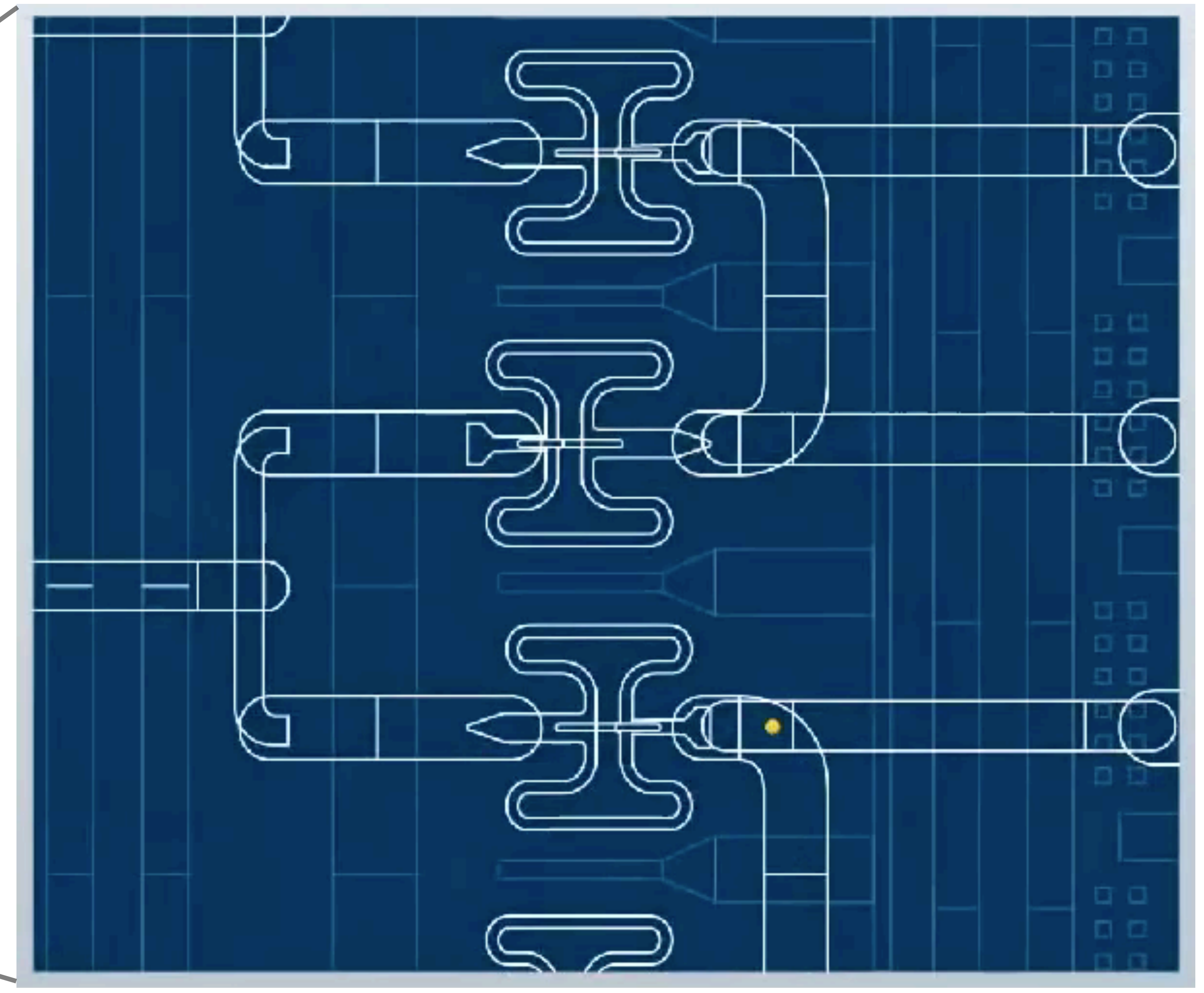
SINGLE CELL SEQUENCING - CAPTURING



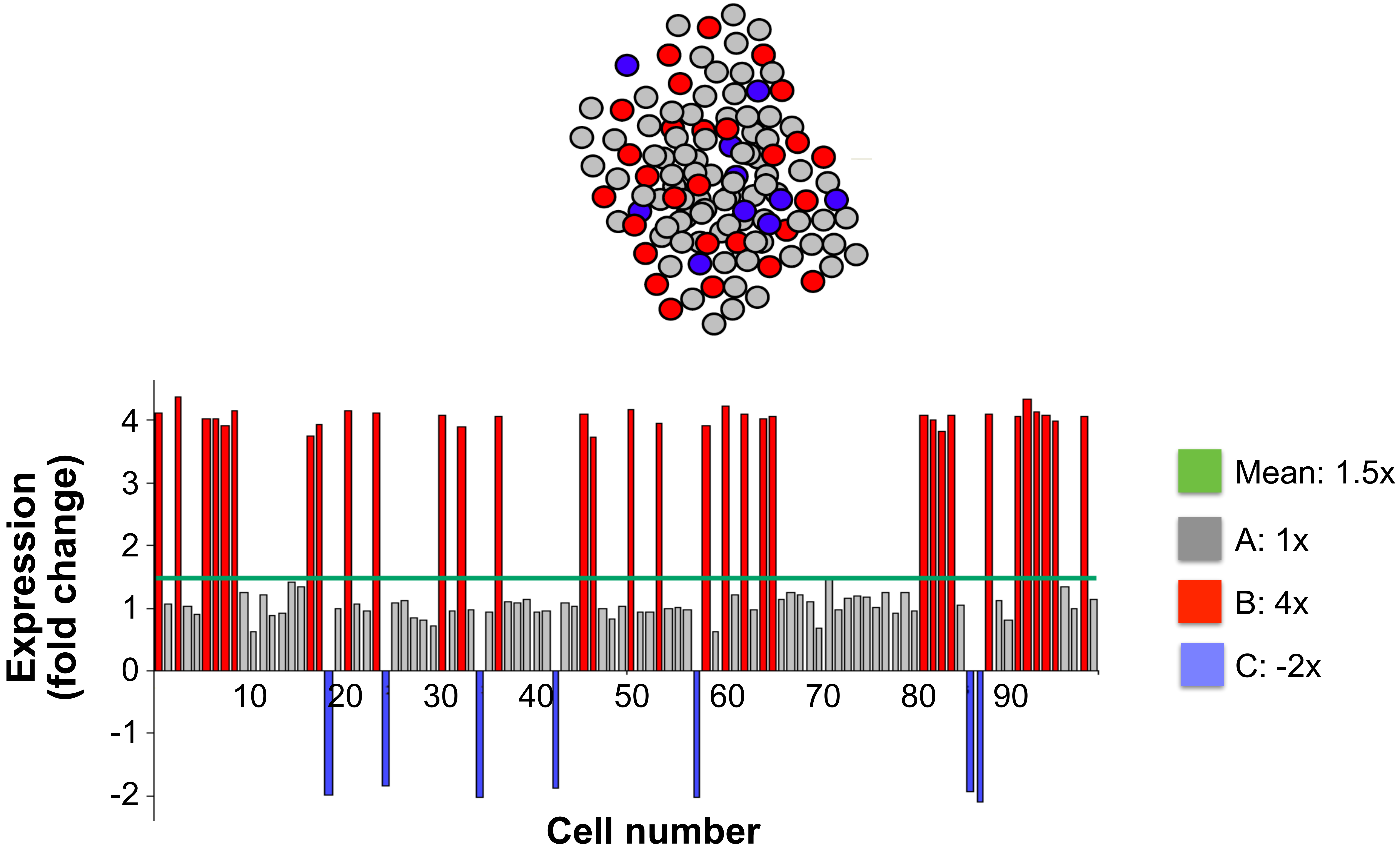
Microfluidics

cell capture

commercial systems (nano traps)



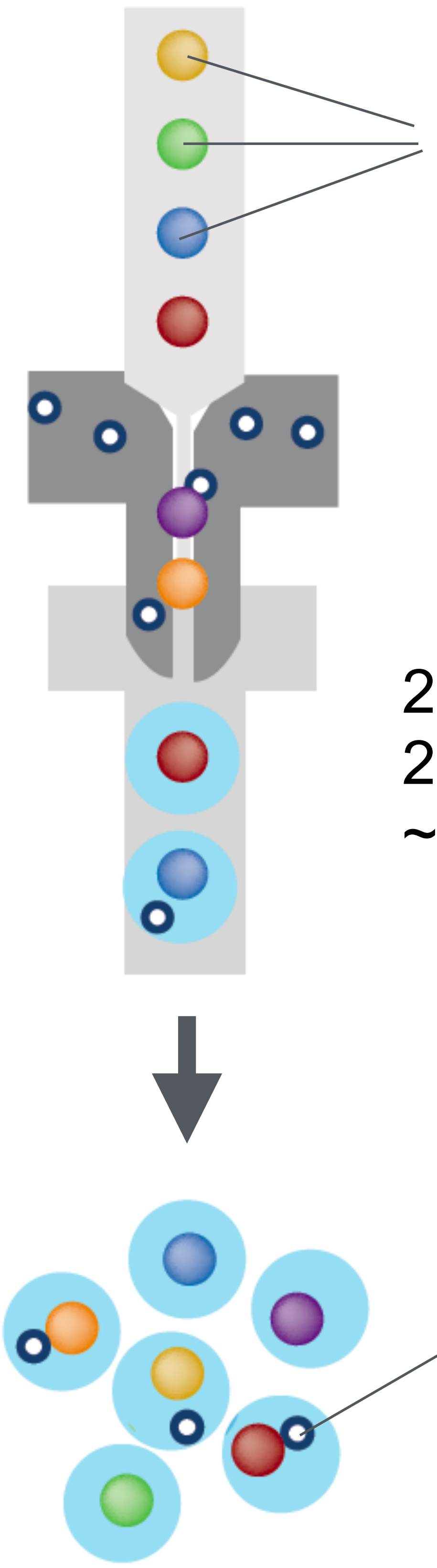
Single cell sequencing to dissect tumor heterogeneity by transcriptome and (epi)genome analysis



Scheme adapted from Fluidigm

Single cell RNA sequencing of cell suspensions
(bone marrow aspirate from multiple myeloma patients)

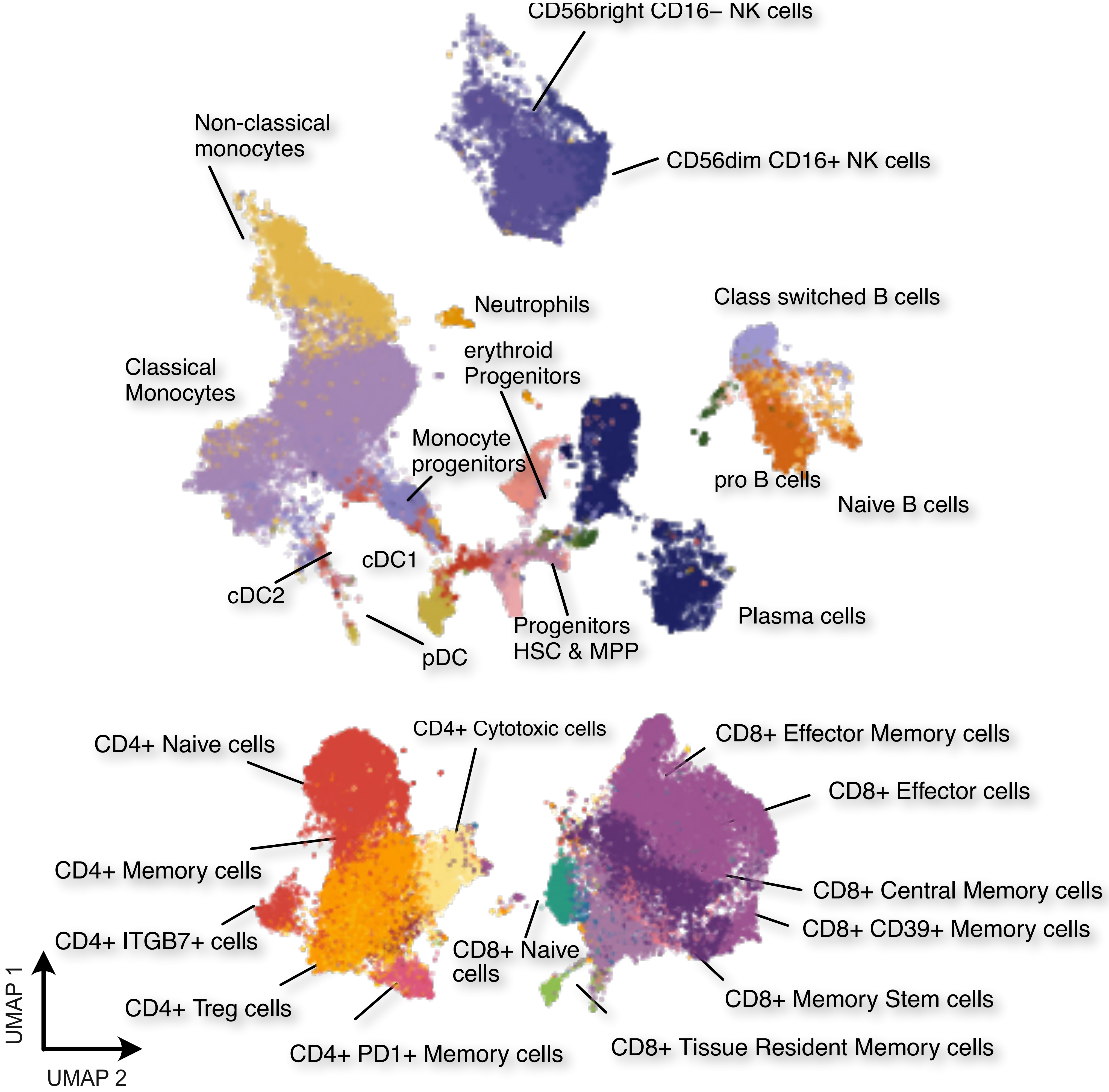
single cell RNA-seq
("drop-seq", 10x Genomics)



beads with
unique DNA
barcodes

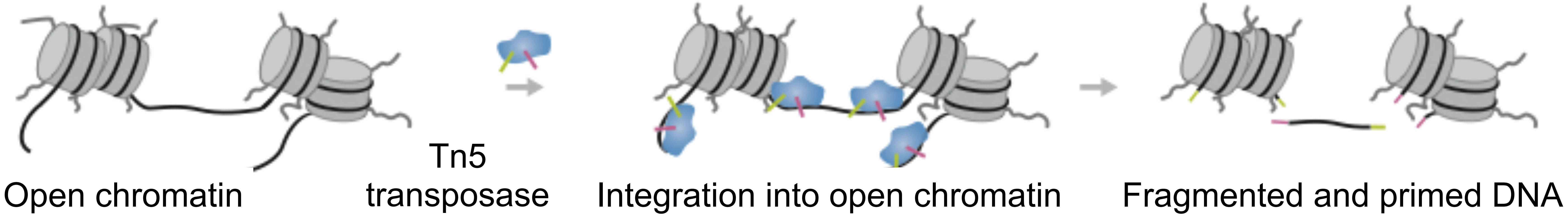
2000-8000 cells/sample
2000-3000 genes/cell
~16 000 different genes

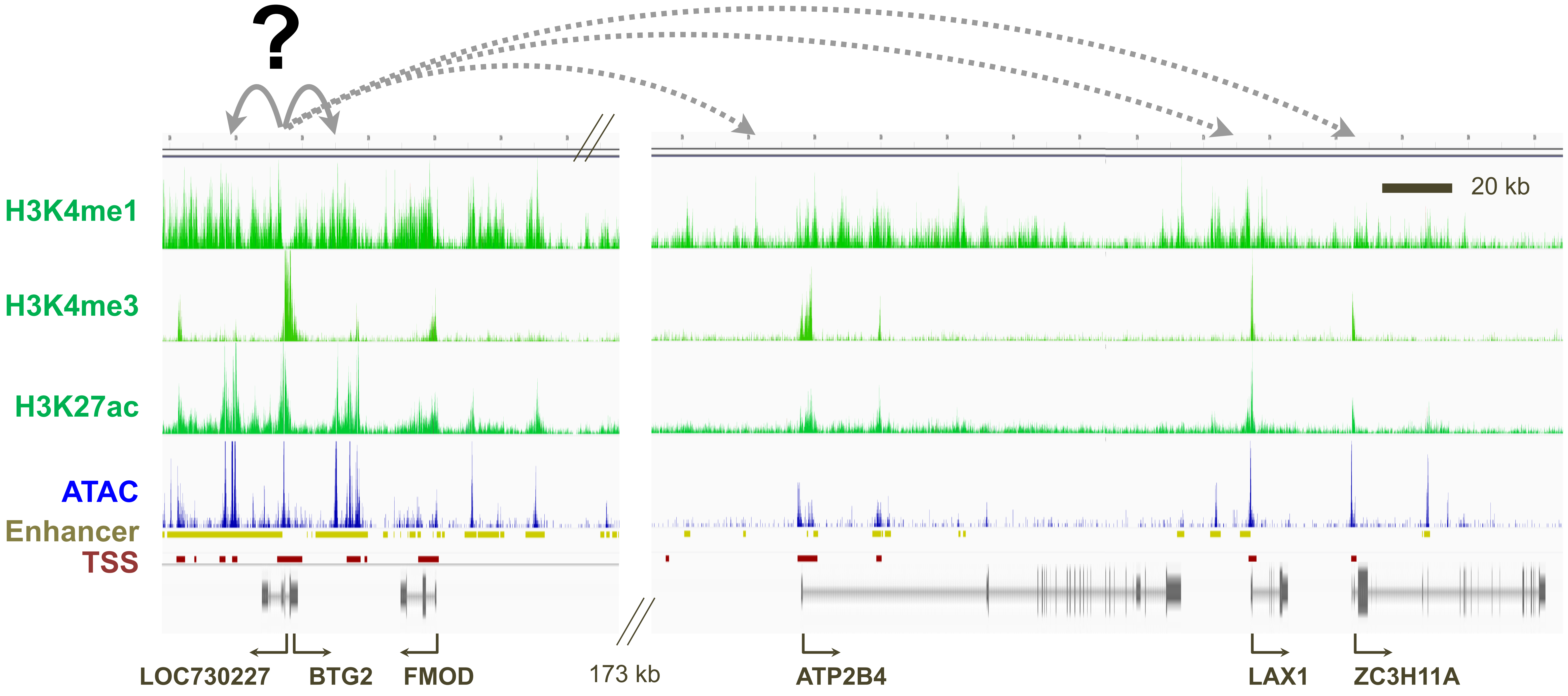
cell encapsulated
in vesicle for
barcoding RNA



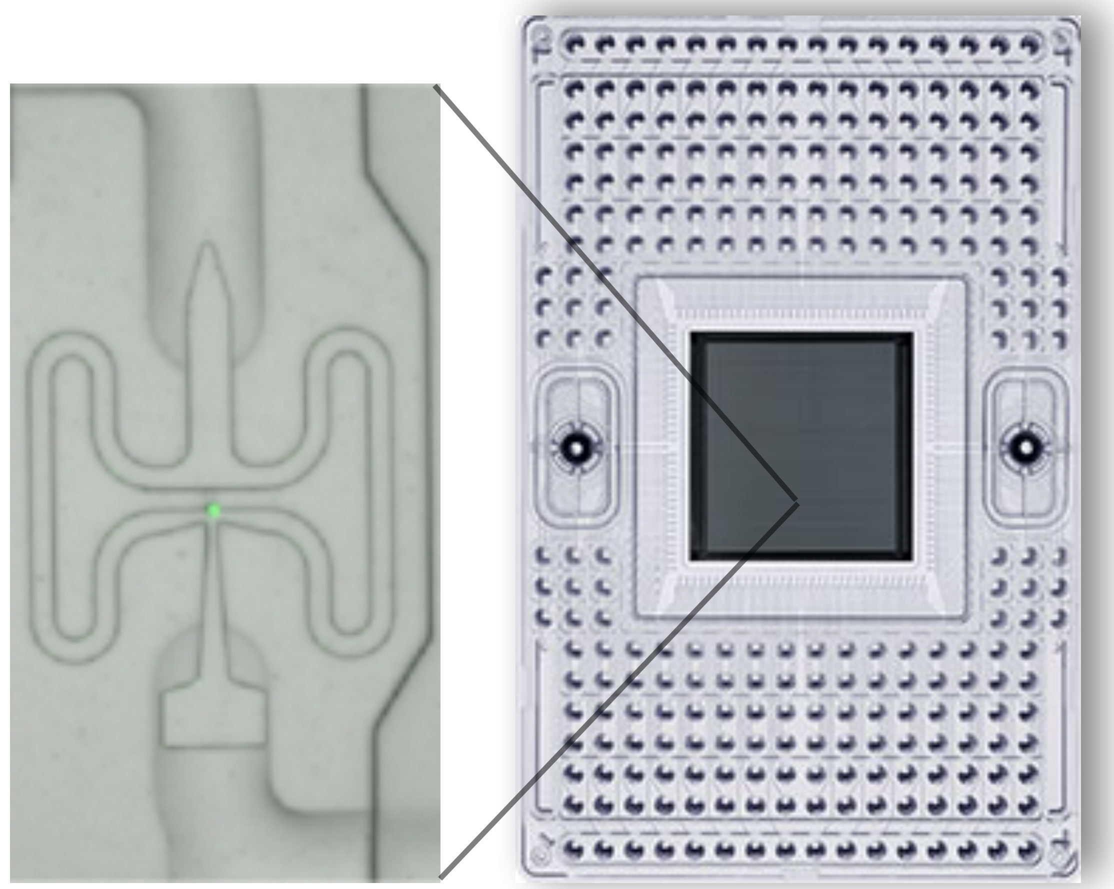
Tirier, Mallm, Goldschmidt, Raab, Rippe et al. (clinical work done at University Hospital Heidelberg)

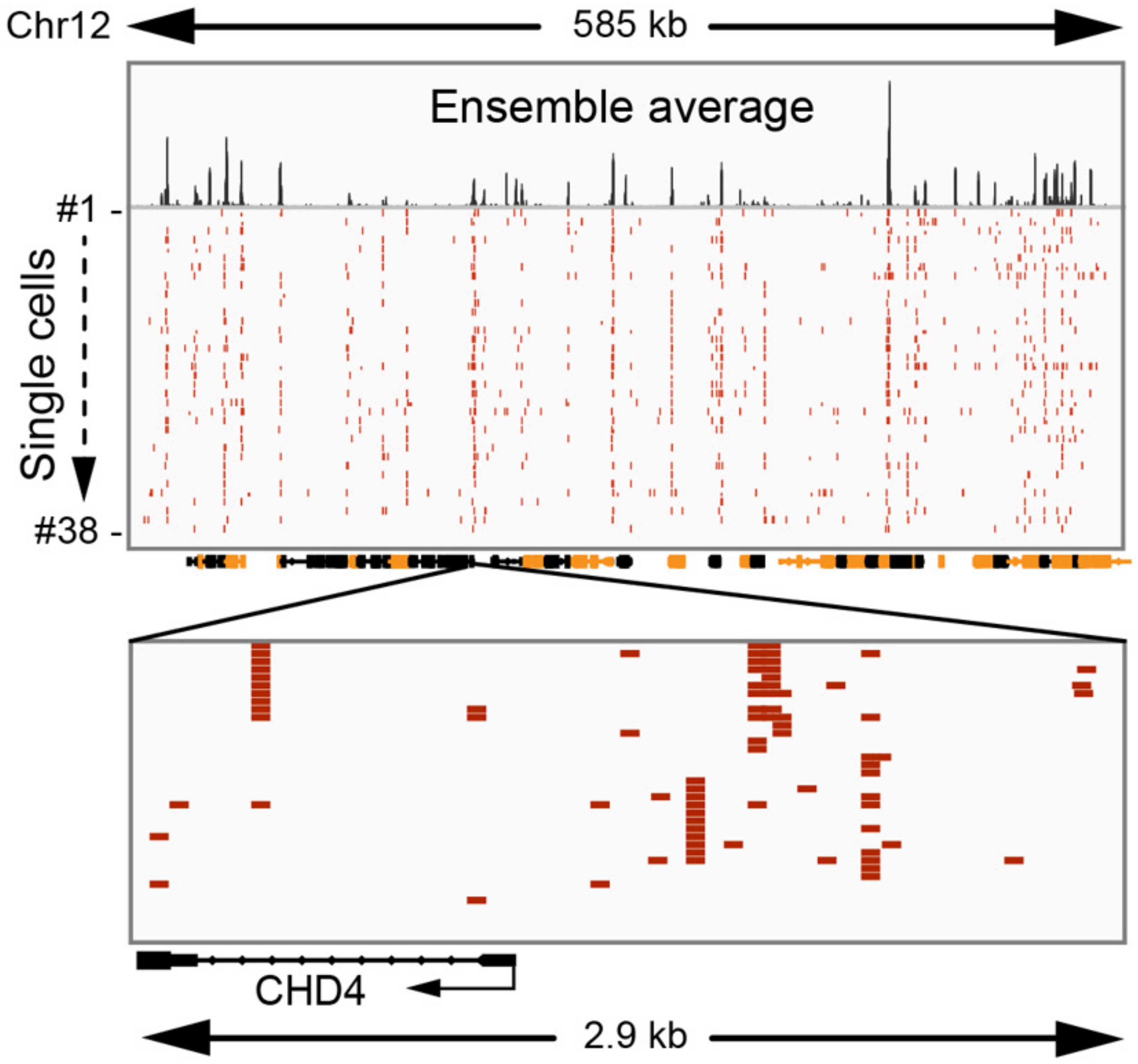
Mapping active enhancer and promoters by ATAC-seq





scATAC-seq analysis in chronic lymphocytic leukemia (CLL)



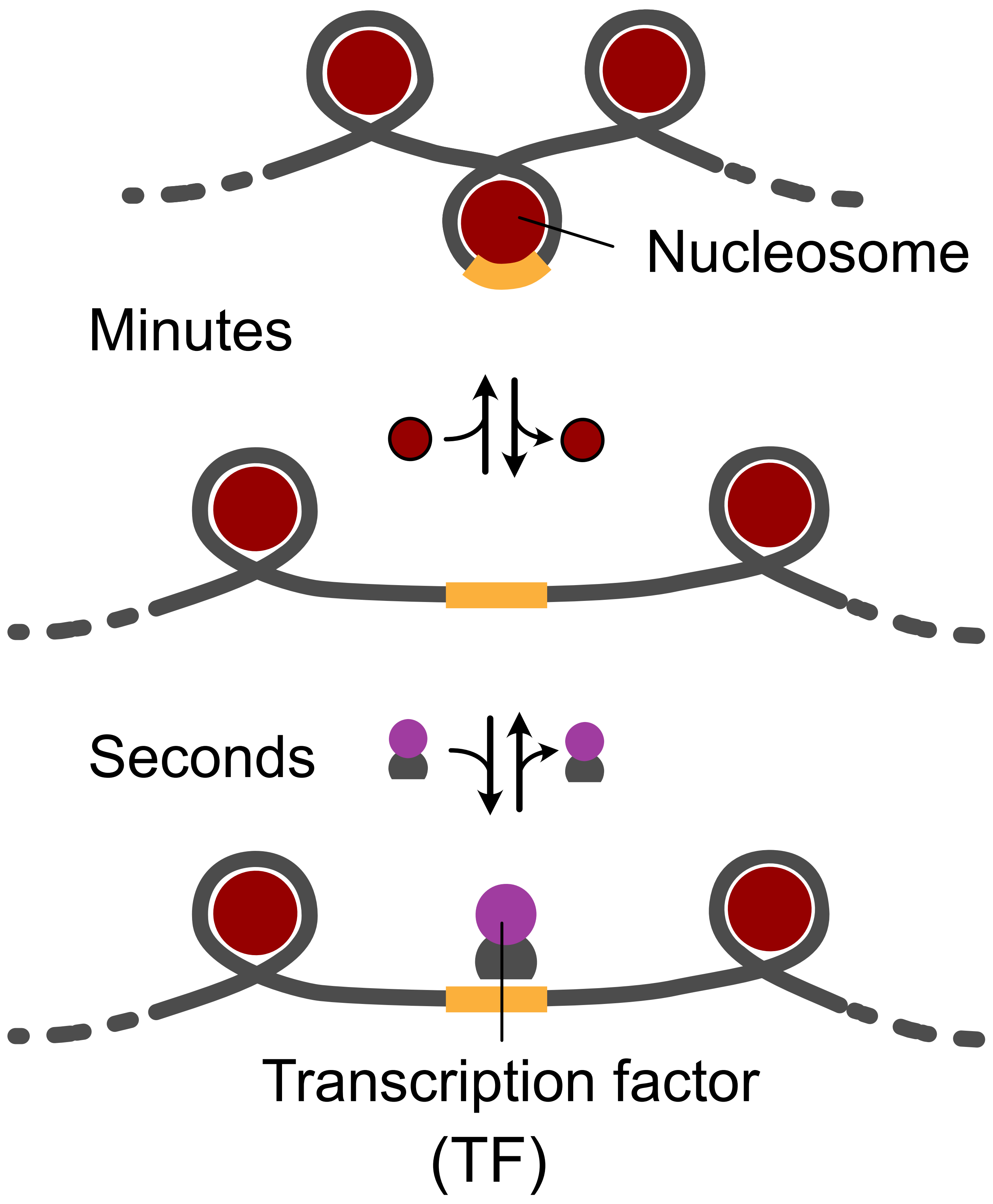


scATAC-seq (Fluidigm C1)

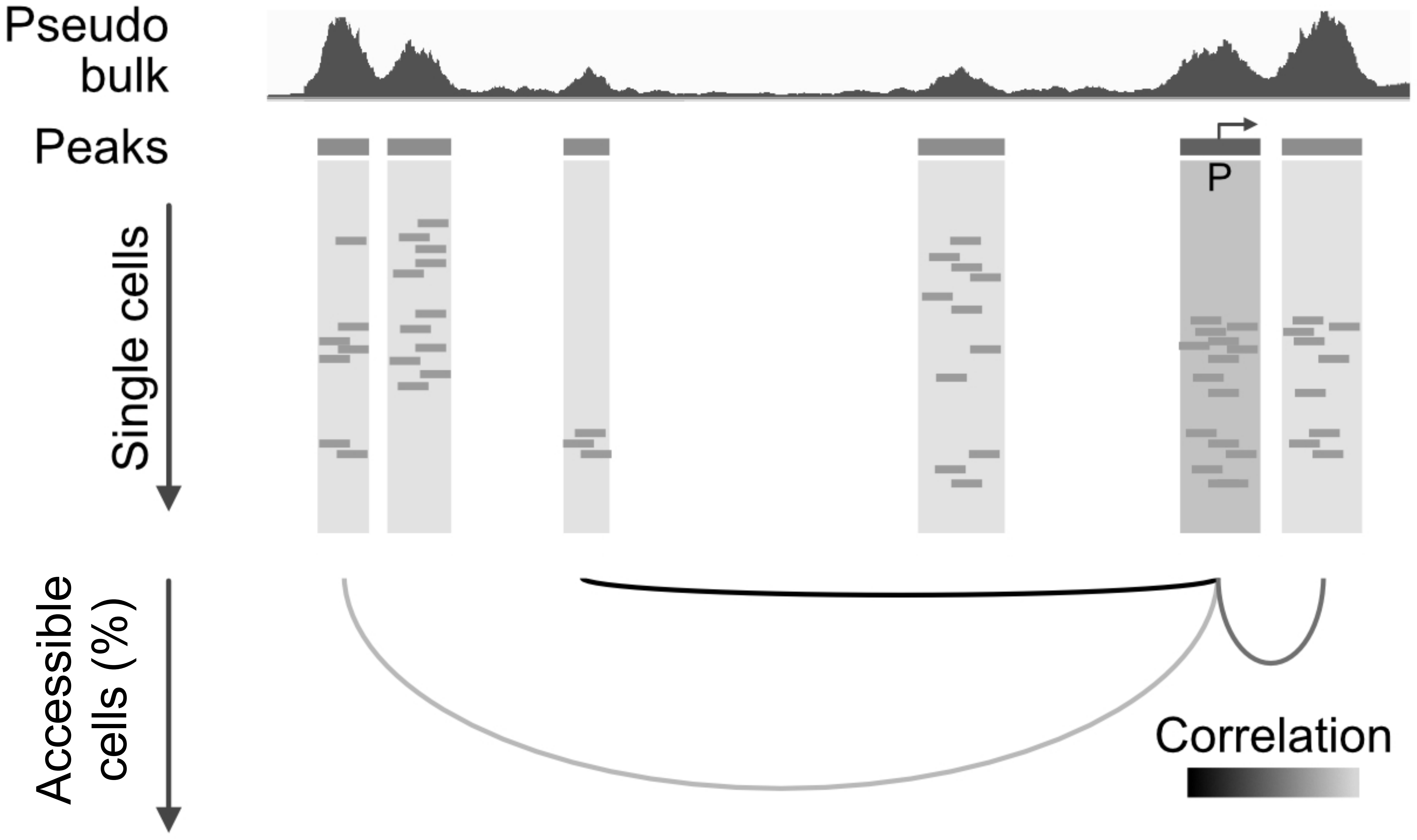
- C19+ B-cells, up to 96 single cells/run
- 40 000-130 000 integrations/cell
- 500-1500 open enhancer/promoters/cell
- 343 non-malignant and 494 CLL
- High-throughput flow cell (2 x 400 cells)

Single-cell ATAC-seq provides information on correlated accessibility changes that reflect transcription activity

Accessibility changes

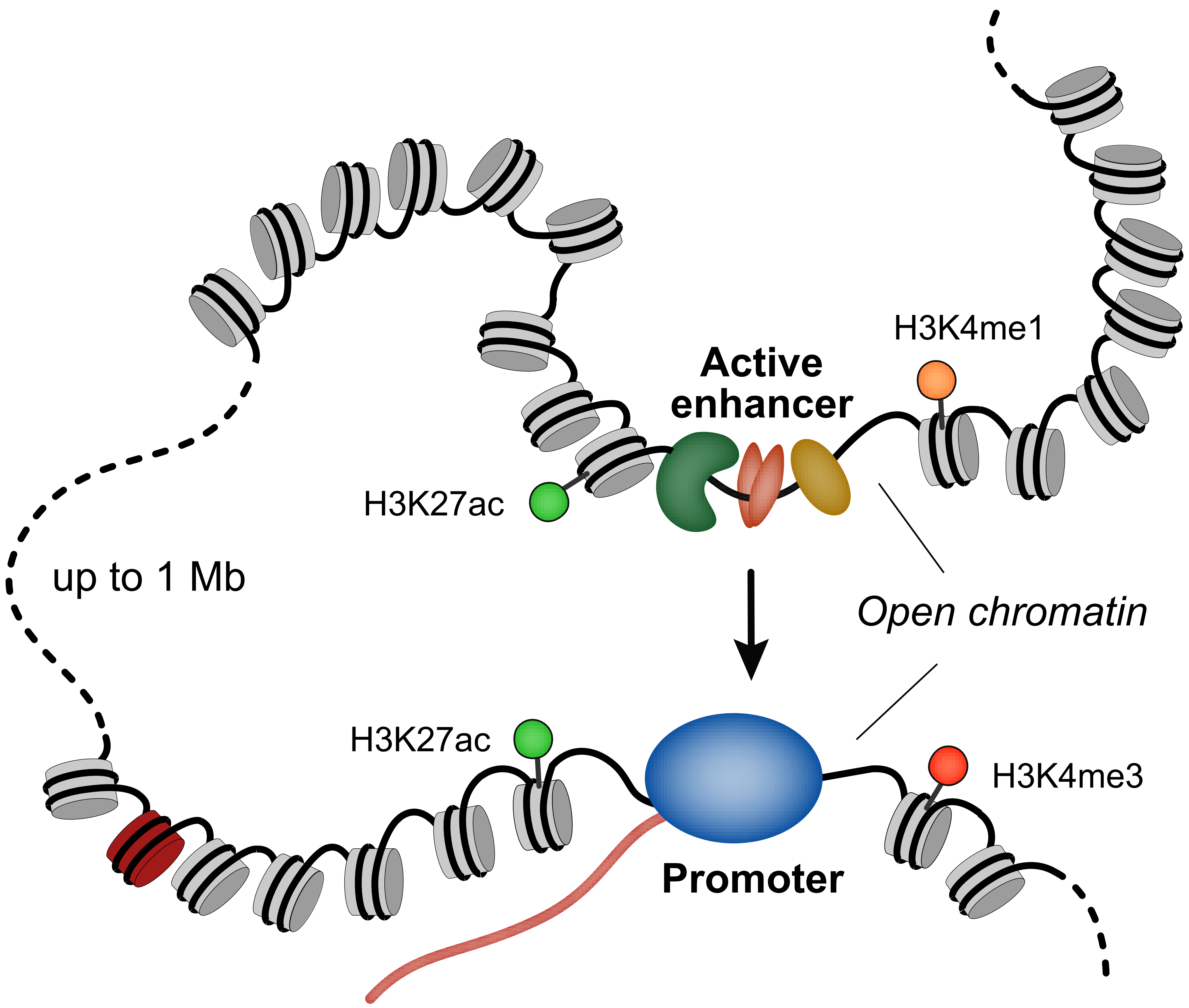


Accessibility correlations

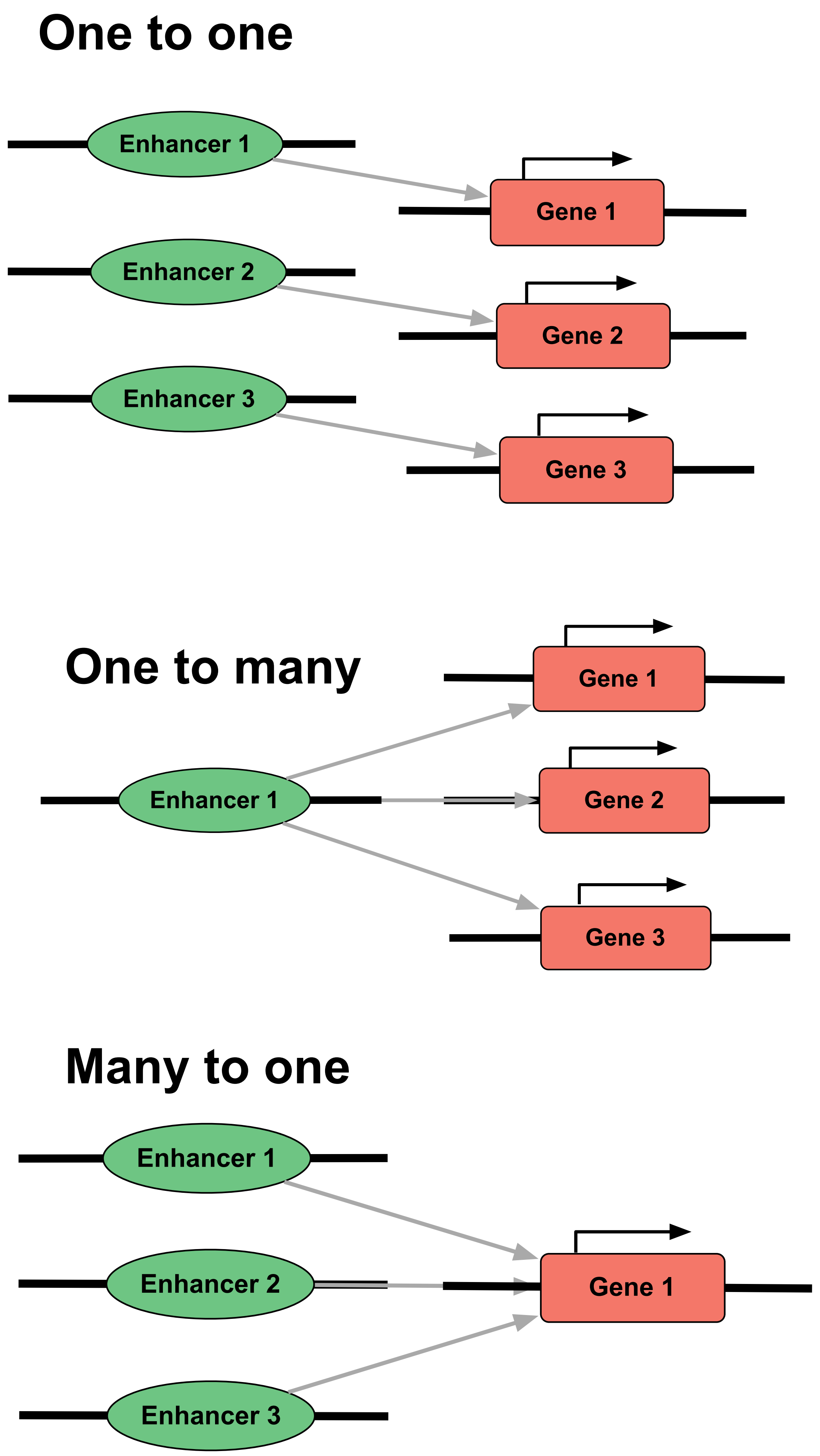


Mallm 2019 *Mol Syst Biol*
Muckenhuber 2023 *Life Sci Alliance*

Changes of chromatin features at promoters and enhancers

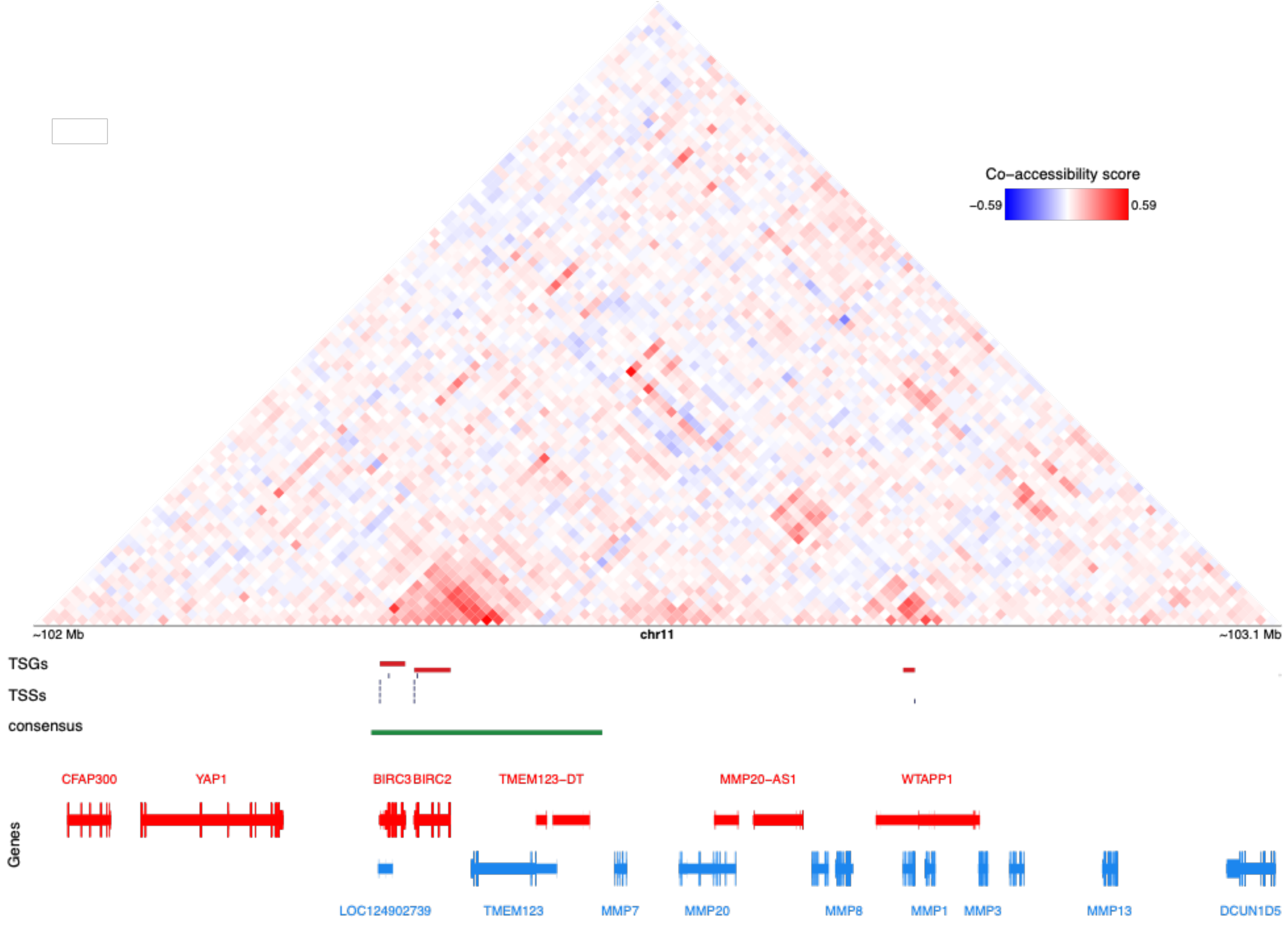


Enhancer-promoter topology



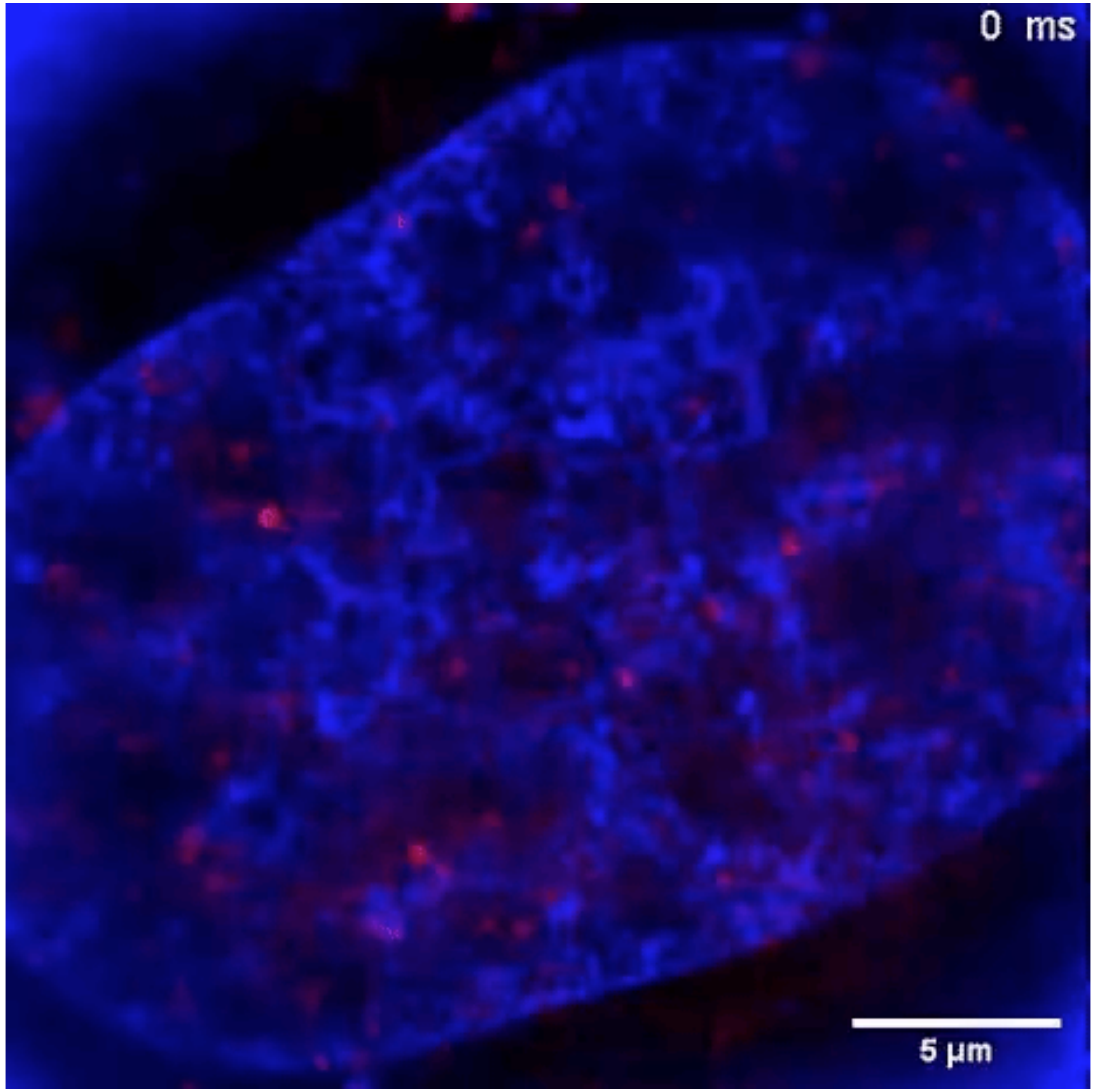
Cell type specific gene regulation in humans
- ~230 000 potential enhancers (ChromHMM)
- ~60 000 promoters (RefSeq)

Single-cell ATAC-seq to identify domains with increased co-accessibility score and transcription factor binding activity



Seufert 2024

Locally enriched activity of NF-kB stimulated with TNFα in the cell nucleus



Mazza et al. Nat Commun 2023

Tissue context information is lost in sequencing analysis

“Tissue” (fruit pieces = cells)

Spatially resolved transcriptomics

Bulk sequencing

Bulk sequencing after sorting

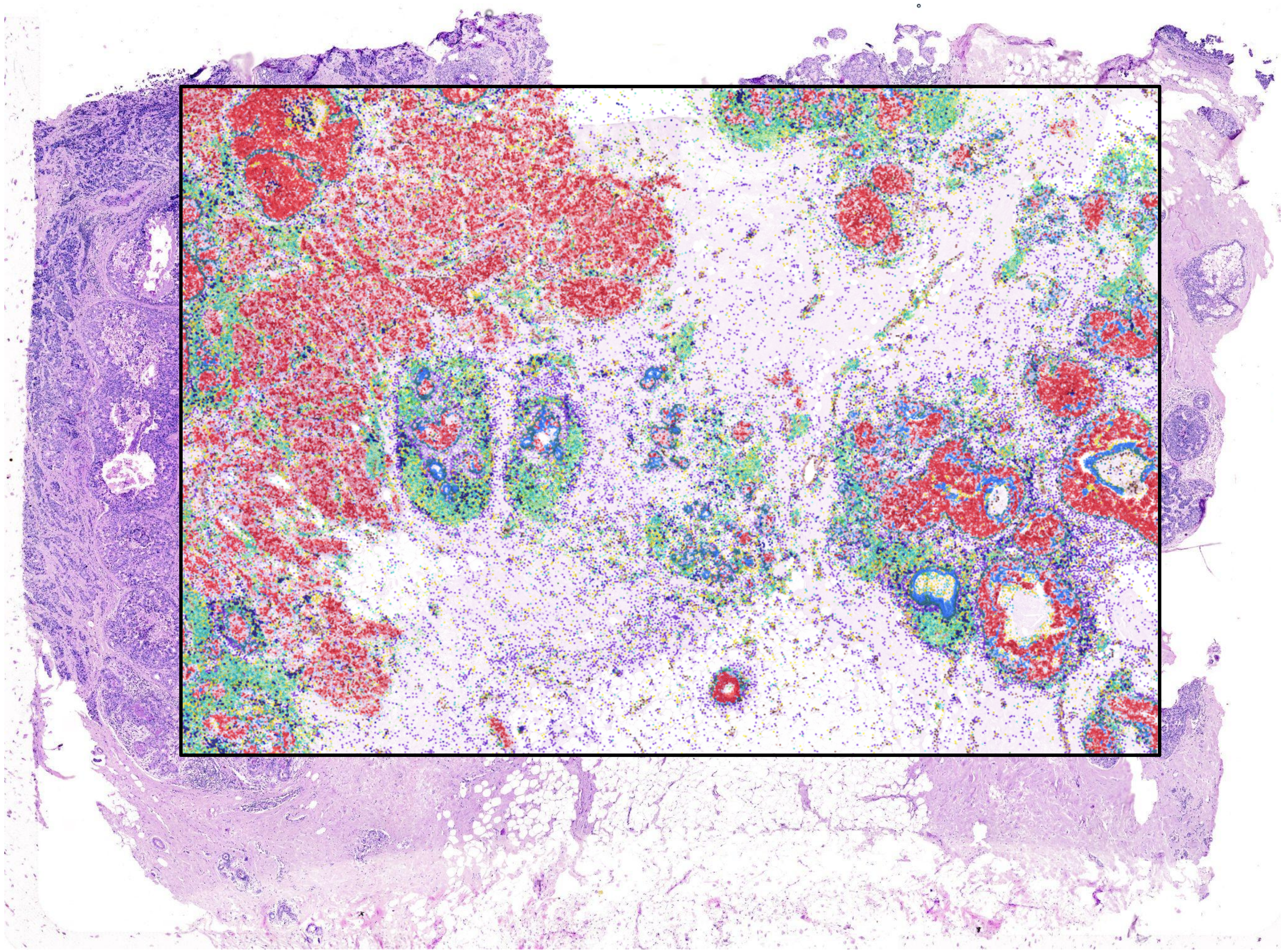
Single cell sequencing

- Link histological and molecular markers
- Resolve tumor heterogeneity in tissue
- Interplay of cancer cells and microenvironment
- Features of tumor boarder lines/invasive fronts

Spatial transcriptomics of a breast cancer tissue section

Hematoxylin & eosin staining (H&E): 2 colors
- pink: protein (any)
- purple: DNA

Morphology
= cell type



Spatial omics:
50-1,000 markers
- specific proteins
- specific transcripts

Cell type

- ● Endothelial RGS5
- ● Endothelial Lymphatic LYVE1
- ● Endothelial ACKR1
- ● Endothelial CXCL12
- ● CAFs MSC iCAF–like
- ● CAFs myCAF–like
- ● PVL Immature
- ● Cycling PVL
- ● PVL Differentiated
- ● B cells Memory
- ● B cells Naive
- ● T cells CD8+
- ● T cells CD4+
- ● Cycling T–cells
- ● NK cells
- ● NKT cells
- ● DCs
- ● Cycling_Myeloid
- ● Macrophage
- ● Monocyte
- ● Mature Luminal
- ● Myoepithelial
- ● Luminal Progenitors
- ● Plasmablasts
- ● Cancer Basal SC
- ● Cancer Cycling
- ● Cancer Her2 SC
- ● Cancer LumB SC
- ● Cancer LumA SC

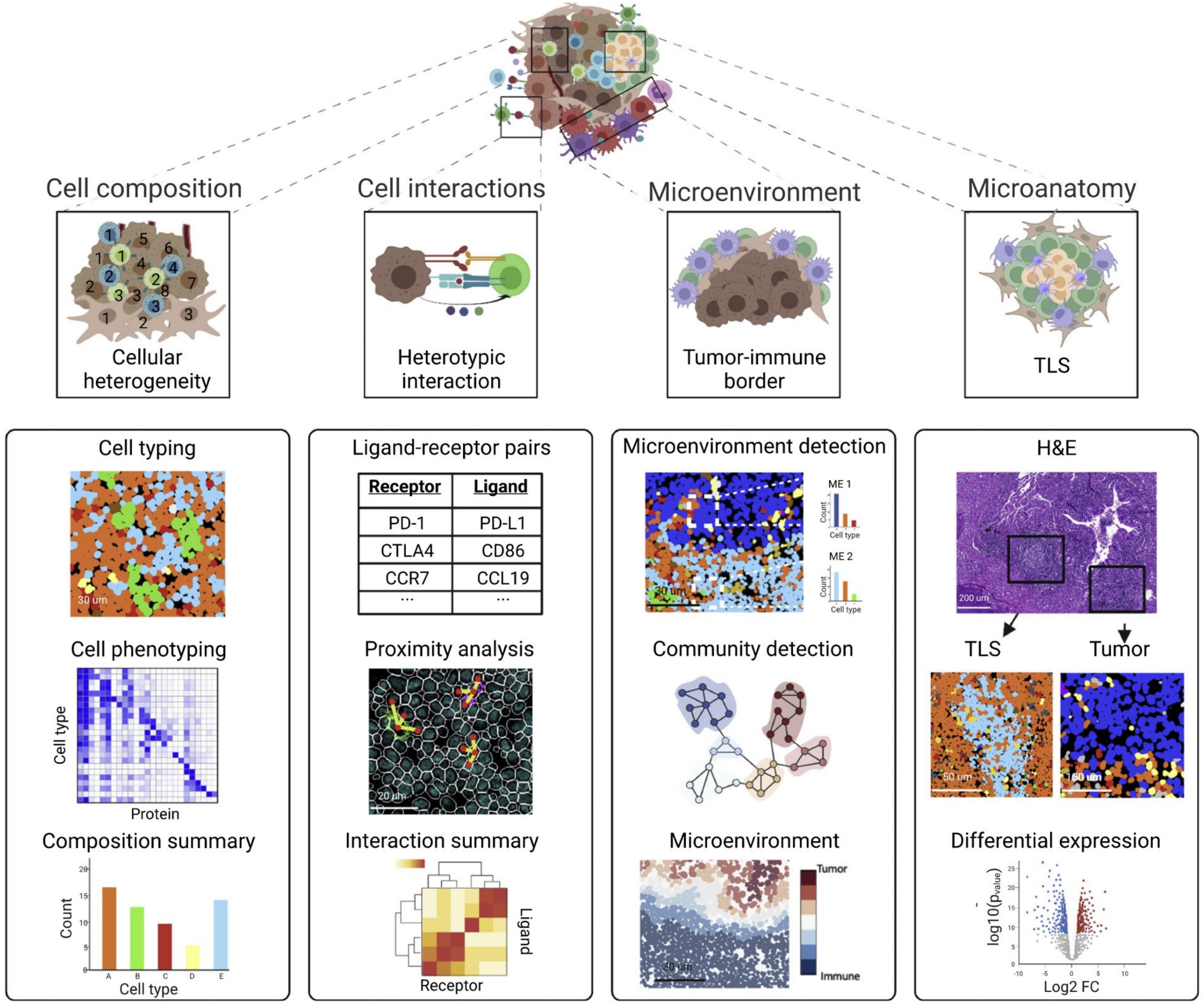
Cell types & states defined by molecular characteristics

Use AI to characterise 1,000,000 cells/slide

Image by Moritz Gerstung

Spatial cancer biology and oncology

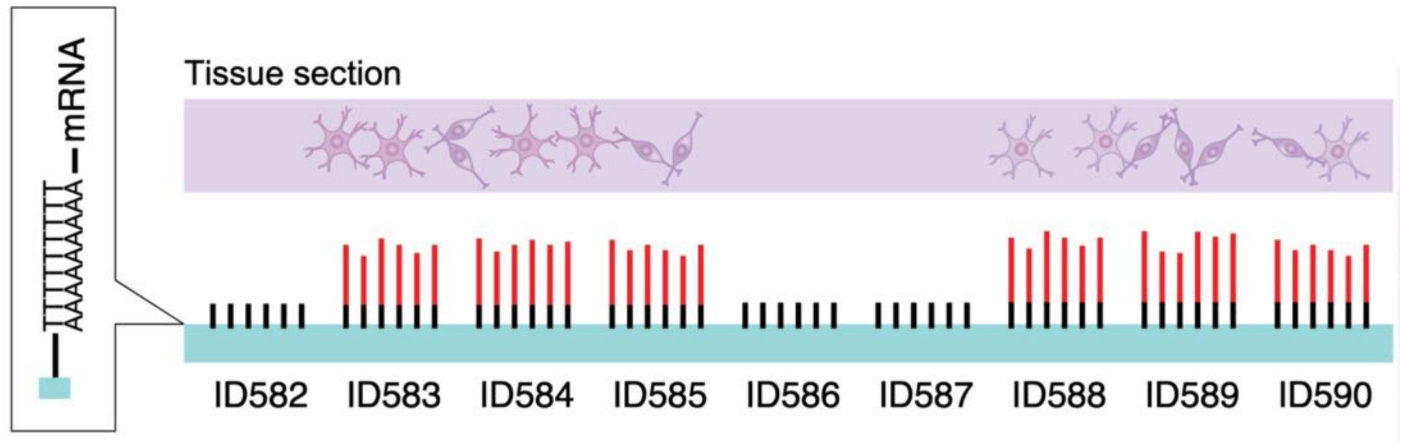
- **Cell composition** of the tumor microenvironment:
  → ~50% of cells are normal
- Resolve **intra-tumor heterogeneity** & **subclones**
  → Mechanisms of progression and resistance
- **Interactions with immune and other normal cells:**
  → Identify drug targets, eg immuno-oncology
- Characterize **tumor microanatomy:**
  → Role of tissue structure

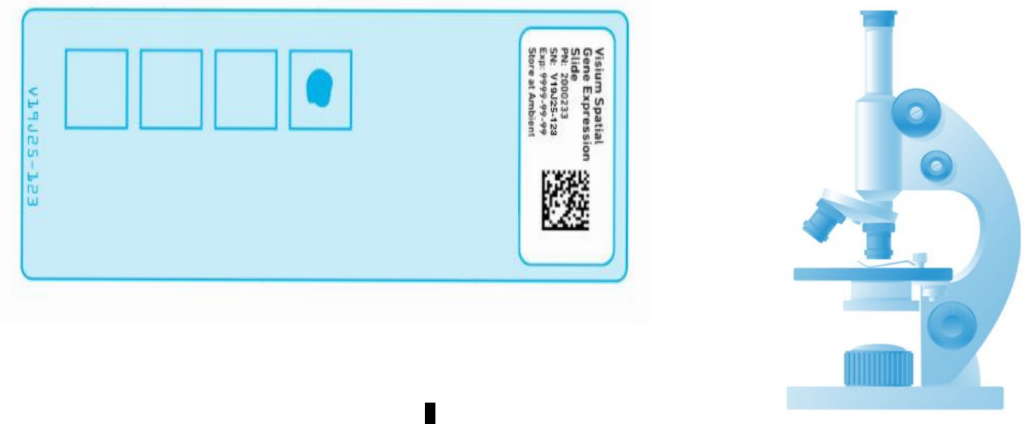


Elhanani 2023 *Cancer Cell*

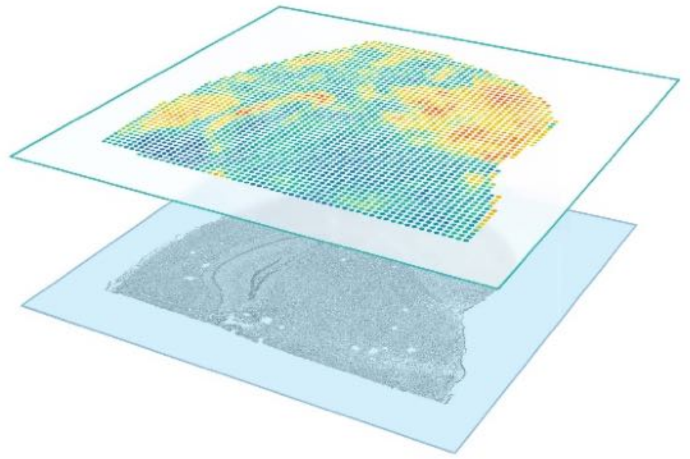
Sequencing and hybridization-based methods for spatial transcriptomics

Sequencing-based methods (unbiased)



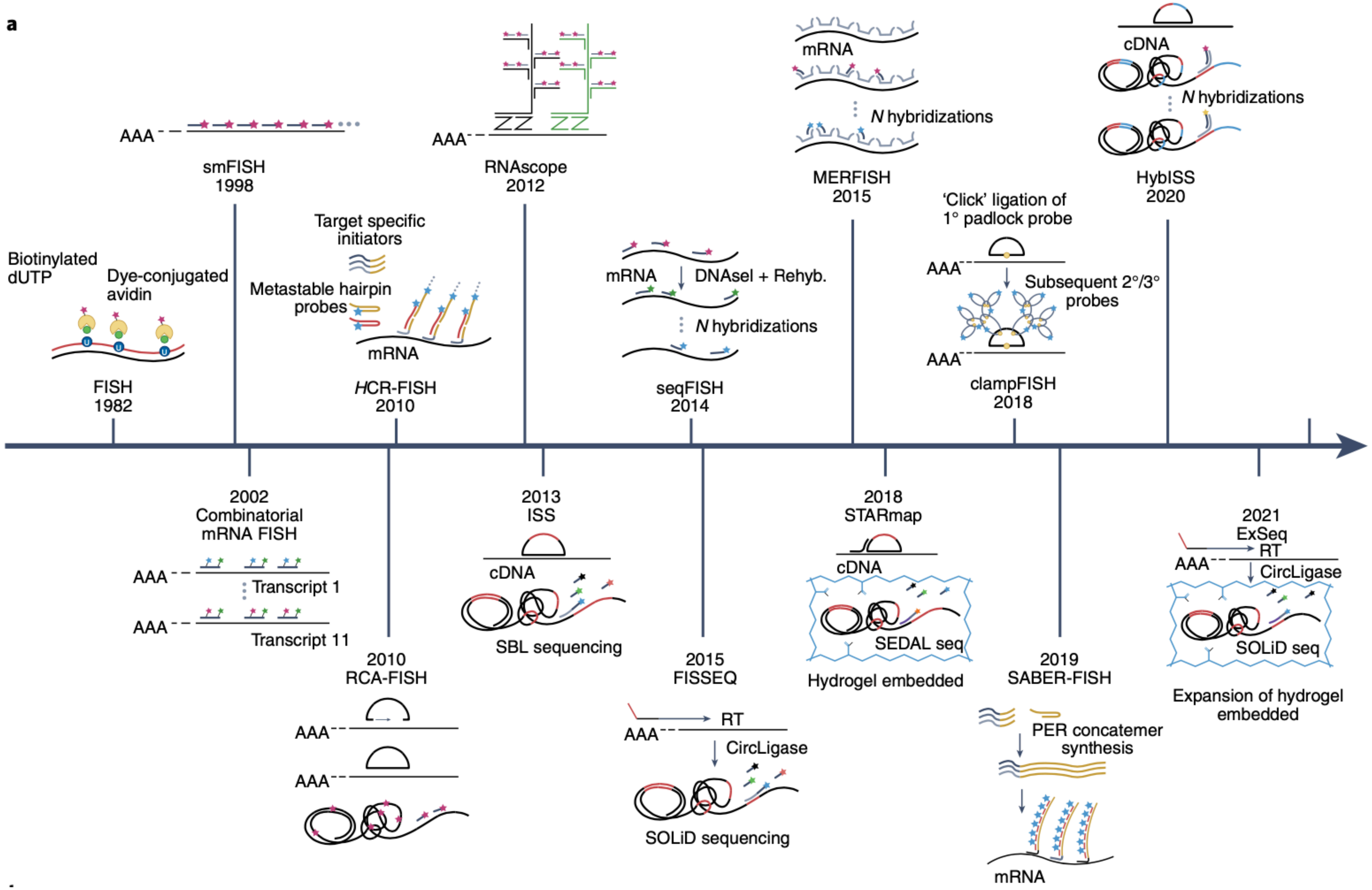


Sequencing of captured RNAs



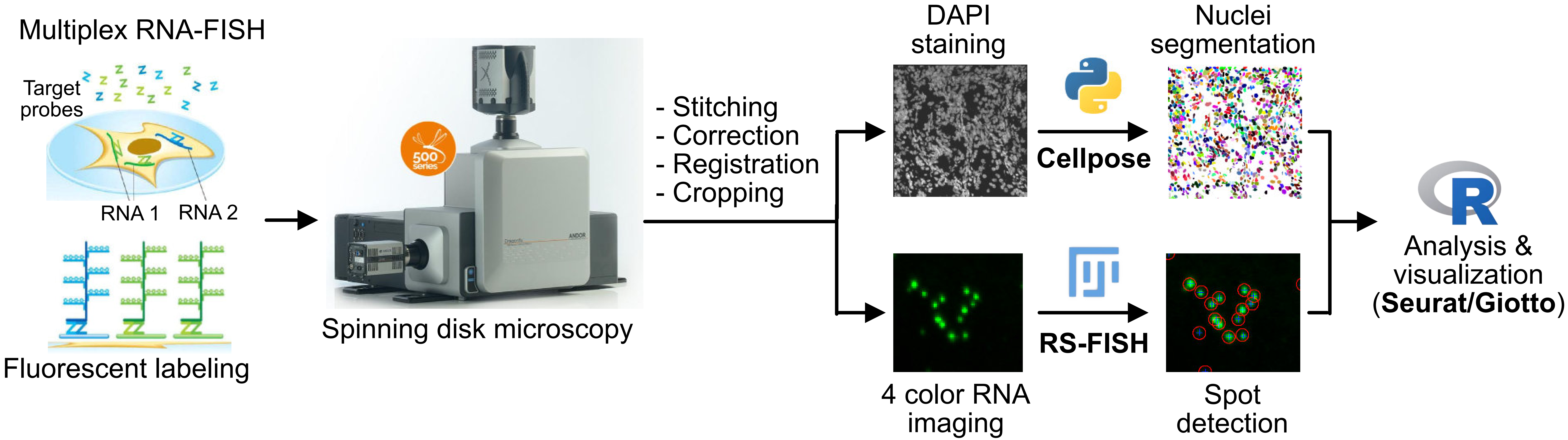
- Visium
- Seq-Scope
- Stereo-seq

Hybridization-based methods (targeted)



Le, P. et al. (2022) NCB

RNAScope with 12-48 probes provides subcellular resolution



Multiplex RNA-FISH

Target probes

RNA 1 RNA 2

Fluorescent labeling

Spinning disk microscopy

- Stitching
- Correction
- Registration
- Cropping

DAPI staining

Cellpose

Nuclei segmentation

4 color RNA imaging

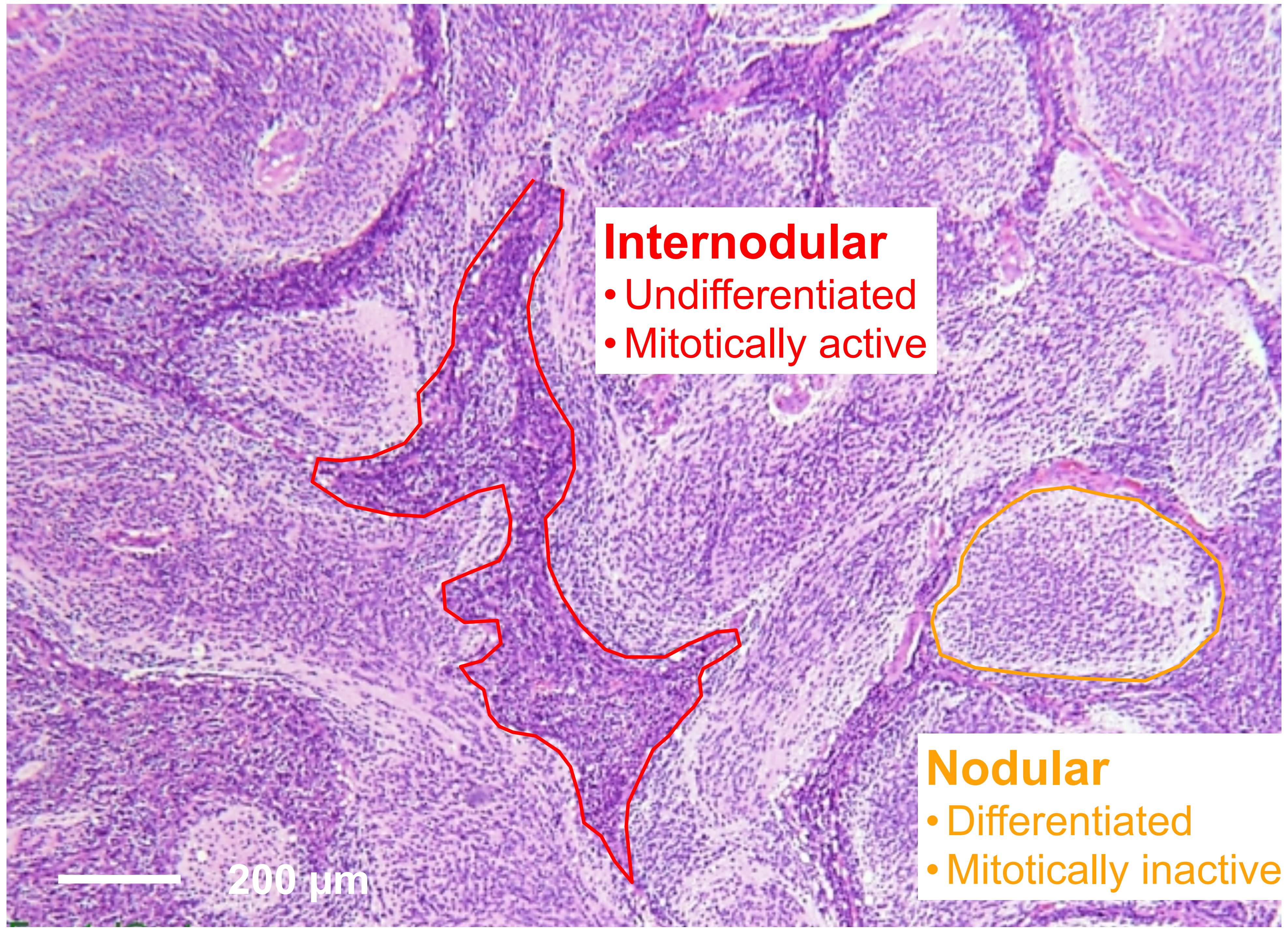
RS-FISH

Spot detection

Analysis & visualization (**Seurat/Giotto**)

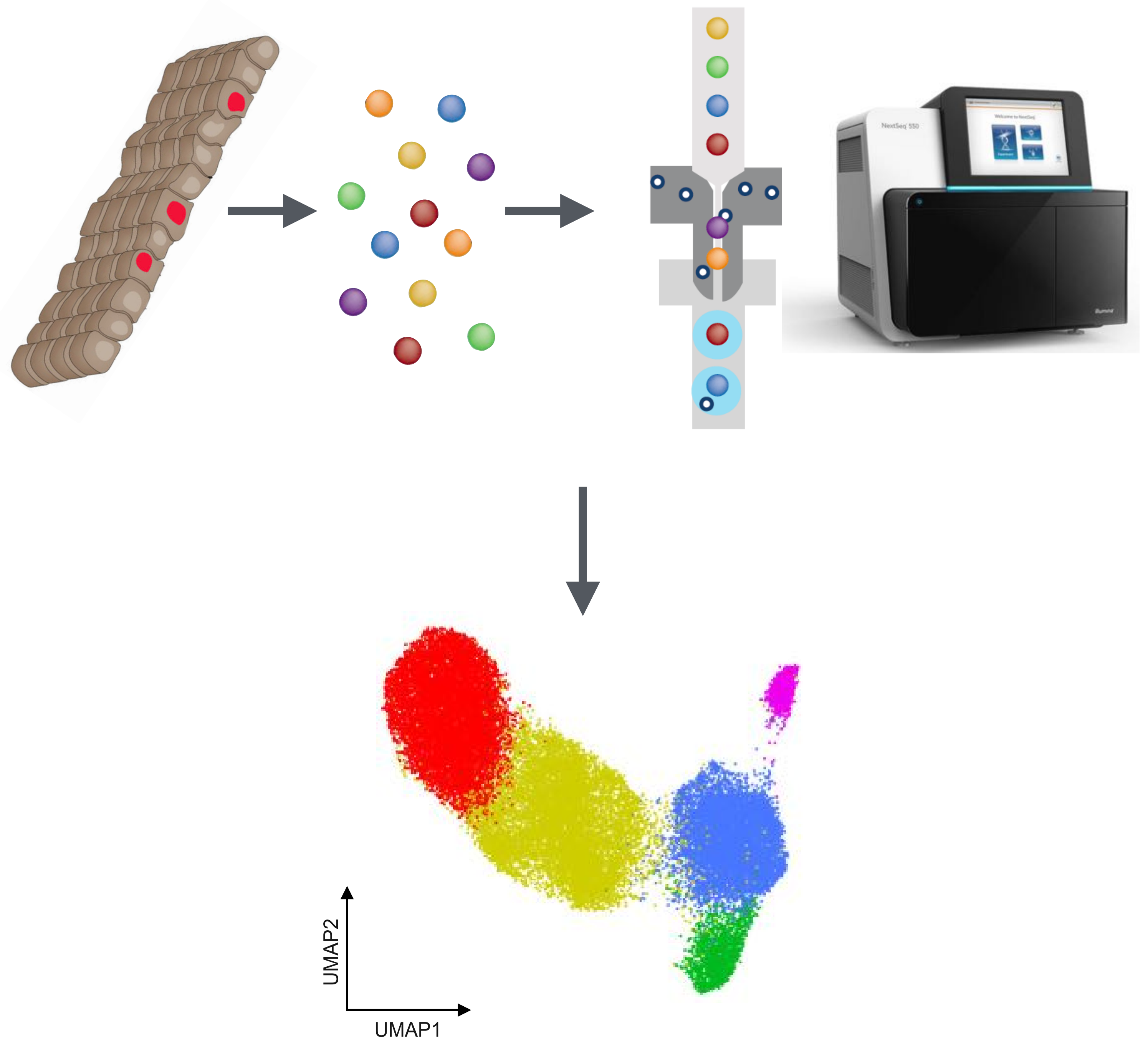
Spatially transcriptomics (ST) to study medulloblastoma with extensive nodularity (MBEN)

Medulloblastoma with extensive nodularity (MBEN)



Internodular
- Undifferentiated
- Mitotically active

Nodular
- Differentiated
- Mitotically inactive


Tissue

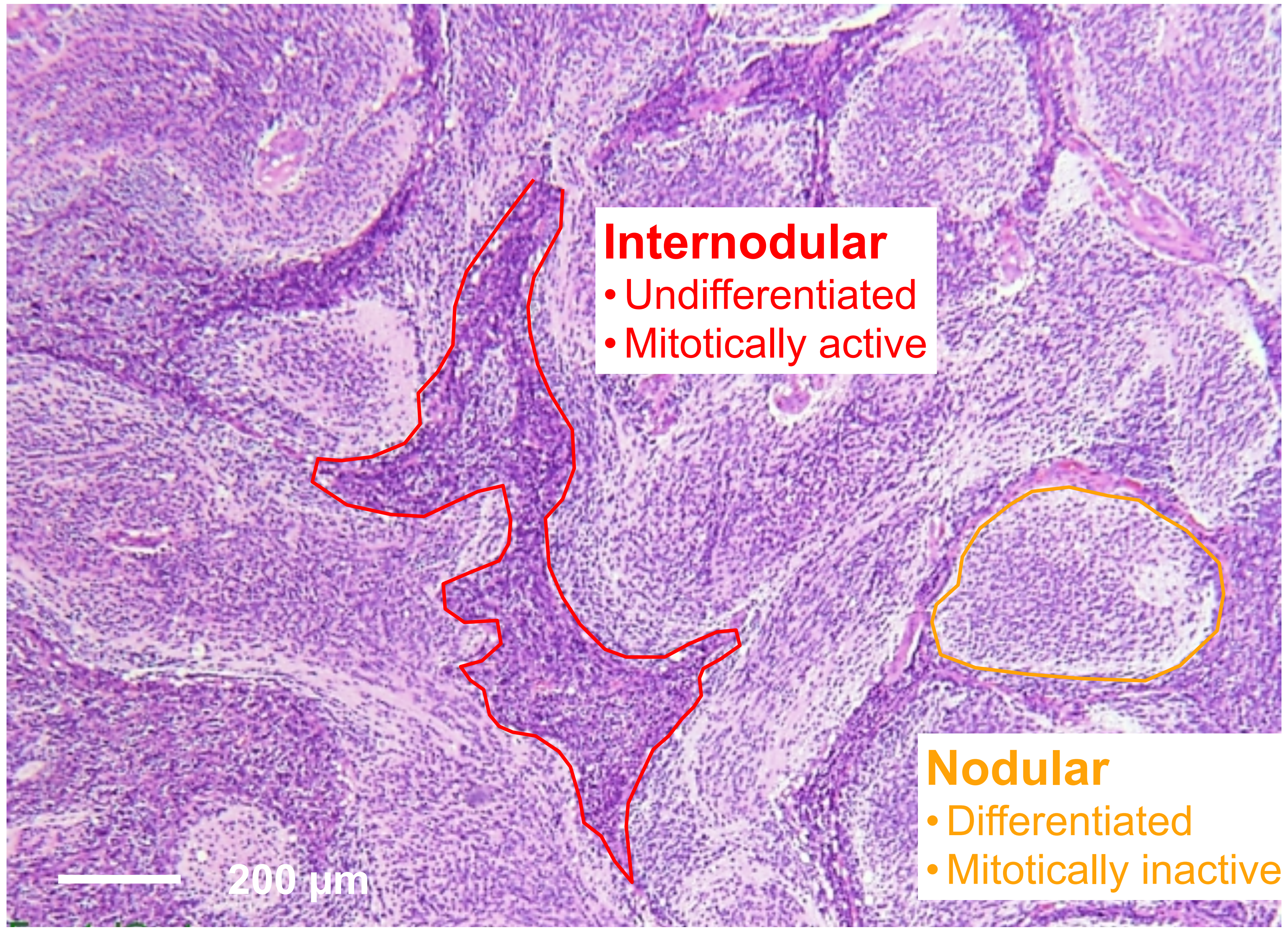
Cell suspension

Single cell RNA-seq

Ghasemi 2024 *Nat Commun*

Assignment of scRNA-seq reference data from cell suspension

Medulloblastoma with extensive nodularity (MBEN)

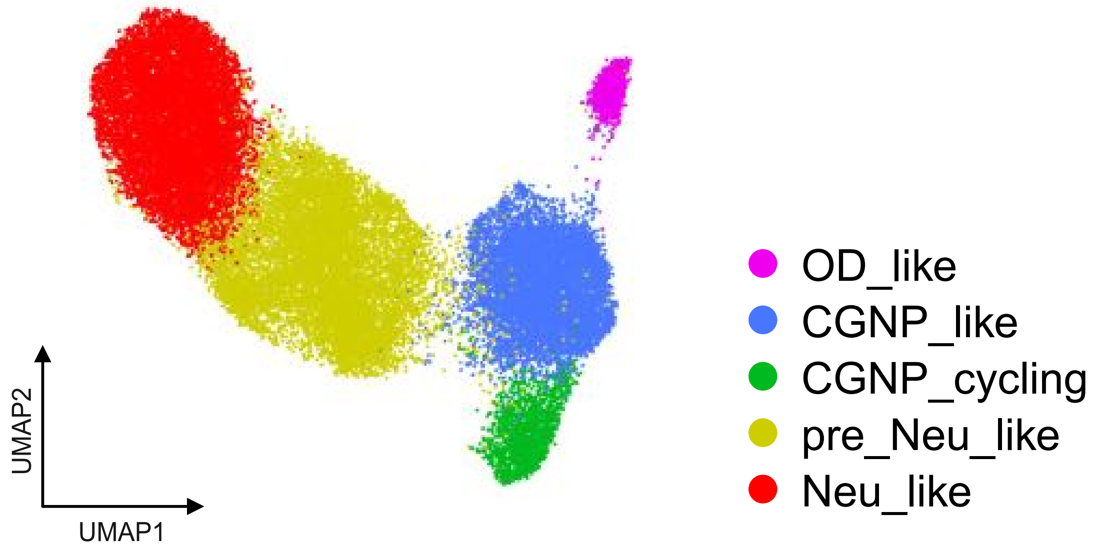


Internodular
- Undifferentiated
- Mitotically active

Nodular
- Differentiated
- Mitotically inactive

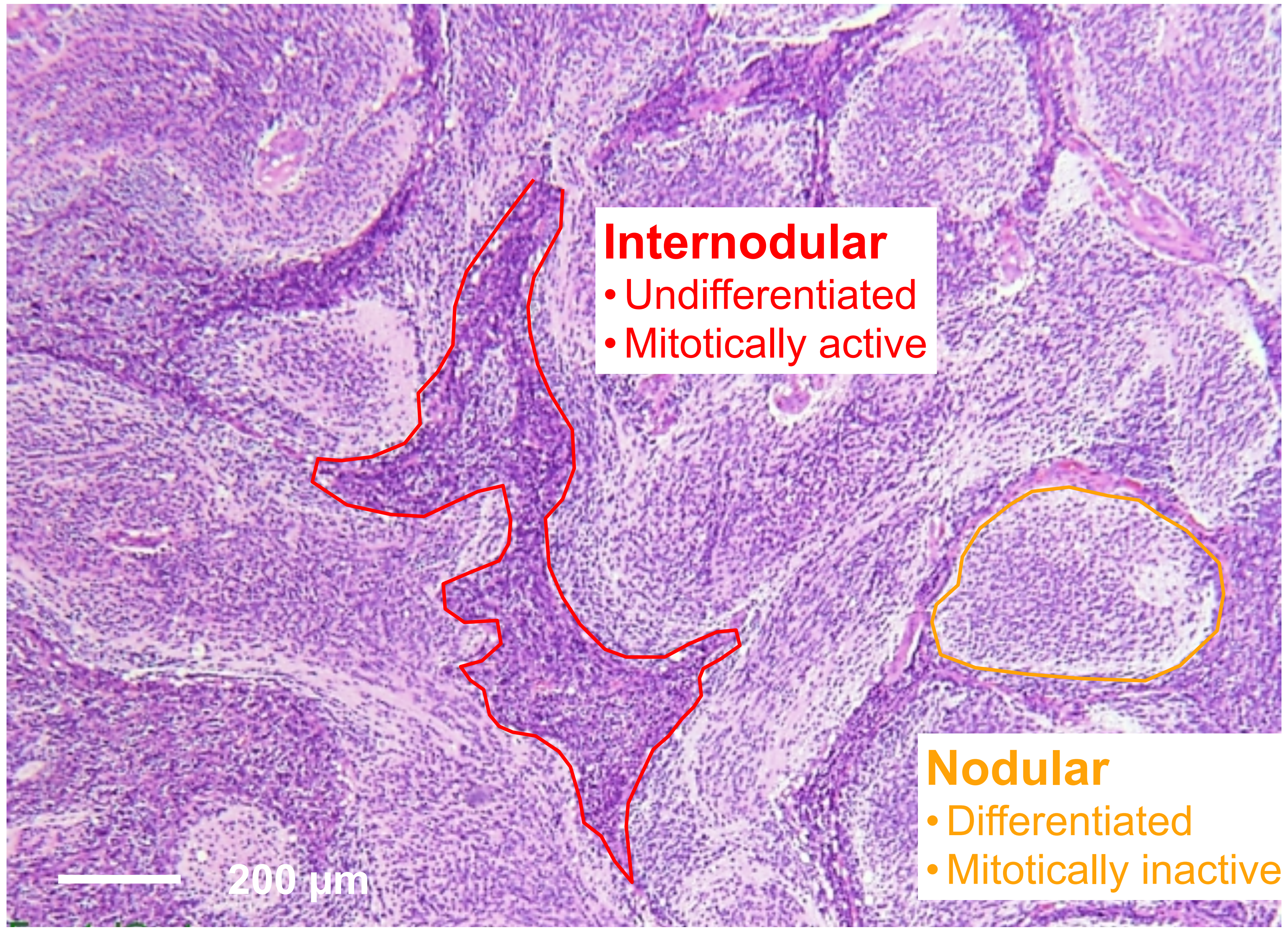
?

scRNA-seq

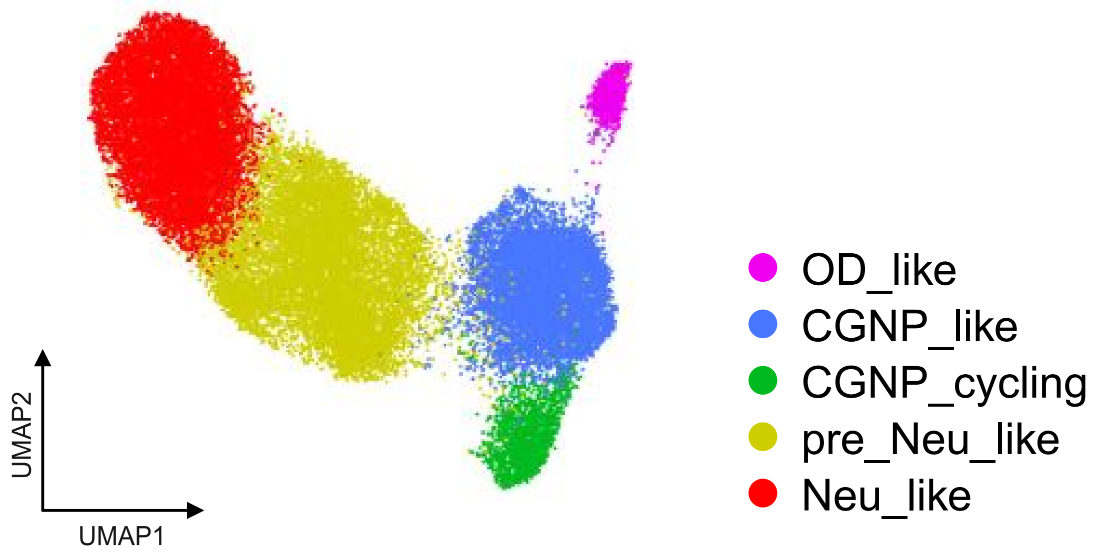


Selection of marker RNAs

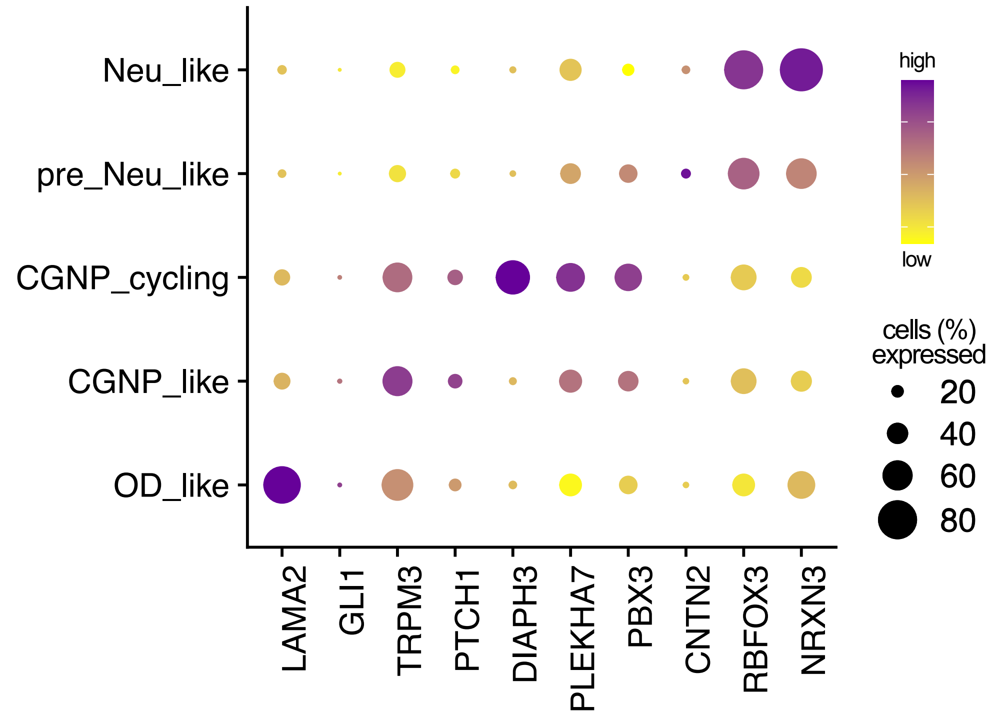
Medulloblastoma with extensive nodularity (MBEN)



scRNA-seq

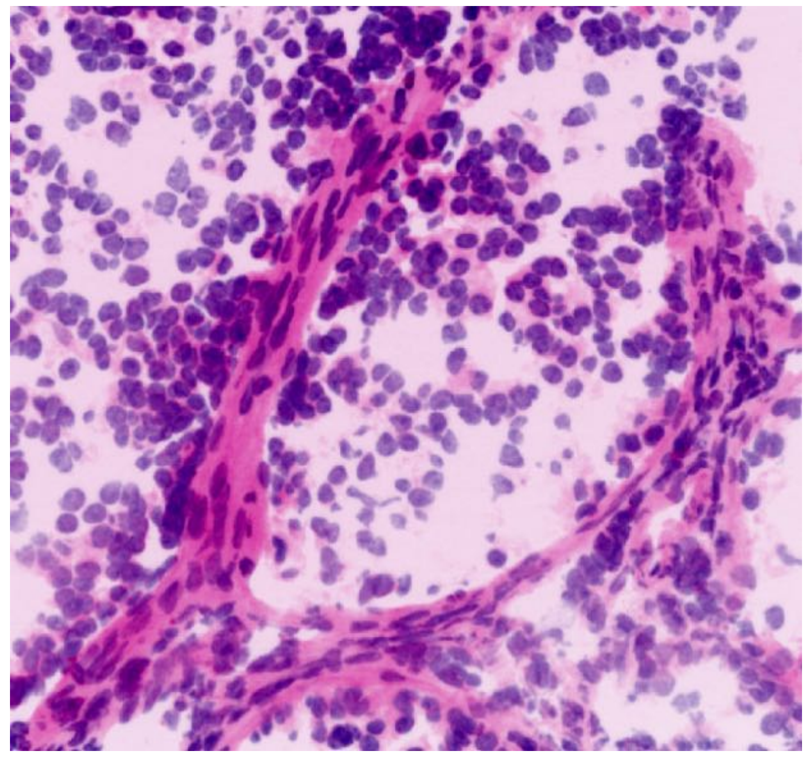


Identification of marker RNAs

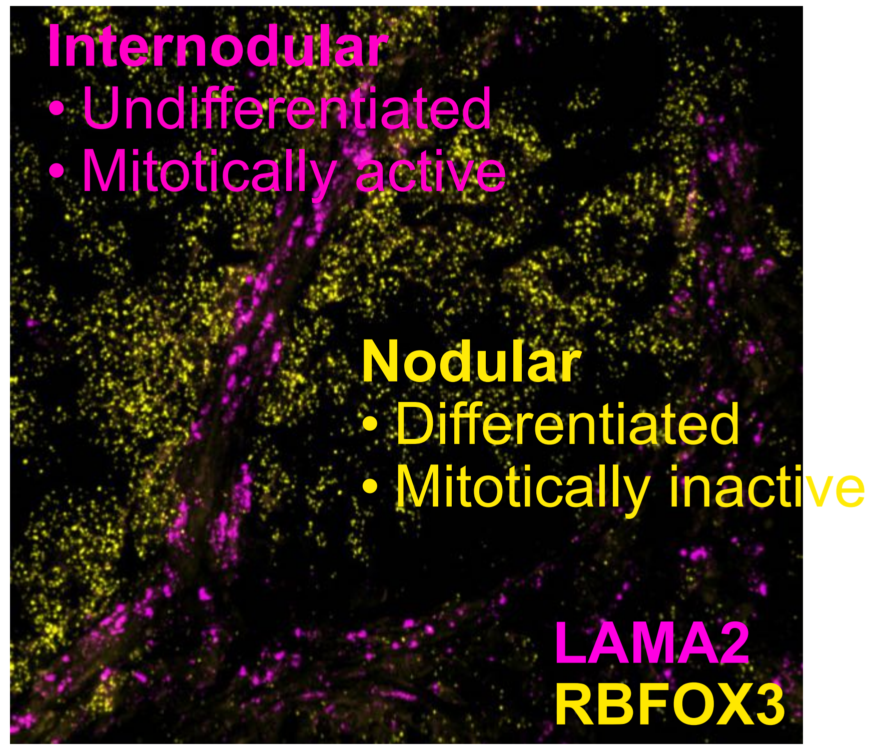


Cell type identification and spatial relations in MBEN

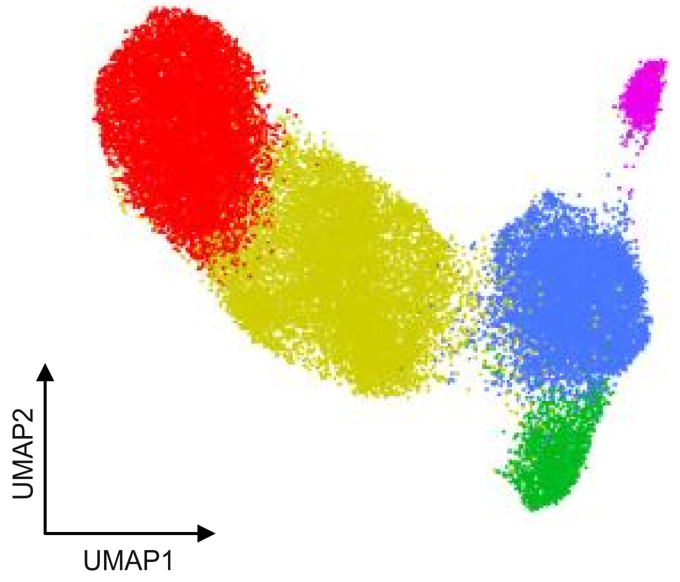
Pseudo H&E



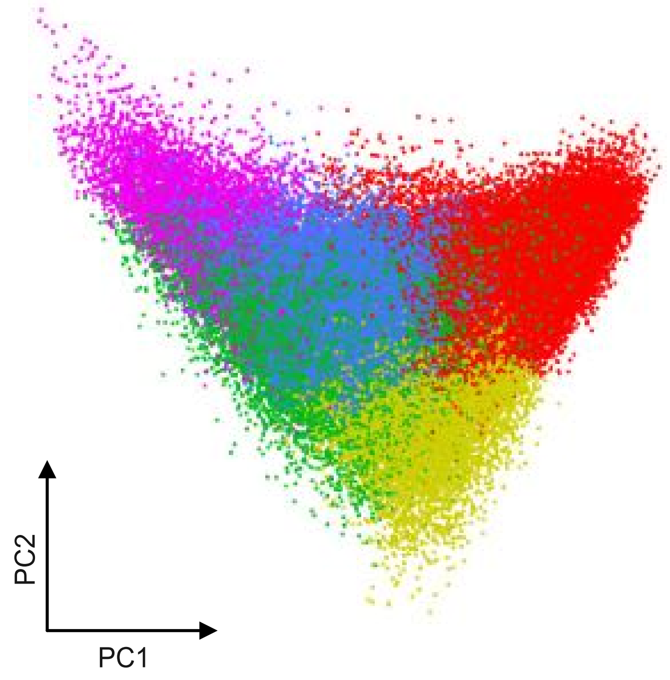
smRNA-FISH signal

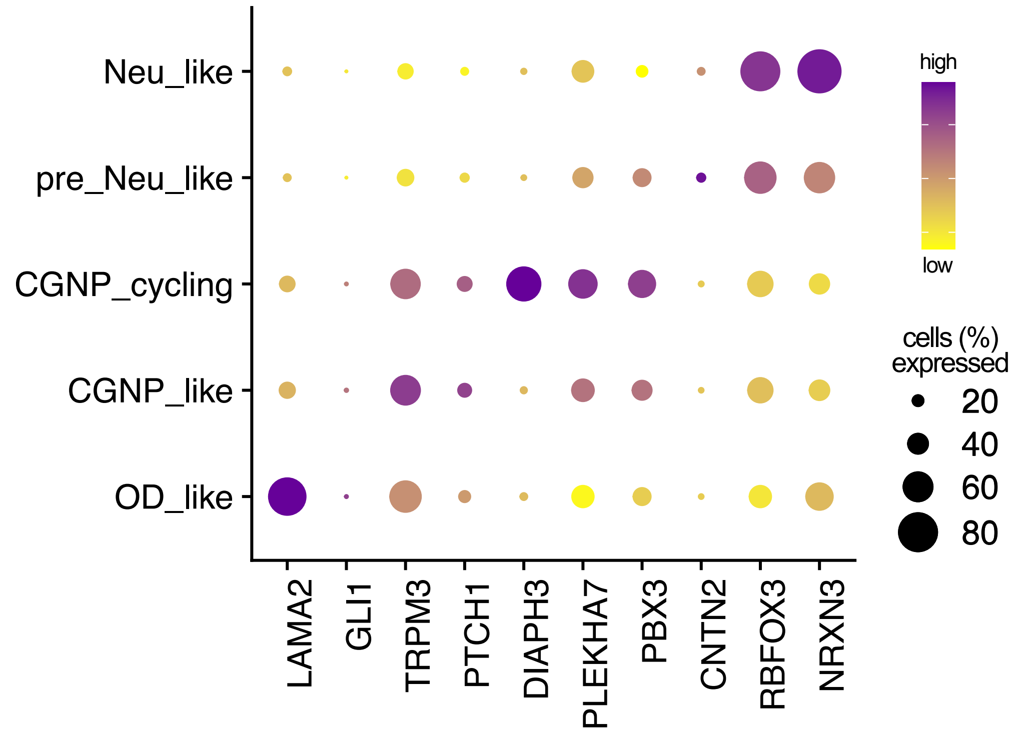


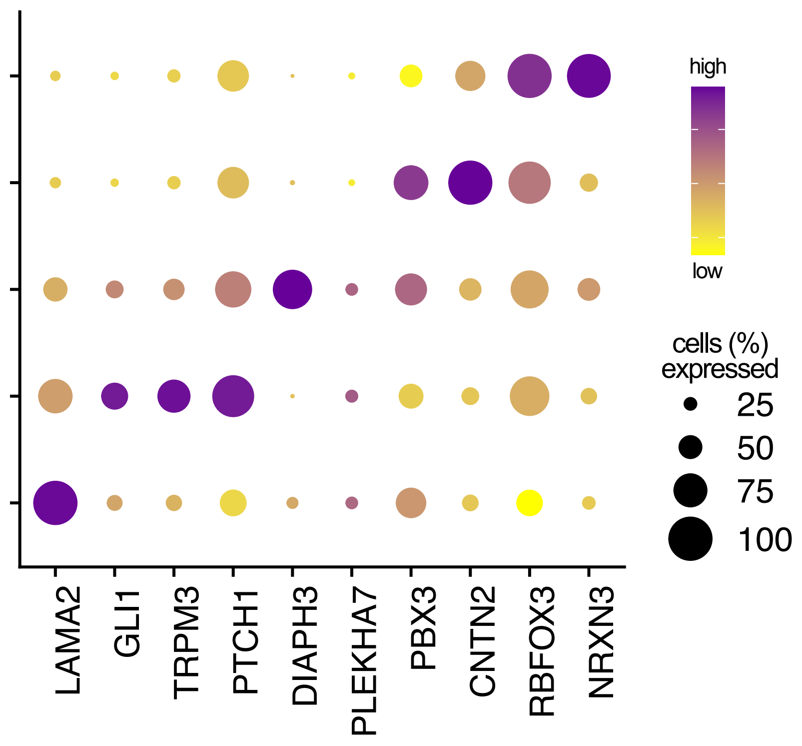
scRNA-seq



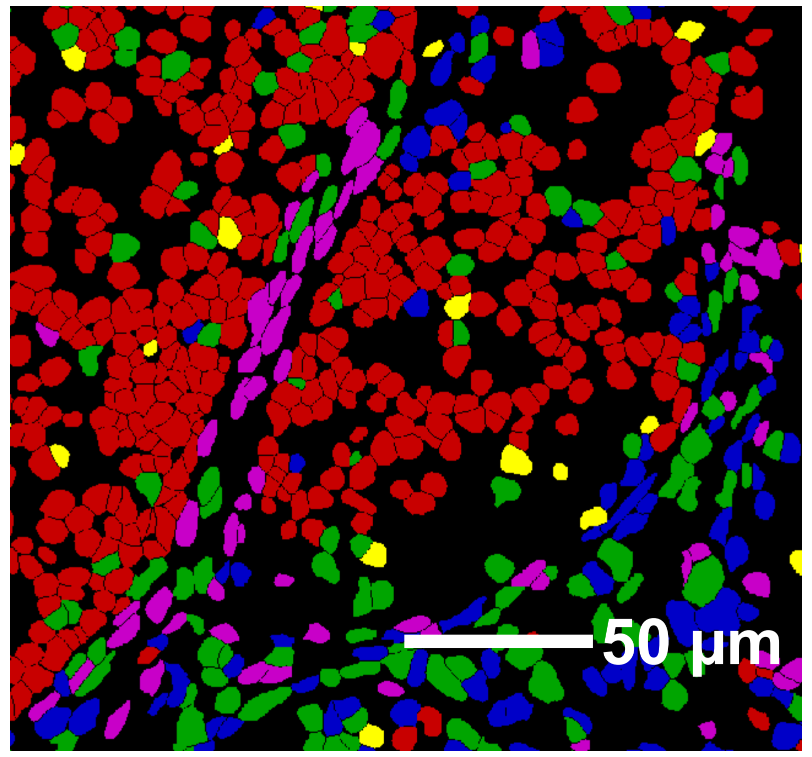
smRNA-FISH



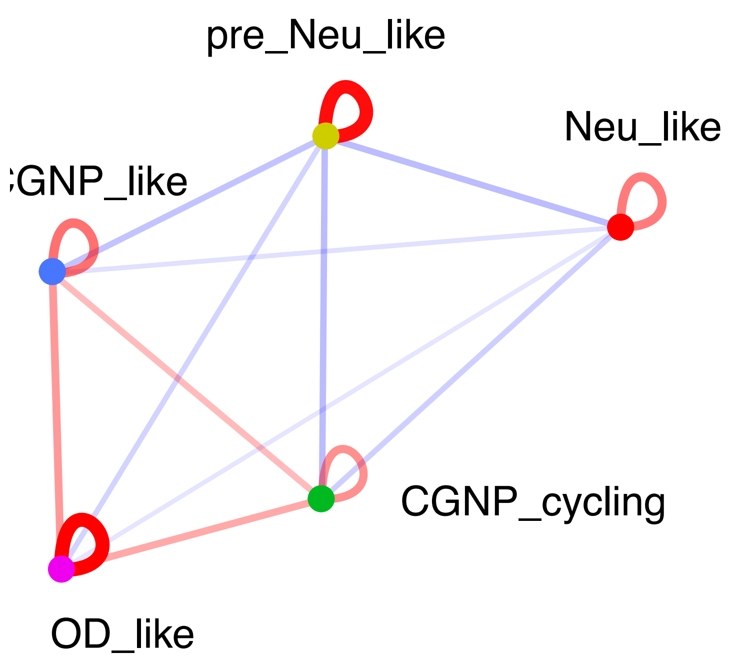




Cell types

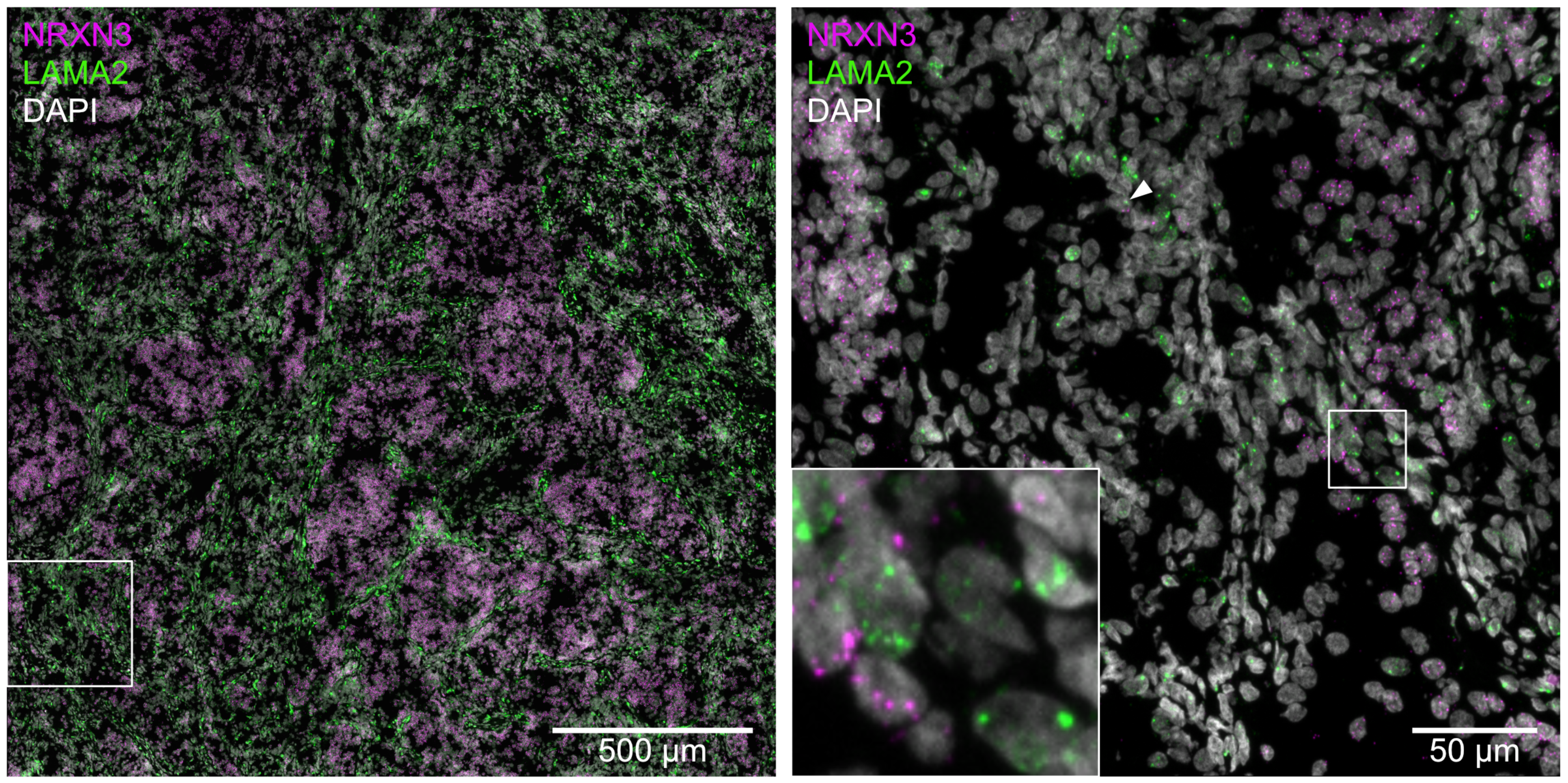


Spatial network

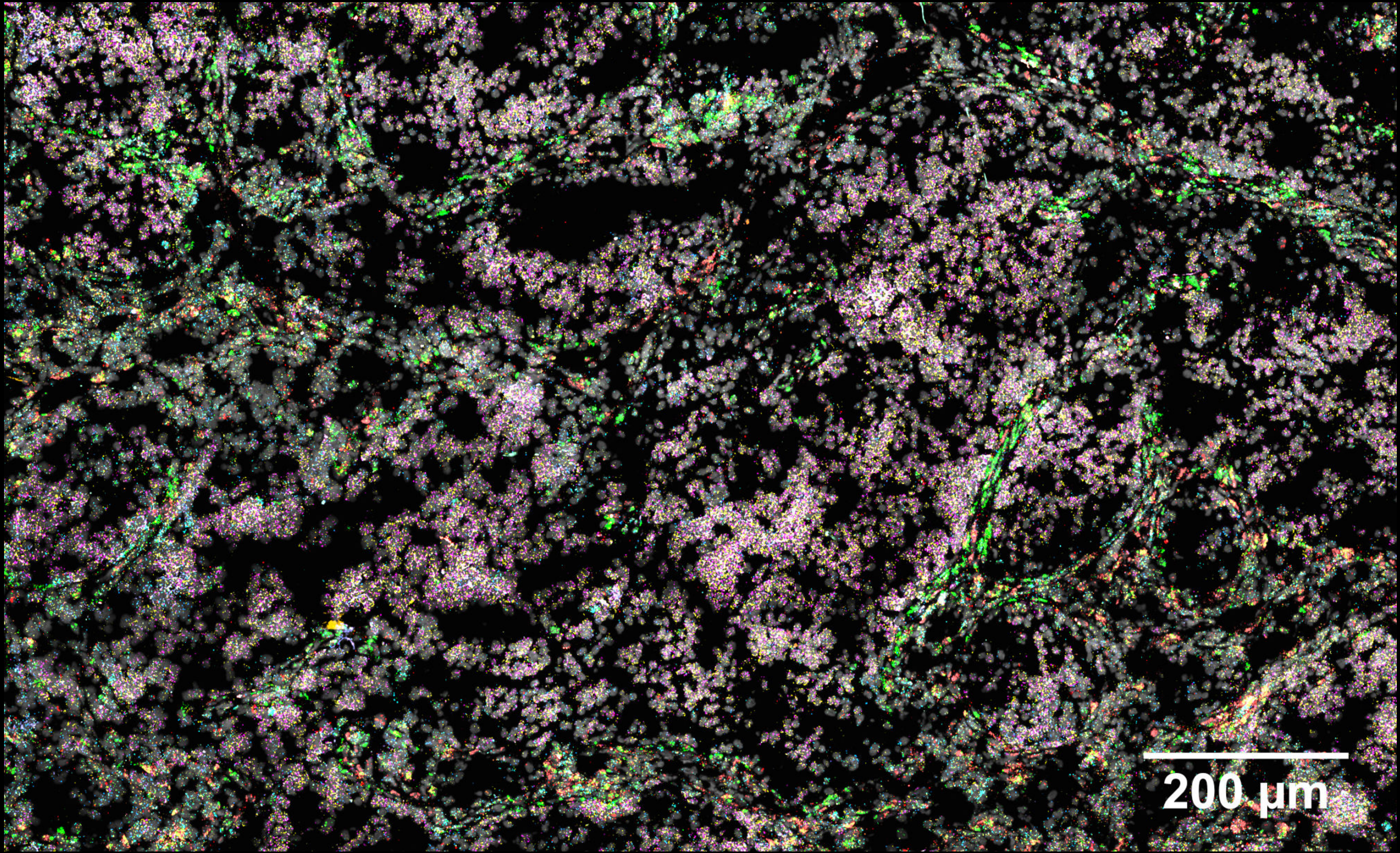


- ● OD_like
- ● CGNP_like
- ● CGNP_cycling
- ● pre_Neu_like
- ● Neu_like

MBEN RNAScope analysis of transcripts provides subcellular resolution



Spatial transcriptomics analysis of MBENs with 12 marker RNAs



DIAPH3
GL1
RBFOX3
PTCH1
NRXN3
CNTN2
PLEKHA7
PBX3
LRRTM4
FOS
LAMA2
TRPM3
DAPI

Spatial transcriptomics analysis of MBENs with 12 marker RNAs

DIAPH3
GL1
RBFOX3
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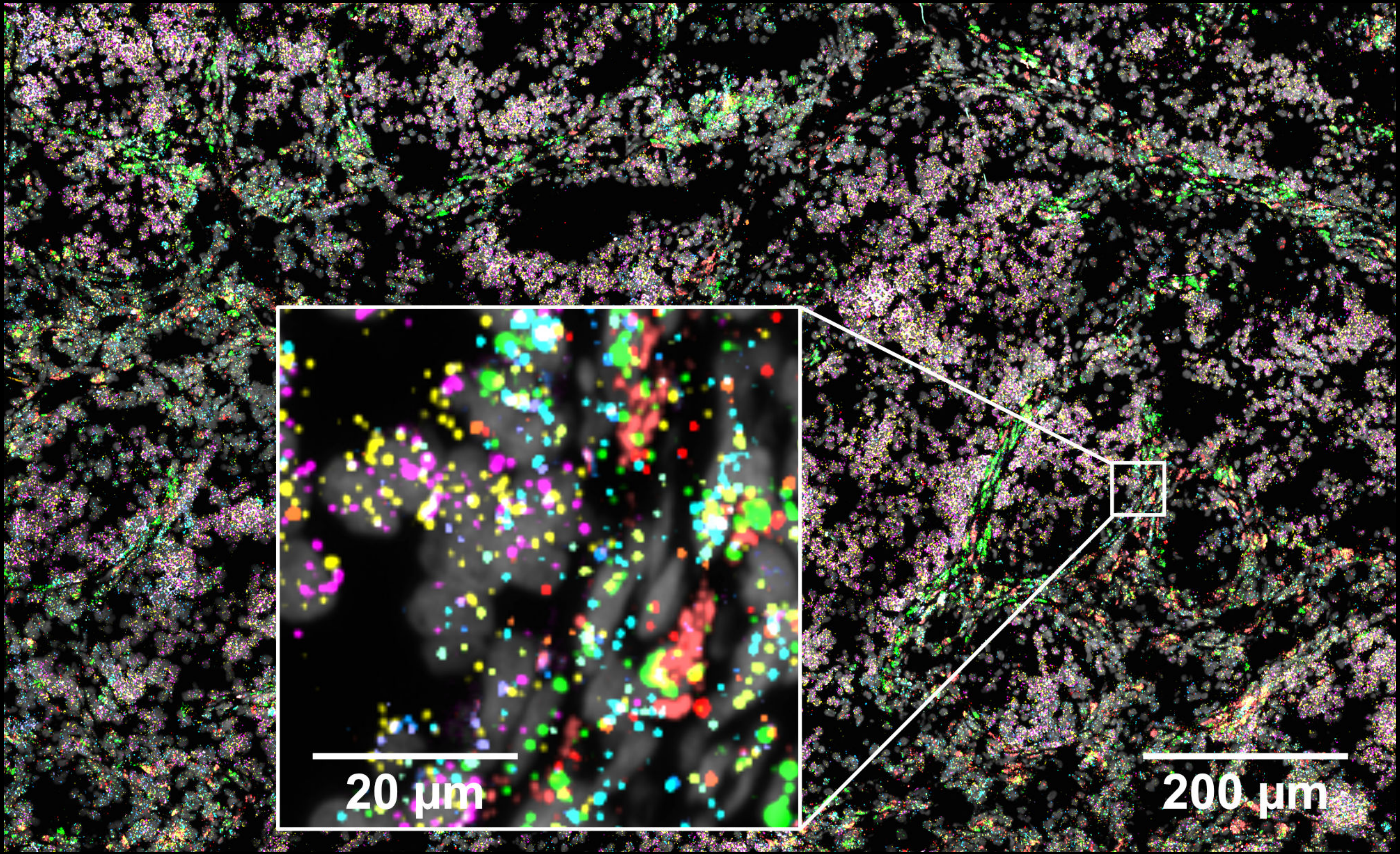
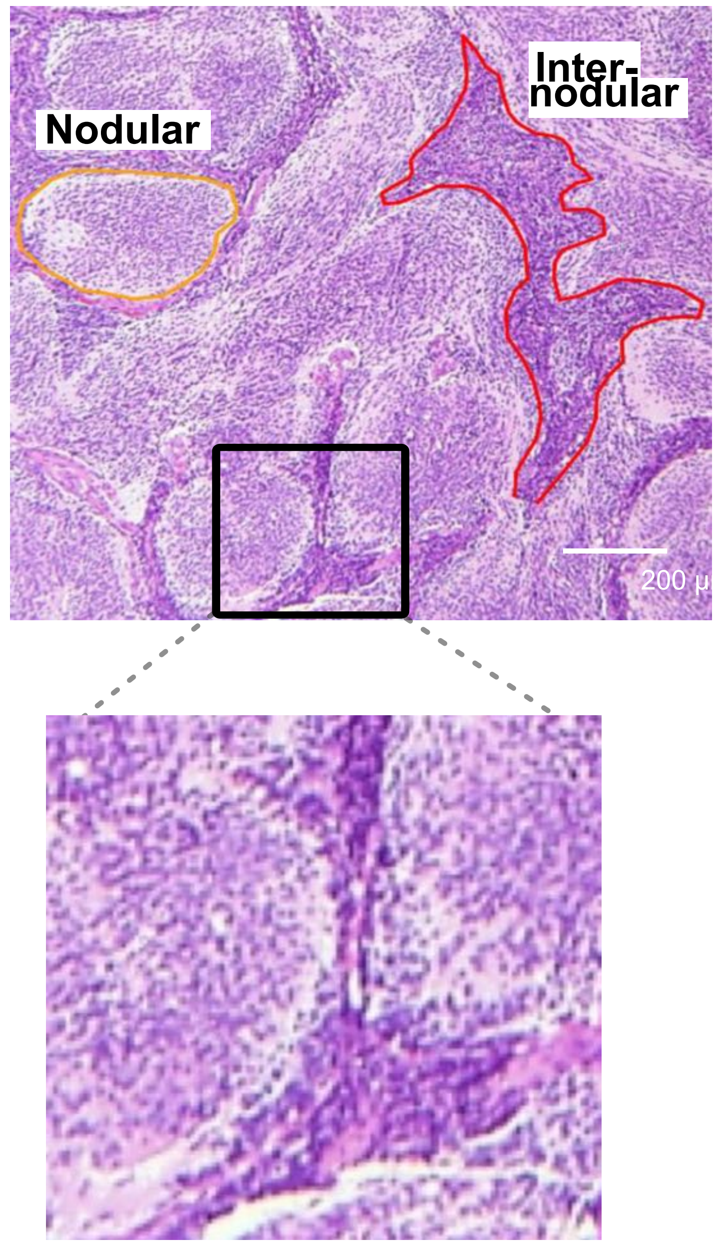
DAPI

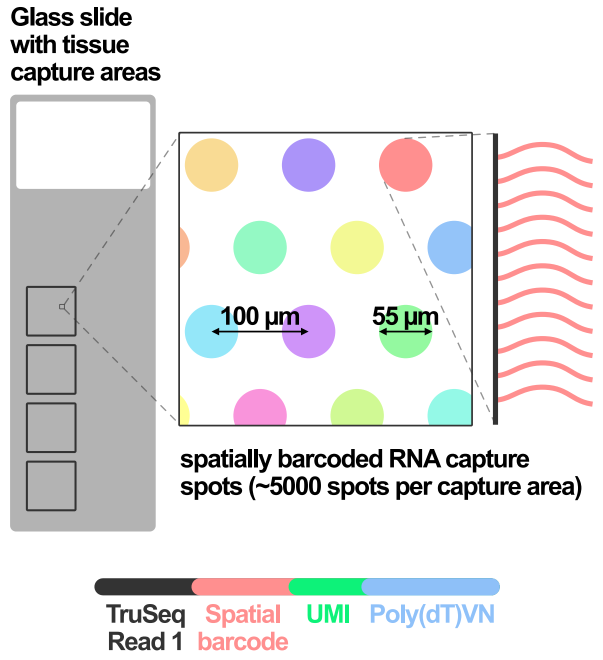


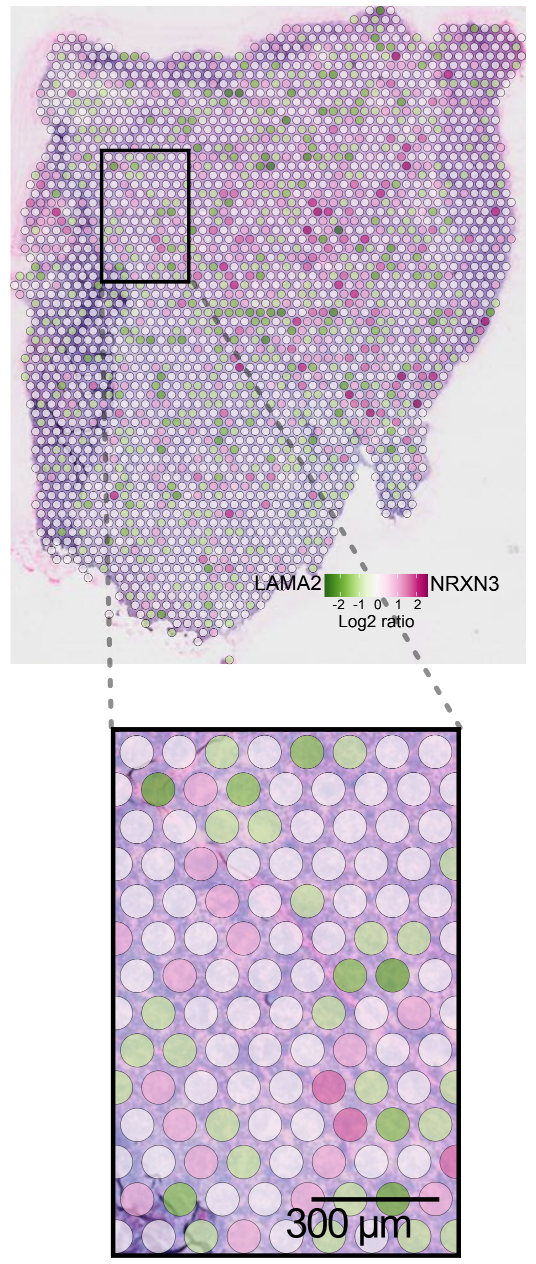
Comparison of different methods for spatial transcriptomics

H&E reference

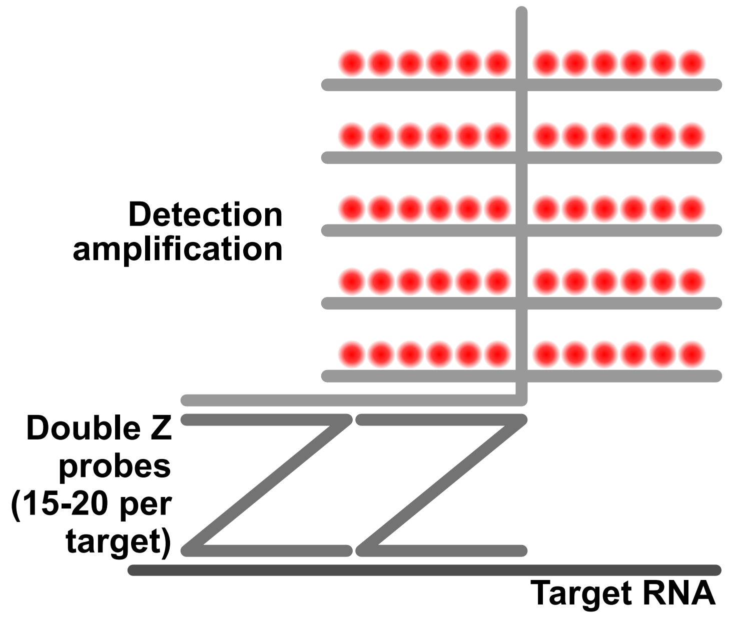


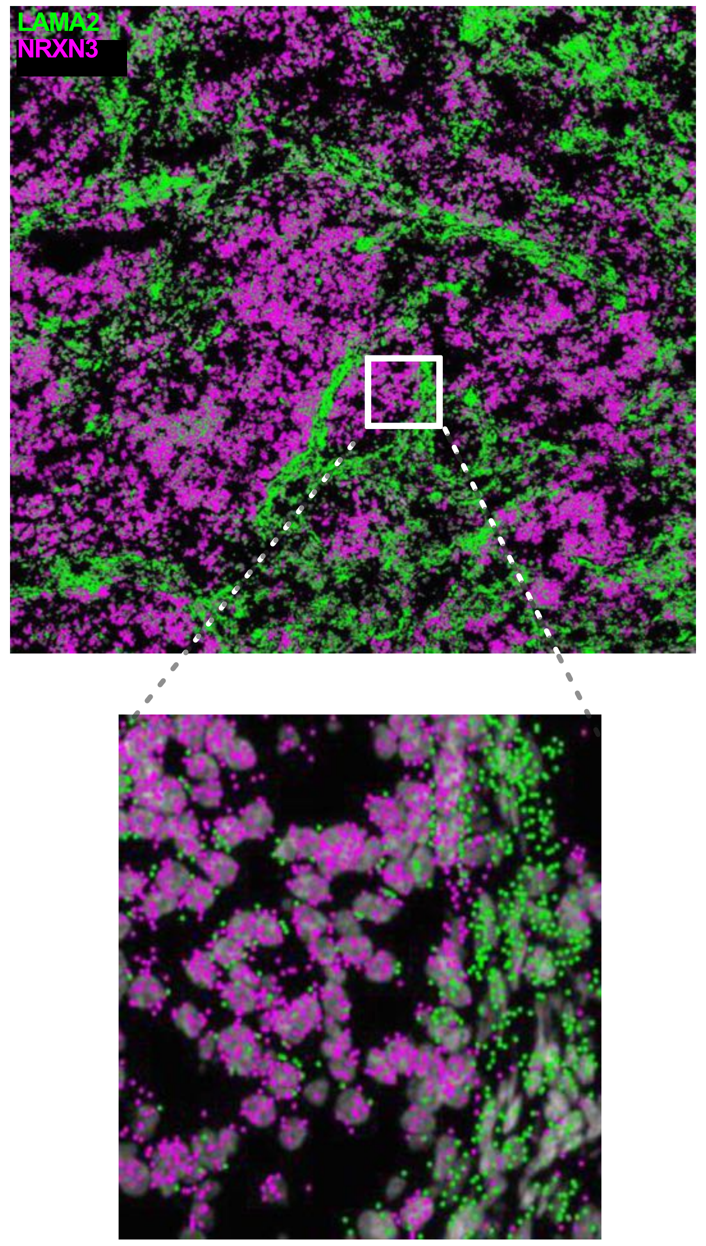
Visium



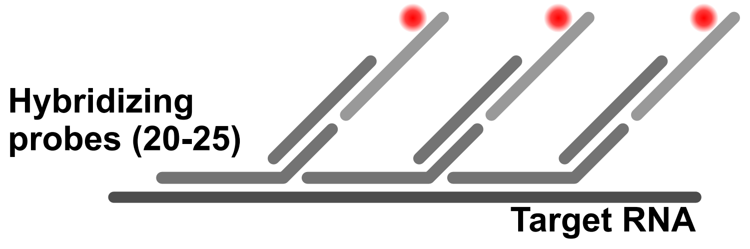


RNAscope HiPlex



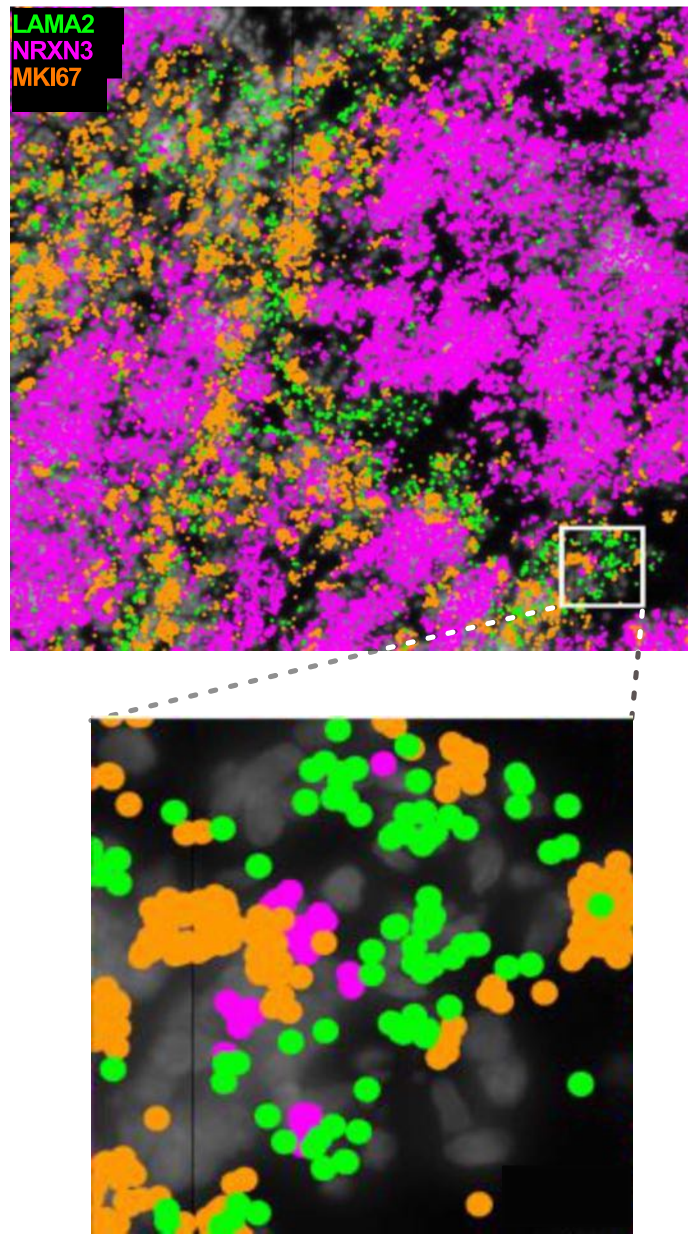


Molecular Cartography

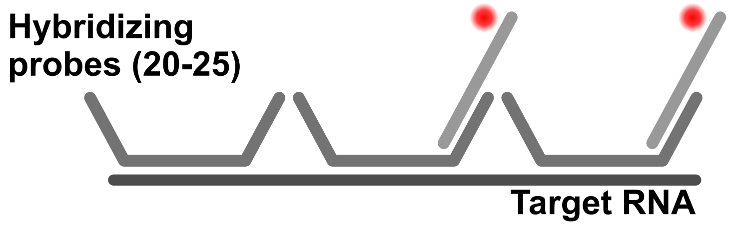


Decoding

	R1	R2	R3	R4	R5	R6	R7	R8
gene X	🔴	🟡		🔴	🔴	🟡	🔴	
gene Y	🔴			🟡	🟡		🔴	🔴
gene Z	🟡	🟡	🔴	🟡		🔴		🟡
...								

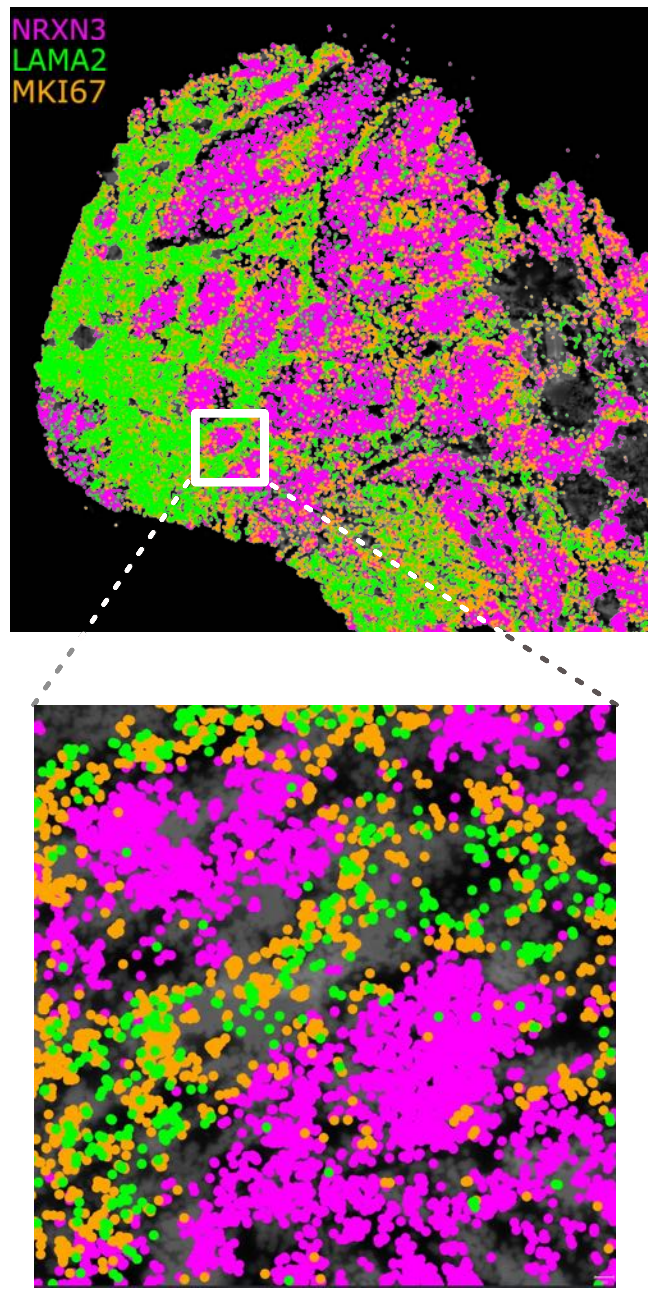


MERFISH (Merscope)



Decoding

	R1	R2	R3	R4	...	...	...	R16
gene X	🔴	🟡						
gene Y				🟤				🔴
gene Z		🟤		🟡				
...								

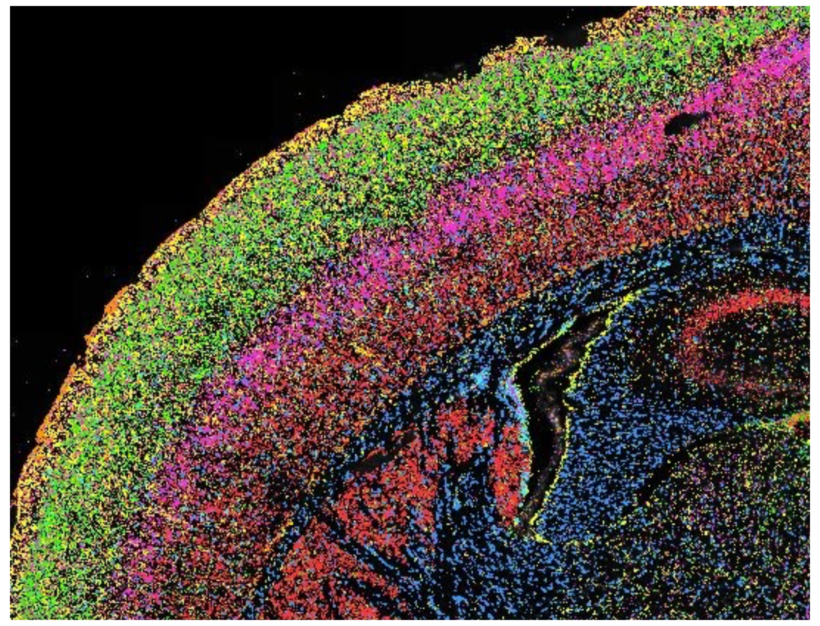


Automated targeted spatial transcriptomics and proteomics

Hybridzation-based transcriptomics

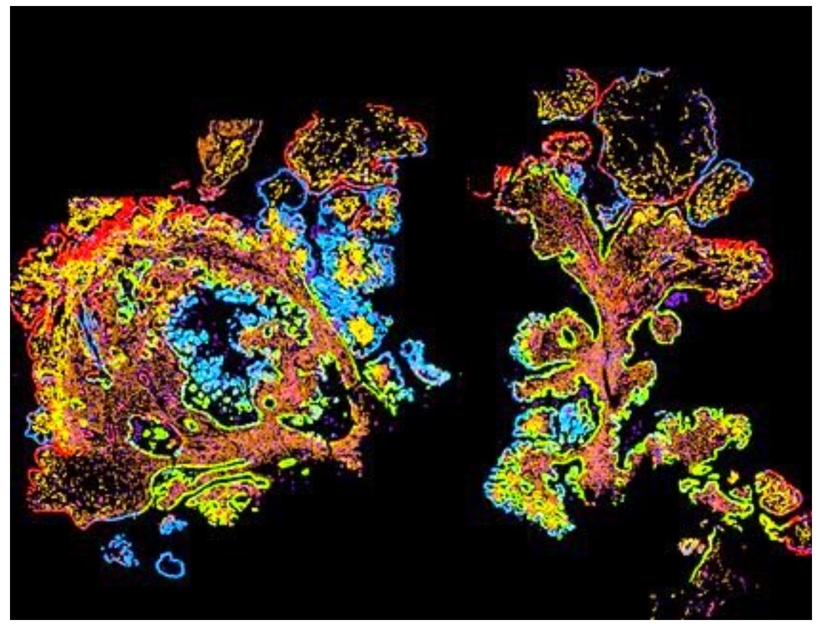
Molecular cartography (Resolve Biosciences)

- smFISH
- 100 targets (200 soon)
- custom gene set
- fresh frozen (FFPE)



Merscope (Vizgen)

- smFISH
- 140, 300, 500 targets
- custom gene set
- tissue clearing
- fresh frozen & FFPE



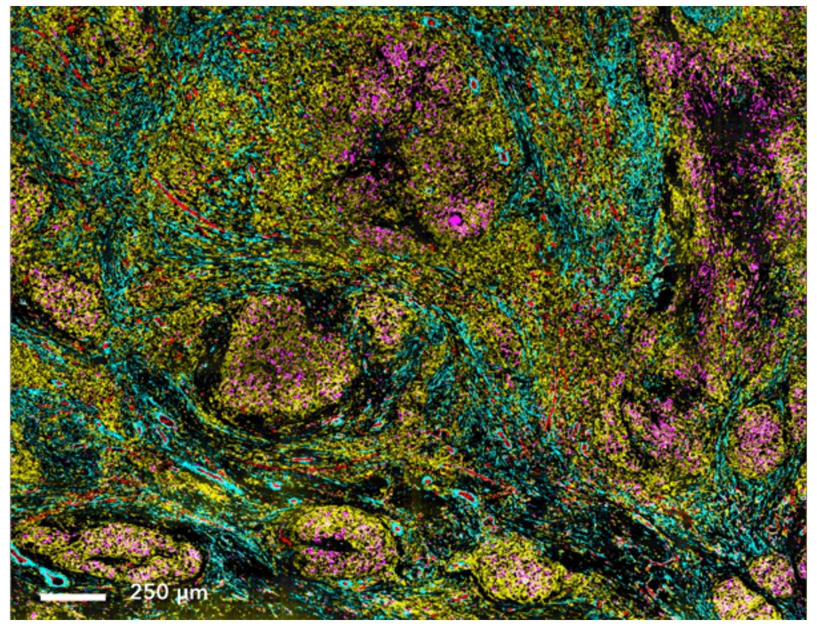
Xenium (10x Genomics)

- RNA “in situ sequencing” (Cartana) technology
- 200-400 targets
- fixed/custom gene set
- FFPE & fresh frozen

Antibody-based proteomics

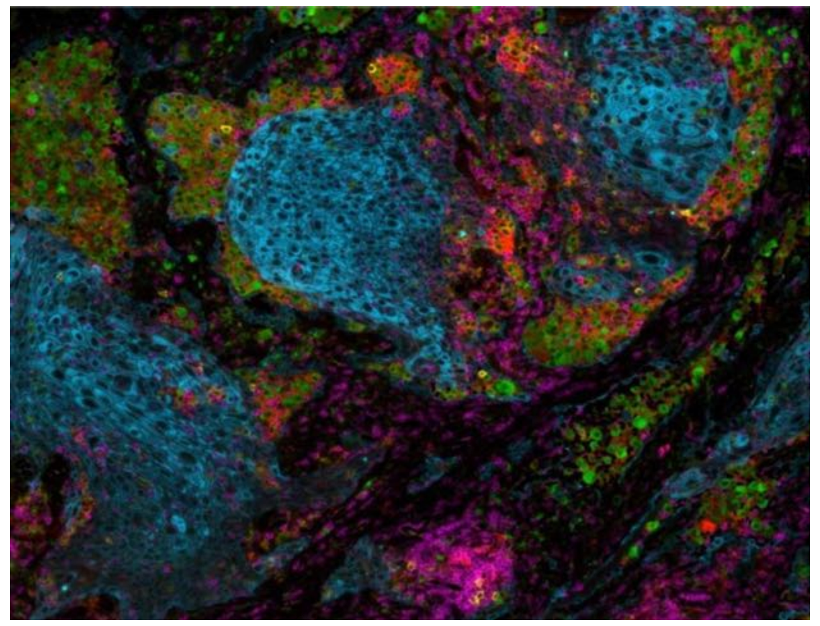
Mibiscope (Ionpath)

- Protein (via mass-spec imaging of Ab)
- ~40 targets
- custom Ab panel
- FFPE & fresh frozen

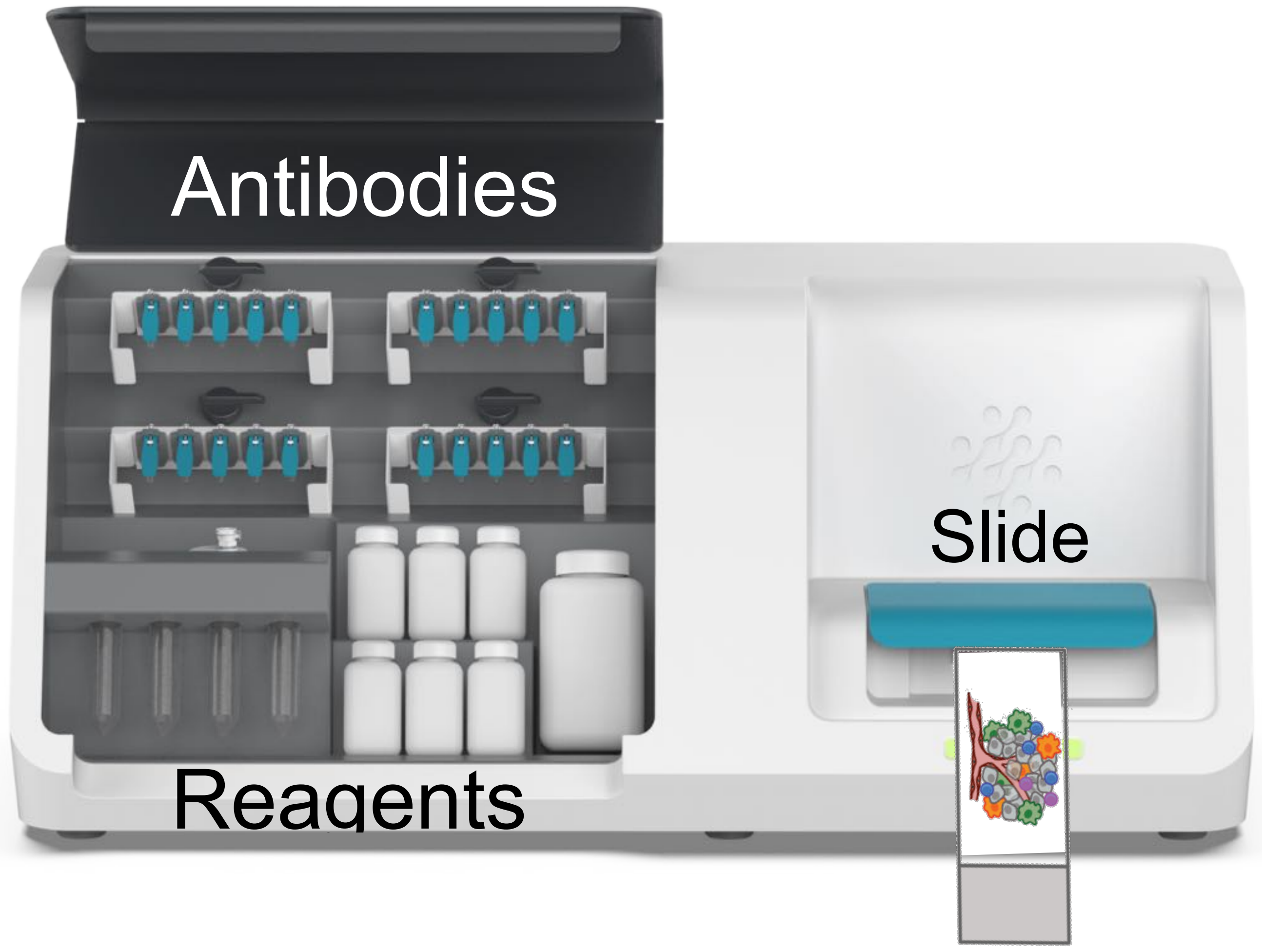


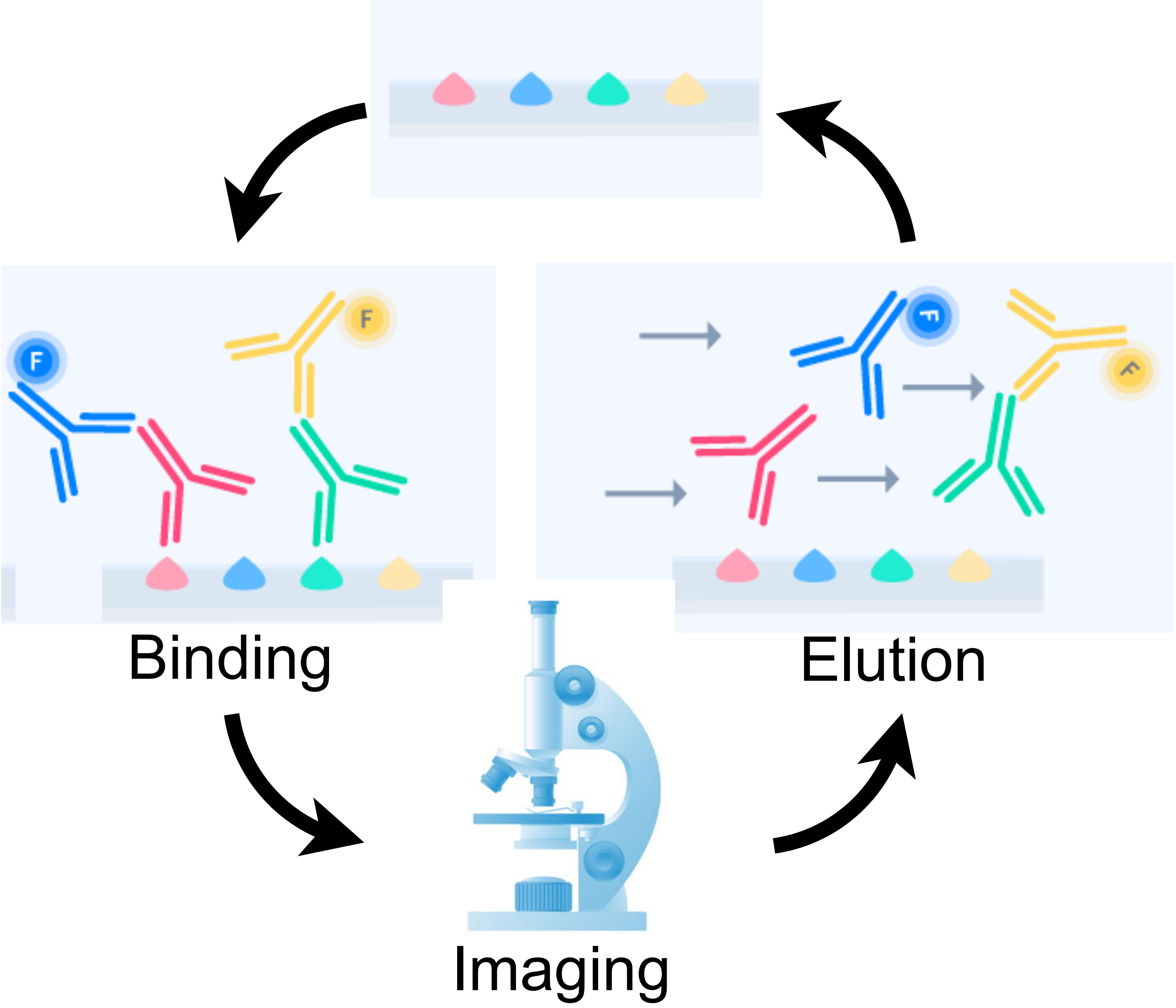
Comet (Lunaphore)

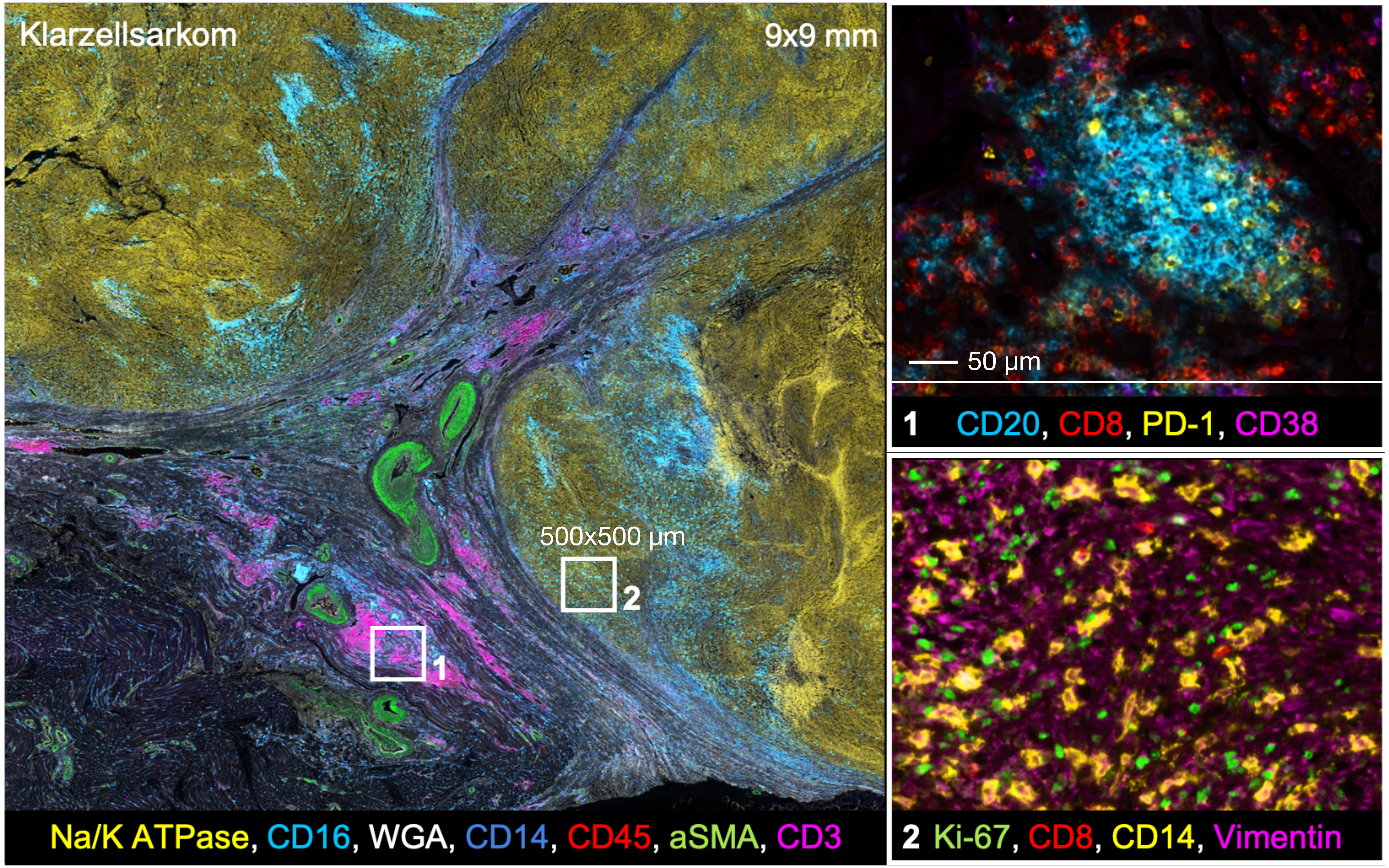
- Protein (via fluorescence imaging of Ab)
- ~40 targets
- custom Ab panel
- FFPE & fresh frozen



Sarcoma immunoenvironment (FFPE) analysis on Lunaphore Comet system



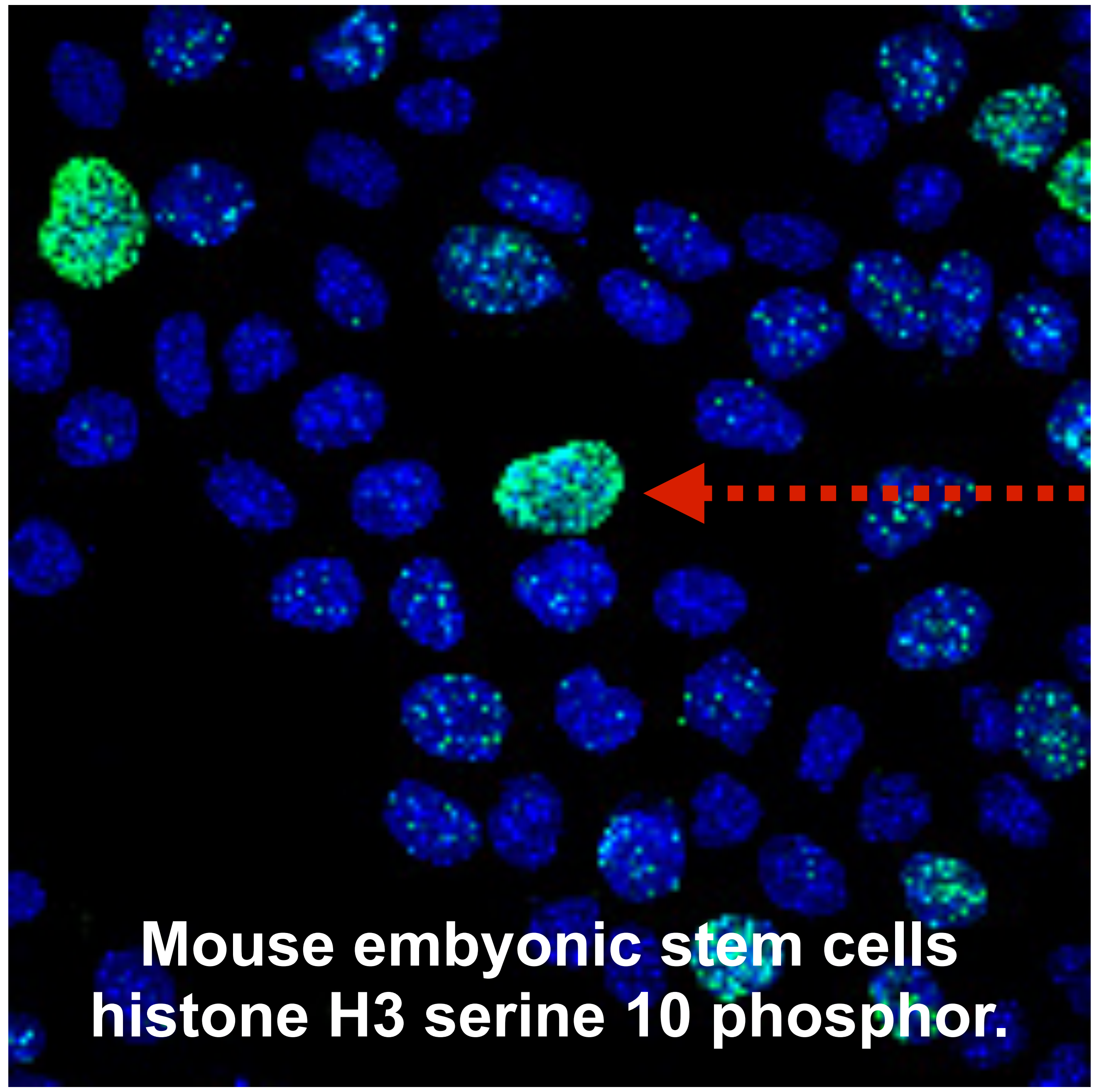




Johanna Wagner (DKFZ & NCT, Fröhling Department, HEROES AYA consortium)

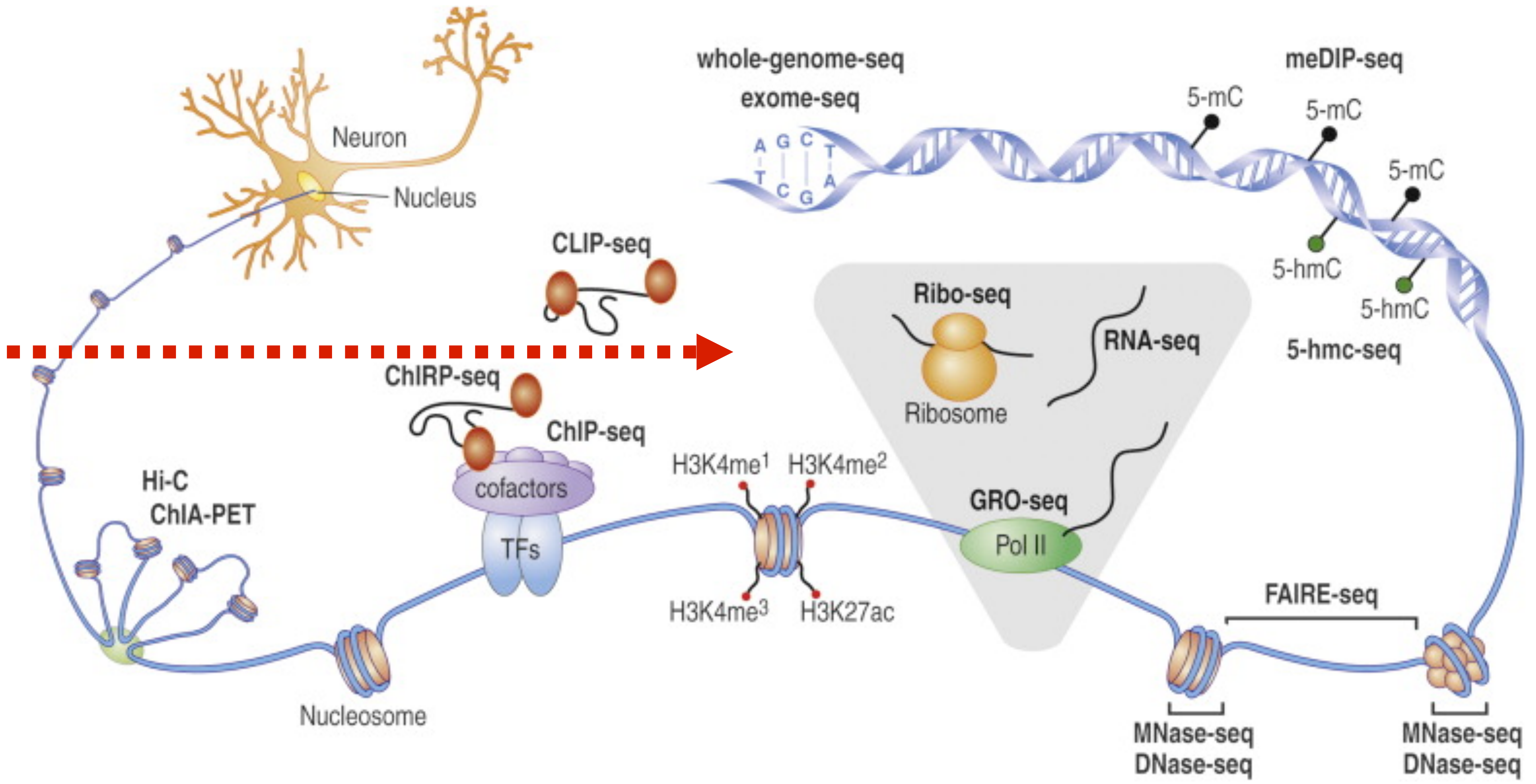
Spatial omics integrates imaging and sequencing based analysis

Fluorescence microscopy

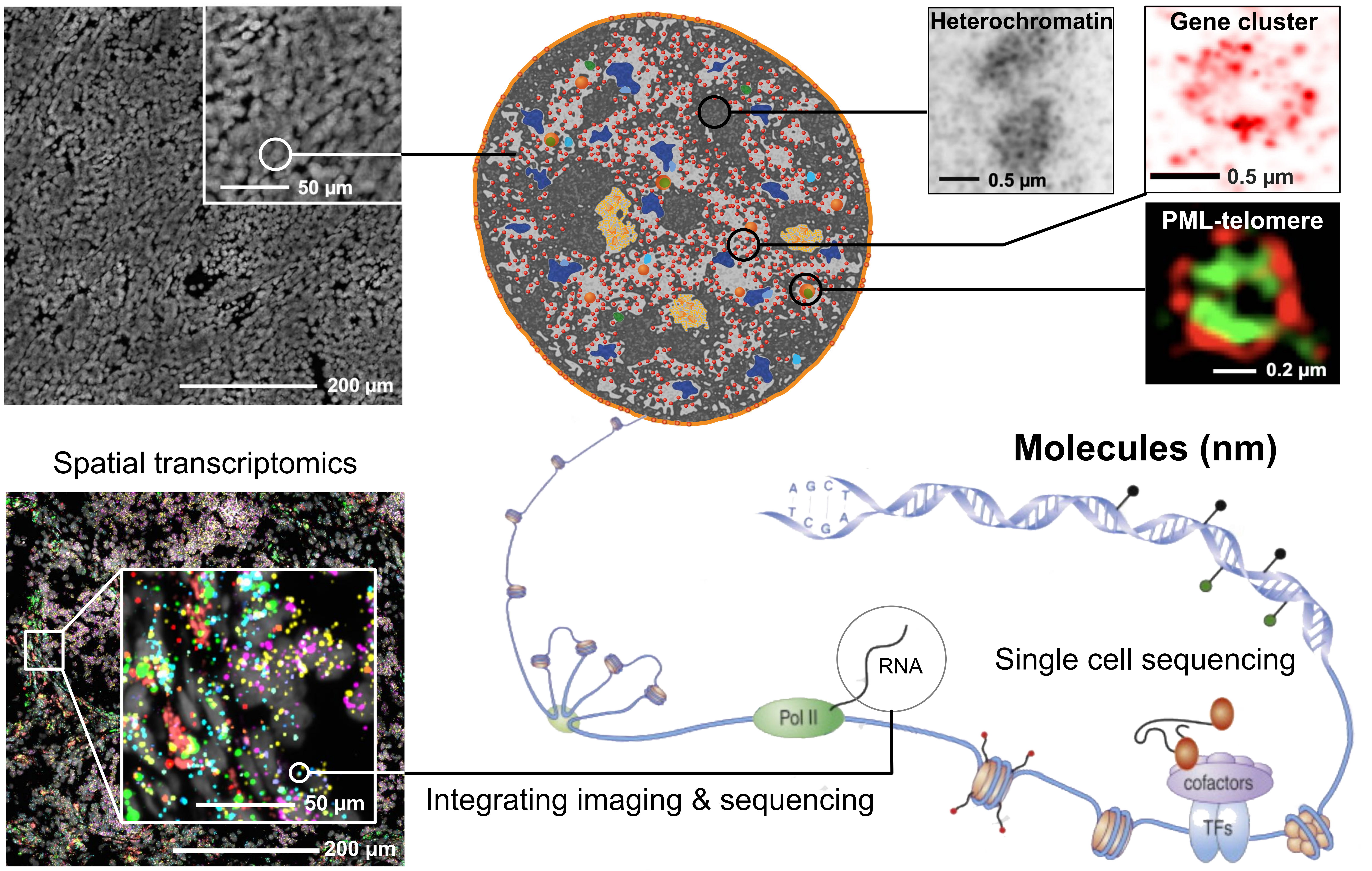


Mouse embyonic stem cells histone H3 serine 10 phosphor.

Sequencing



The future of studying genome structure-function relationships



Tumor tissue (mm)

Fluorescence microscopy DNA

Nucleus (10 µm)

Subcompartments (1 µm)

Super-resolution microscopy

Spatial transcriptomics

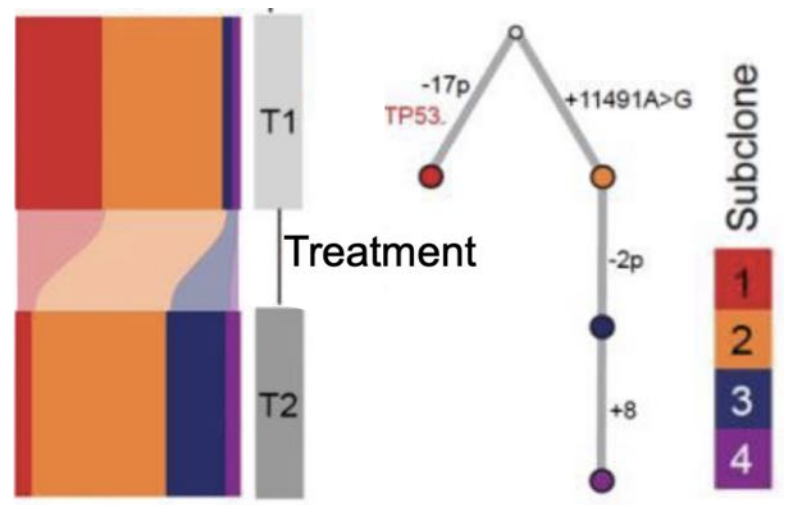
Molecules (nm)

Single cell sequencing

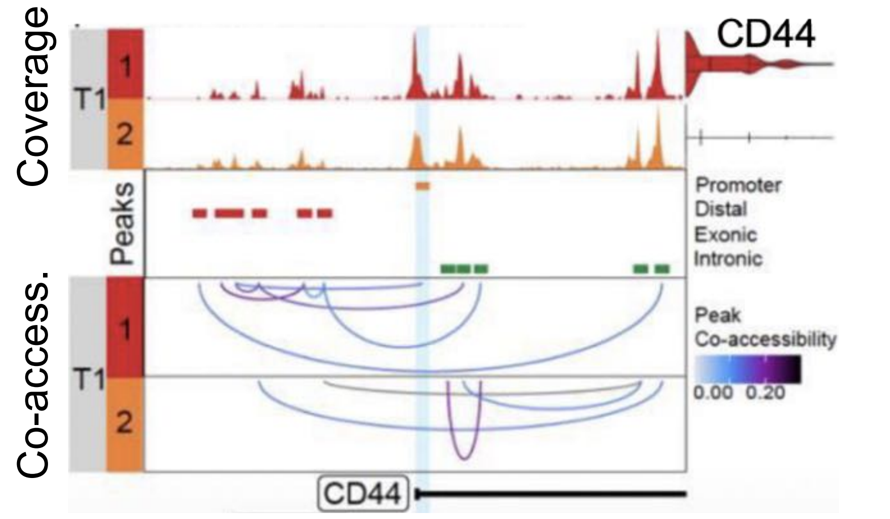
Integrating imaging & sequencing

Tumor heterogeneity

Tumor subclone evolution



Deregulated gene expression



Microenvironment