i-CORE
Israeli Centers of Research Excellence

WORKSHOP

TAU HUJI HH BIU SMC
Gene Regulation in Complex Human Disease

Transcriptional circuitry and the regulatory conformation of the genome

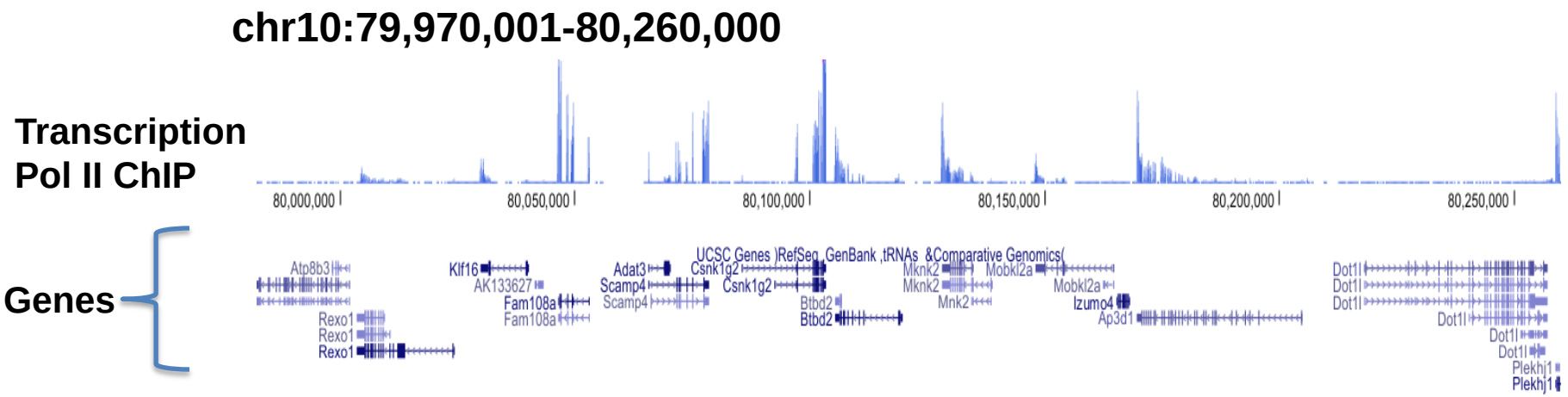
Tradition of Excellence
Bar-Ilan University
אוניברסיטת בר-אילן

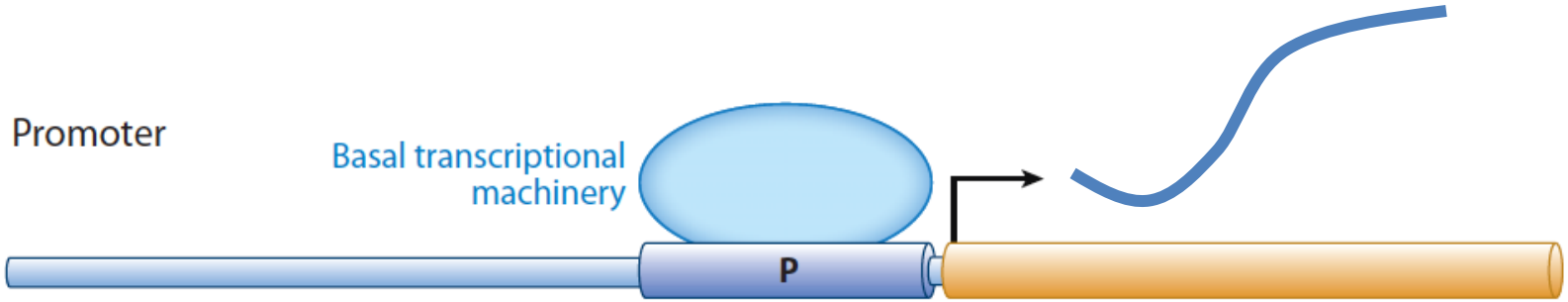
Ofir Hakim

Faculty of Life Sciences

Regulatory Chromatin

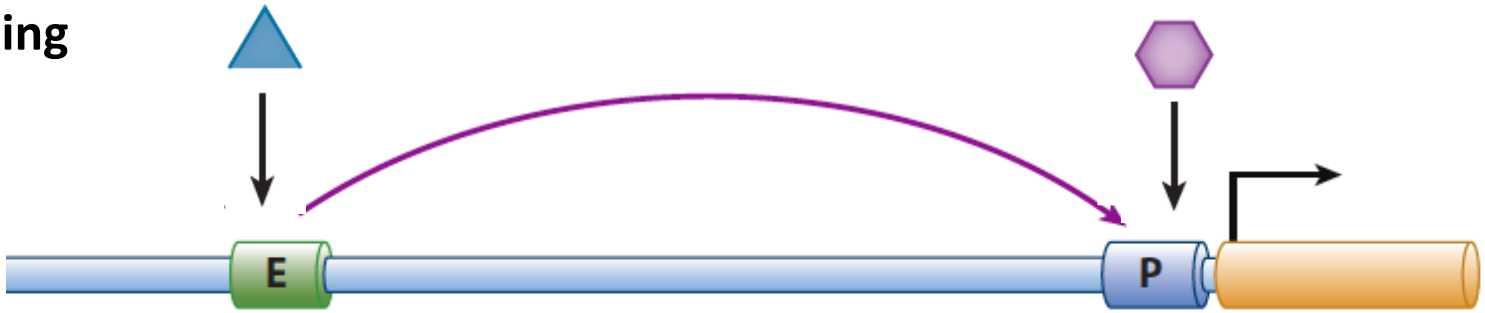
Cell Function is Largely Mediated by Transcription



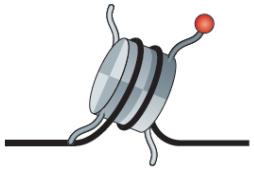


Layers of Genome Regulation

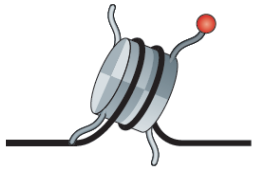
1. TF binding



2. Histone modification



- **H3K27ac**



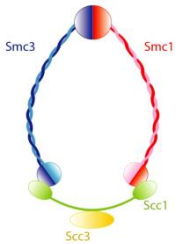
- **H3K4me3**

3. Chromatin modifiers & coactivators

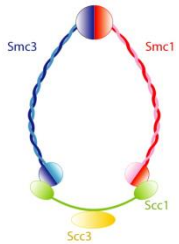
p300

N-CoR

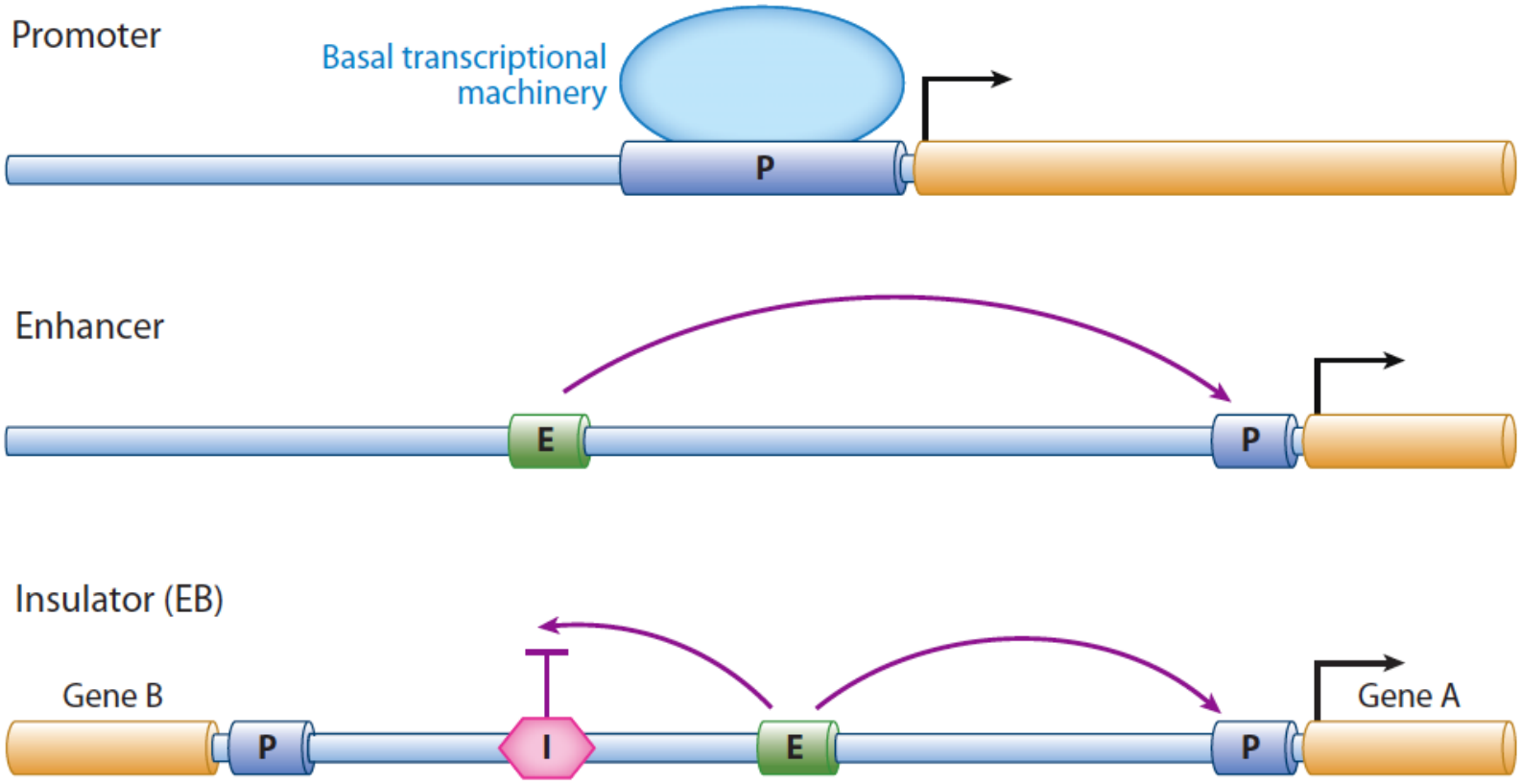
4. DNA looping factors



cohesin

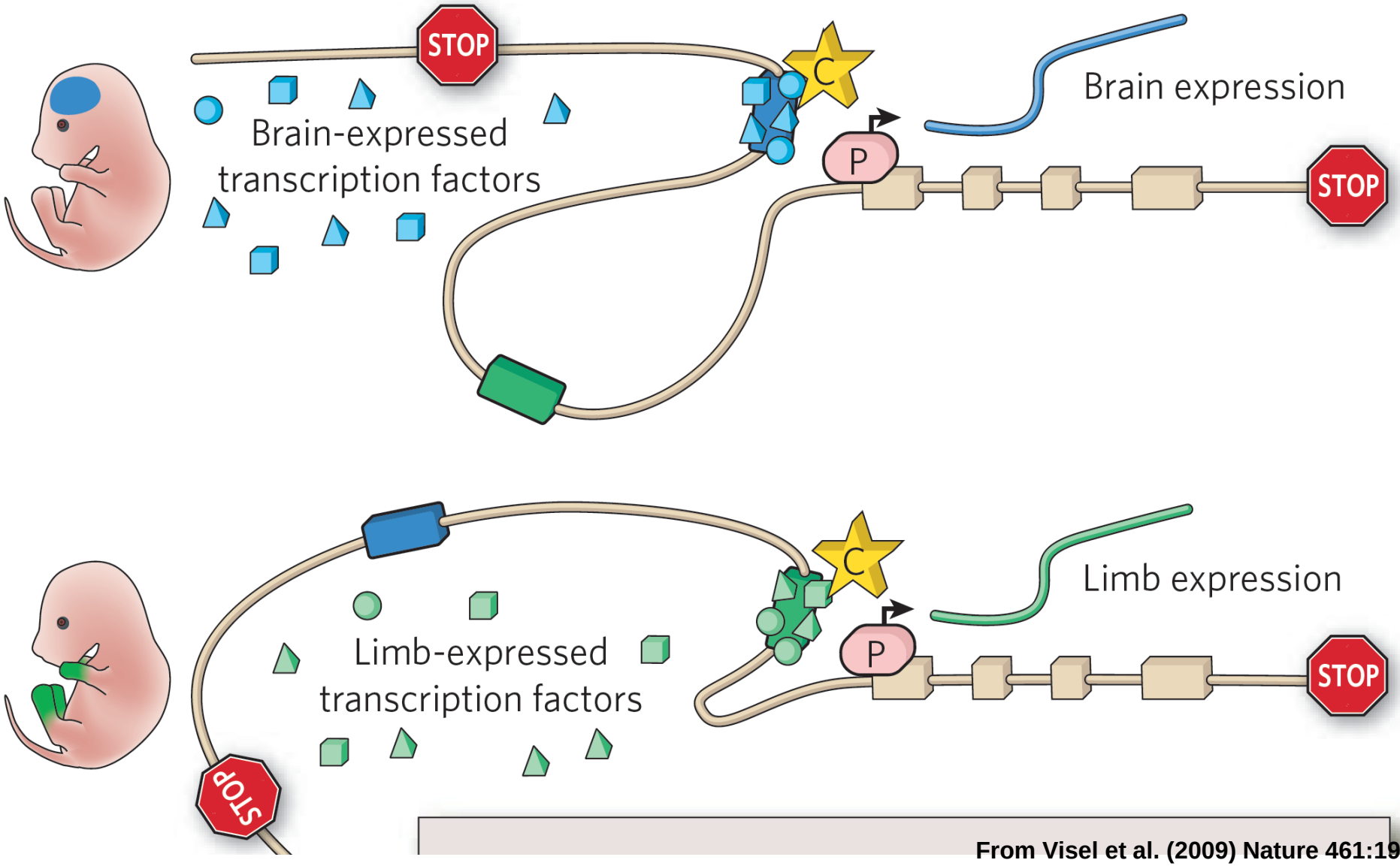


Genome Regulation in 3D



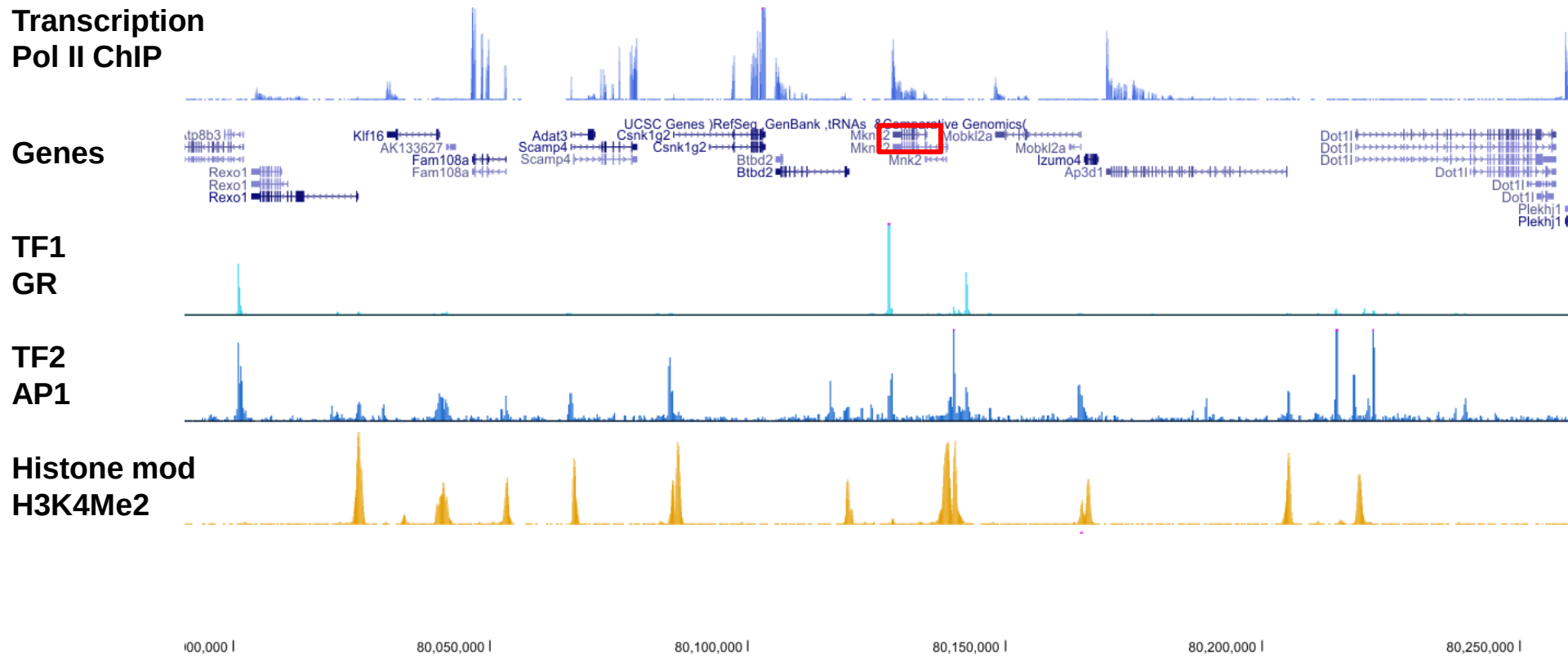
Noonan and McCallion, *Ann Rev Genomics Hum Genet* 11:1 (2010)

Cell-Type Specificity



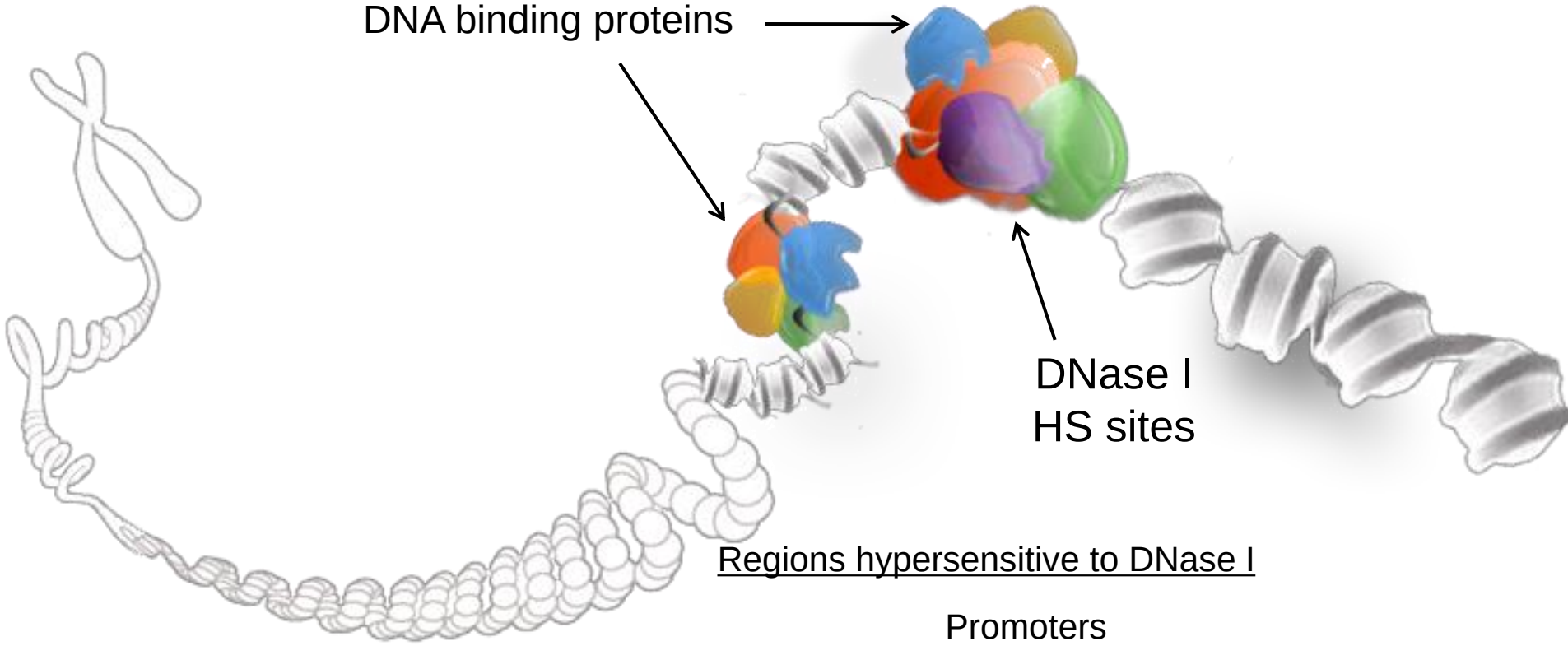
From Visel et al. (2009) Nature 461:199

The Complexity of Genome Regulation



chr10:79,970,001-80,260,000

Regulatory Elements



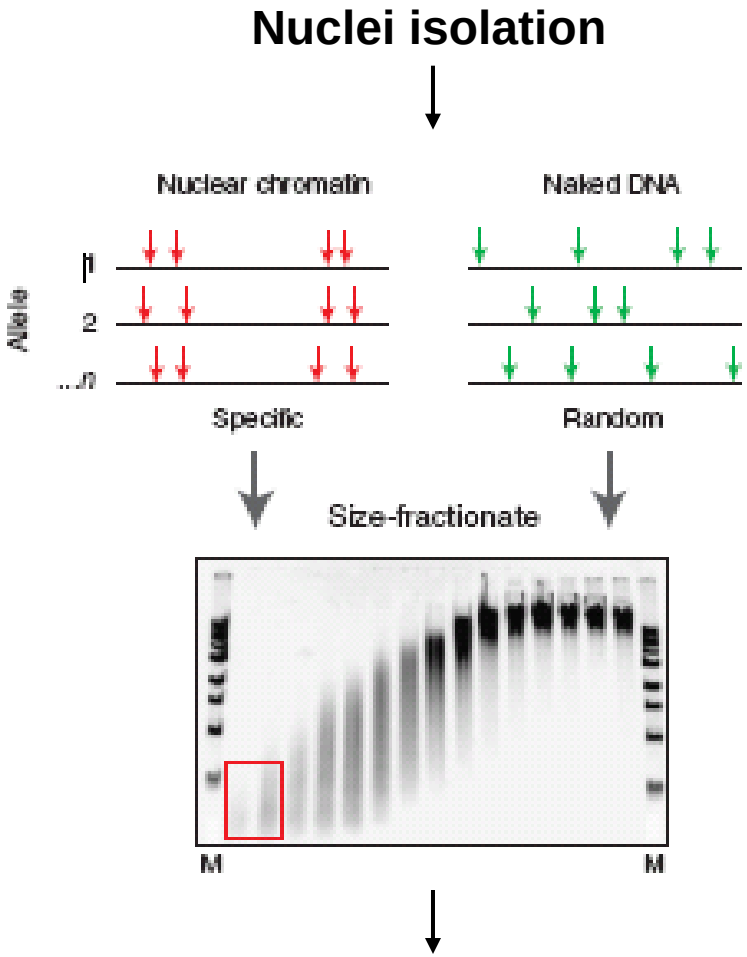
Identify “open” regions of Chromatin

Regions hypersensitive to DNase I

Promoters
Enhancers
Silencers
Insulators
Locus control regions

Image modified from: Greg Crawford, Cold Spring Harb. Protoc., 2010.

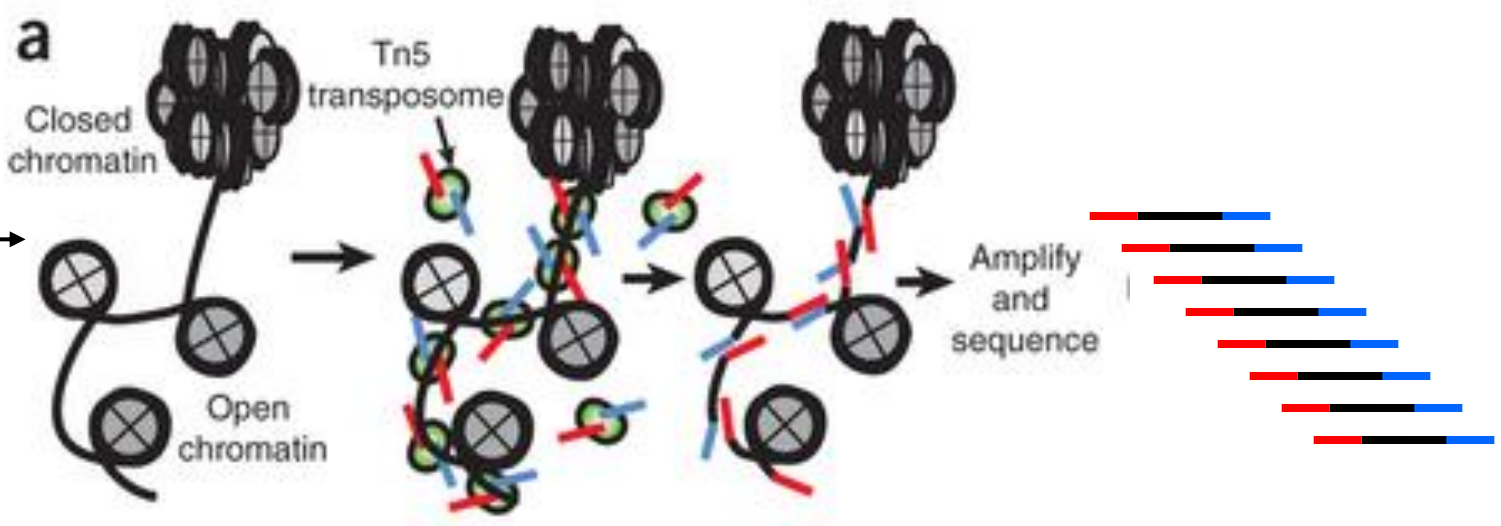
A Strategy To Enrich For DHS



Sabo et al., Nature Methods, 2006

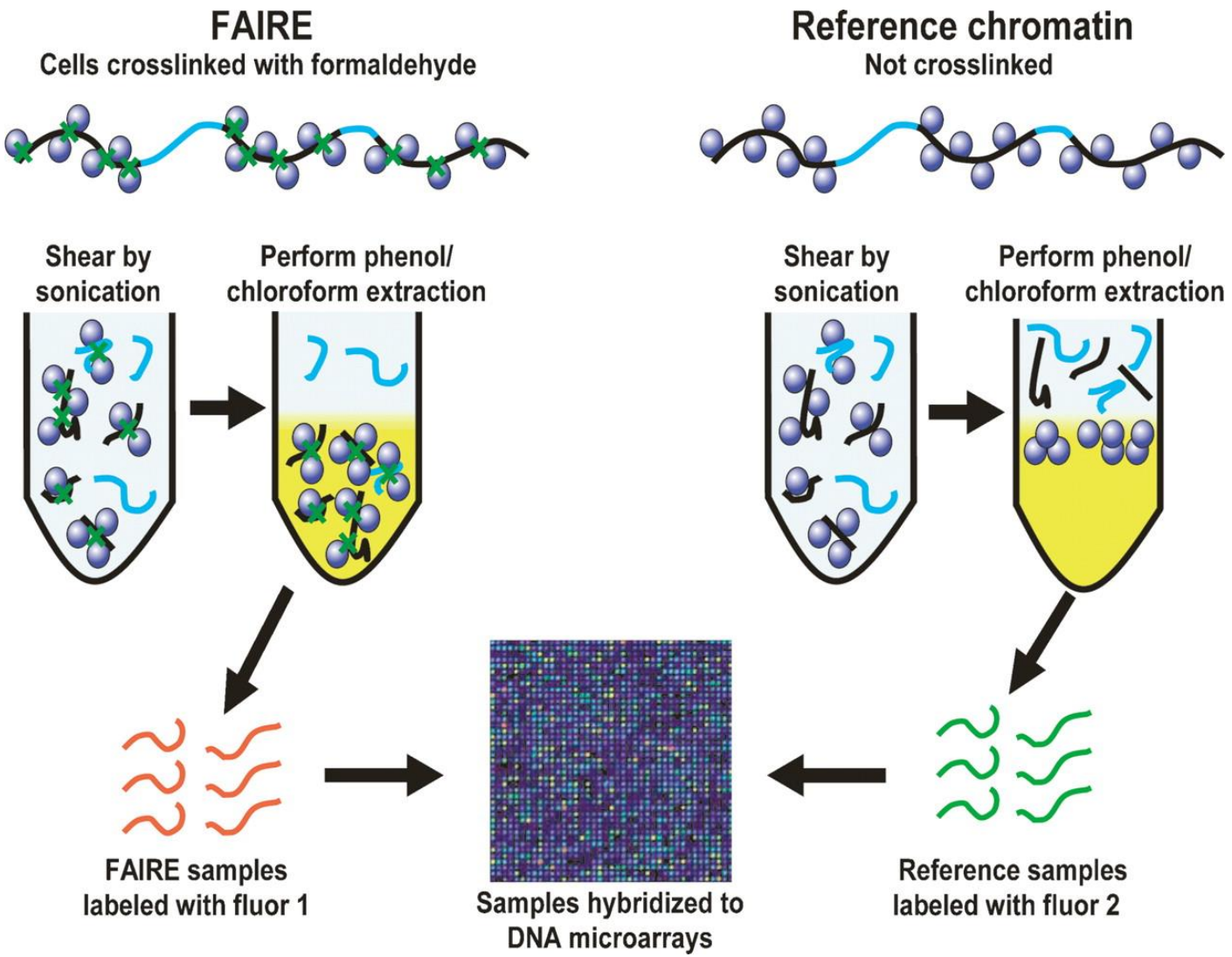
ATAC-seq
Assay of Transposase Accessible Chromatin

Nuclei isolation



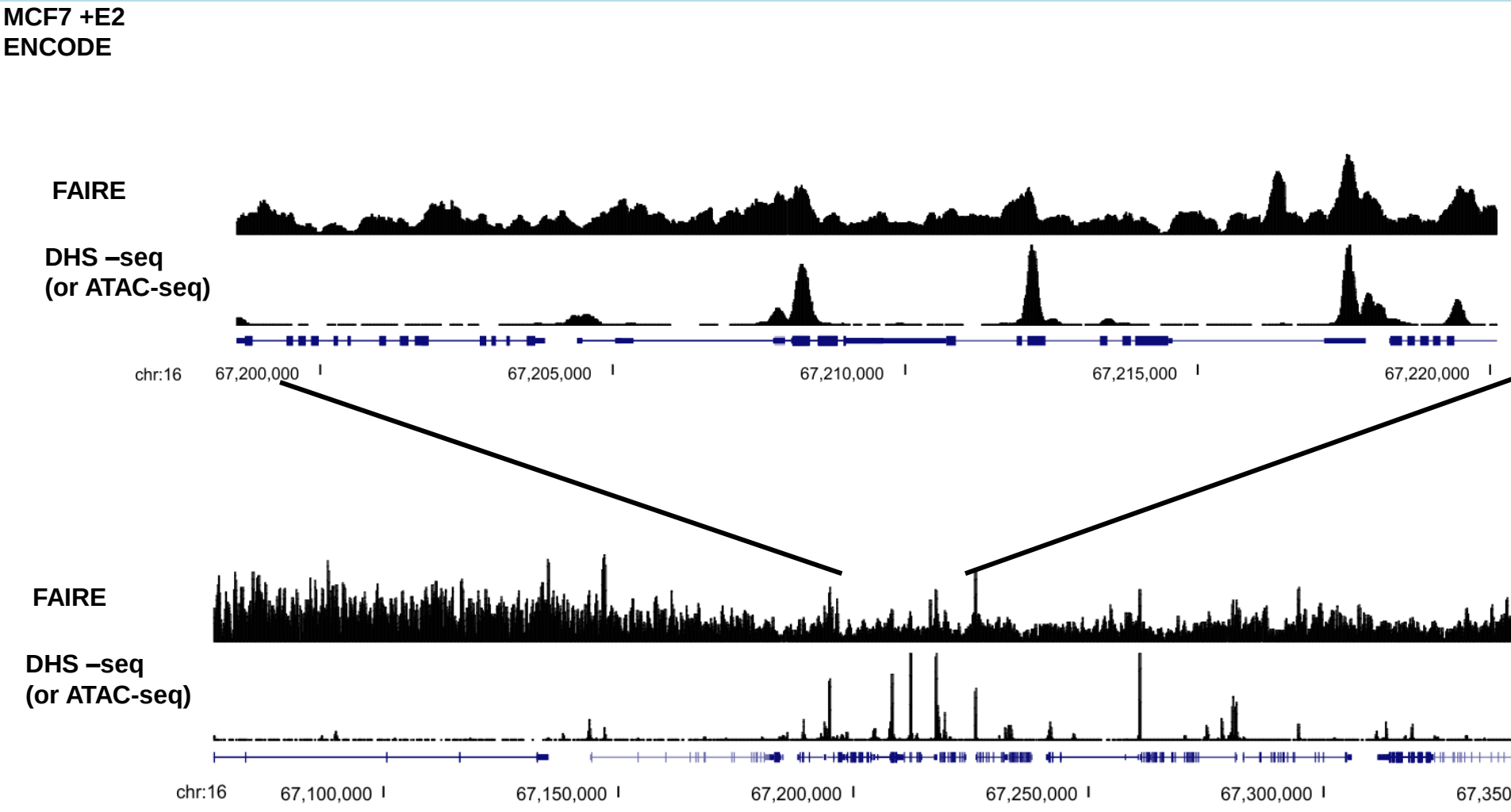
Nature Methods 10, 1213–1218 (2013)

FAIRE-seq
Formaldehyde-Assisted Isolation of Regulatory Elements



Genome Res. Jun 2007; 17(6): 877–885

Resolution and Background



Pros and Cons

Common advantages

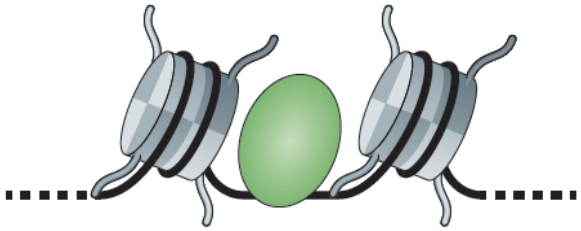
Unbiased
Quantitative
Not require special reagents such as antibodies
Can be applied to any organism and tissue

	DNase-seq	**ATAC-seq**	**FAIRE-seq**
# of cells	10^7-10^8	10^3-10^4	10^6-10^7
Sample pre treatment	Pure nuclei	Pure nuclei	Fixed sample
Experiment time	3-4 days	1 day	3-4 days
Peak resolution	High	High	Low
Motif enrichment and footprint	+	+	-
Required user proficiency and skill	+++	+	+

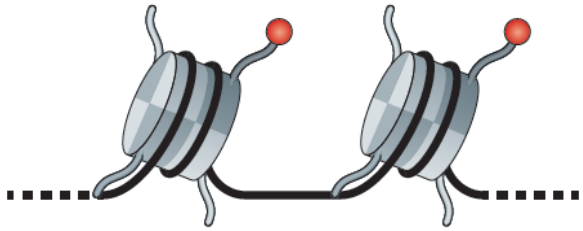
Chromatin Immuno Precipitation - ChIP

Detection of protein-DNA associations *in vivo*

TFs

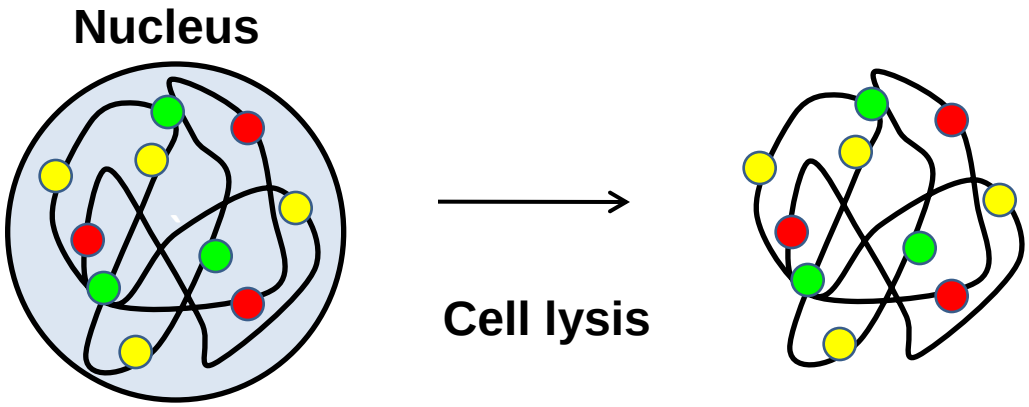


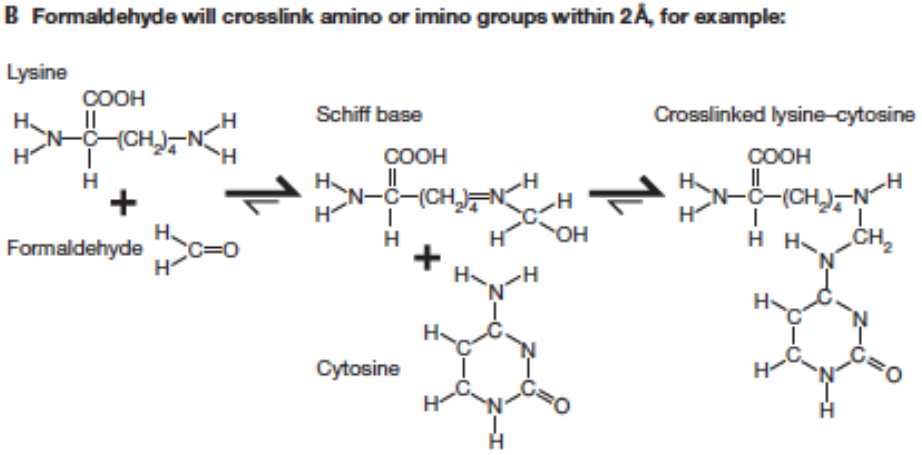
Histone mods



Furey (2012) Nat Rev Genet 13:840

1. Crosslinking With Formaldehyde

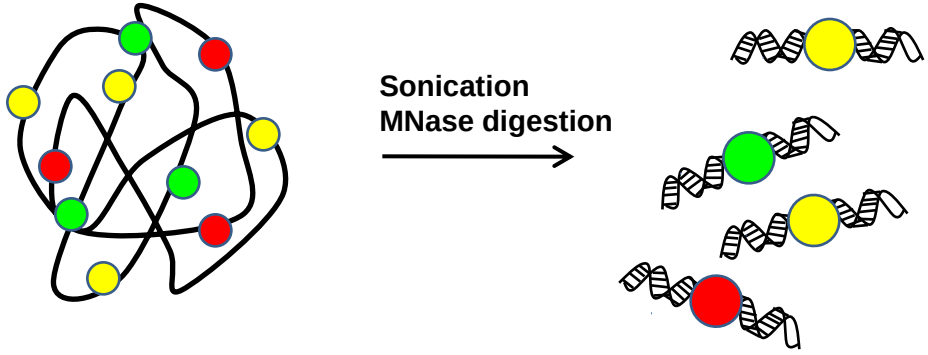




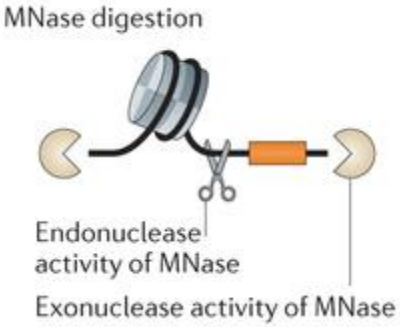
Optimization:

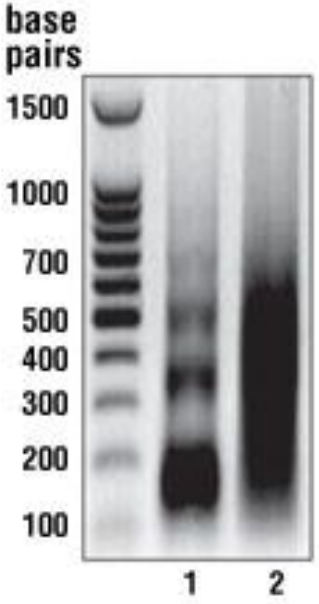
FA%- commonly 1%
Time- commonly 10 minutes
Temperature- commonly 37° C

2. Fragmentation

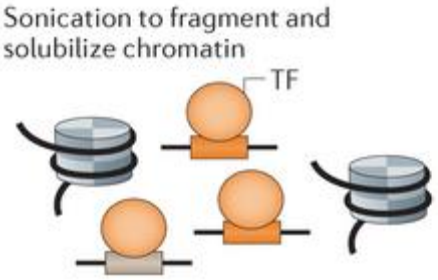


Mnase digestion





Sonication

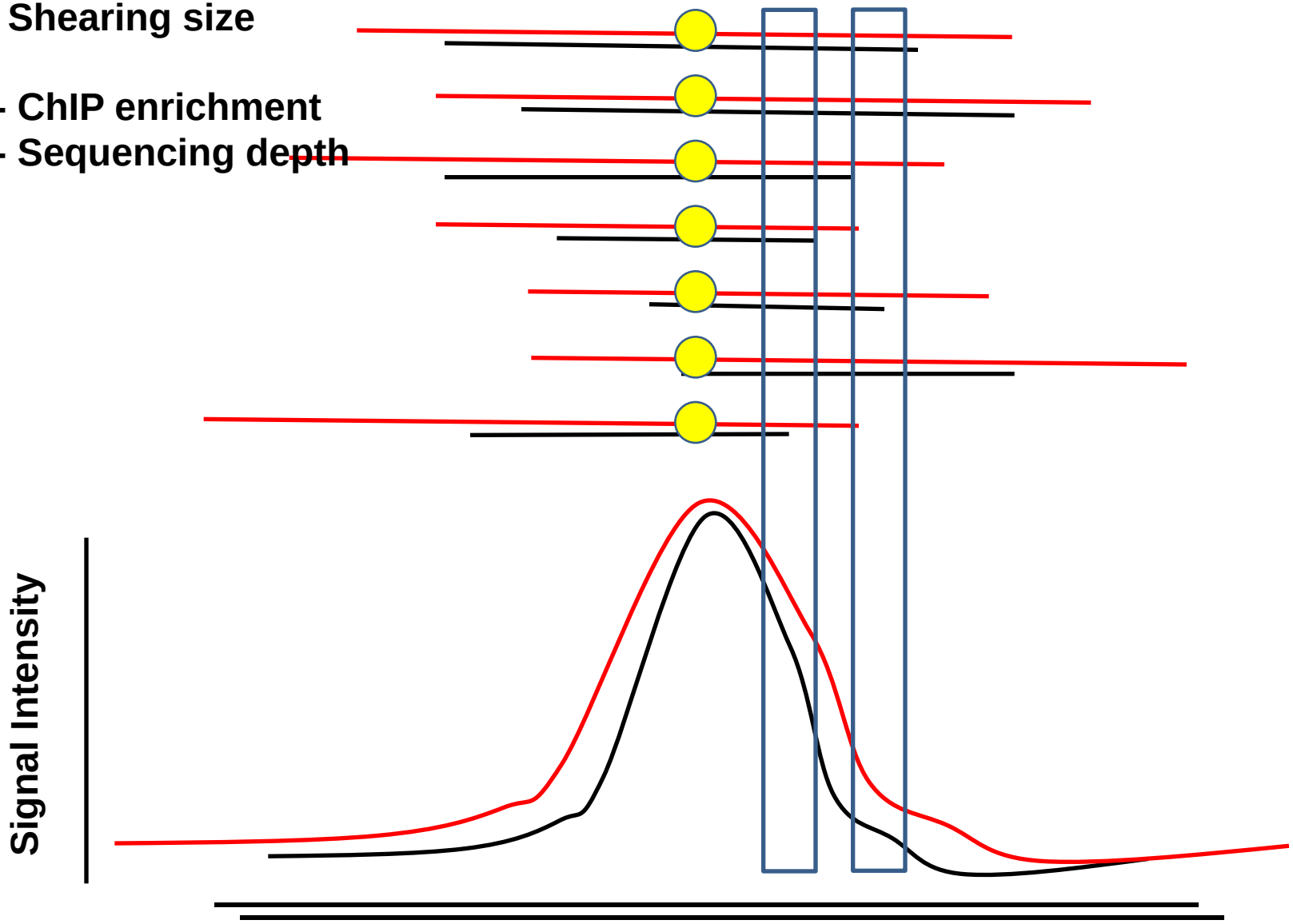


http://www.cellsignal.com/
Nature Reviews Genetics 15, 814–827 (2014)

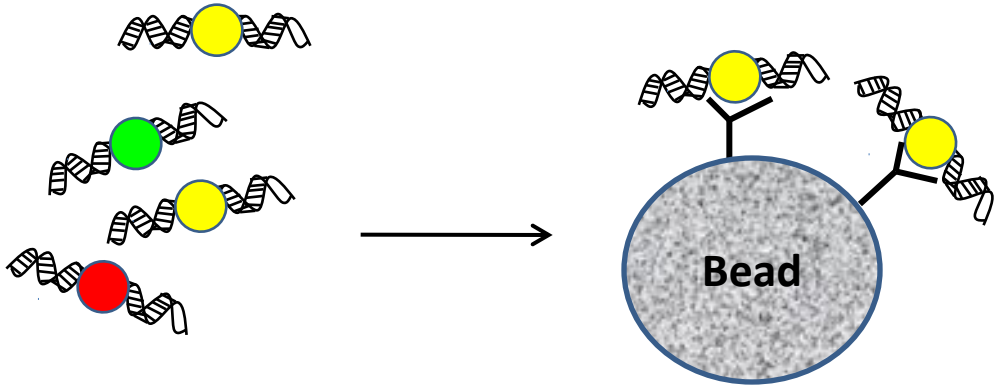
Fragmentation and Peak Resolution

- Shearing size

- ChIP enrichment
- Sequencing depth



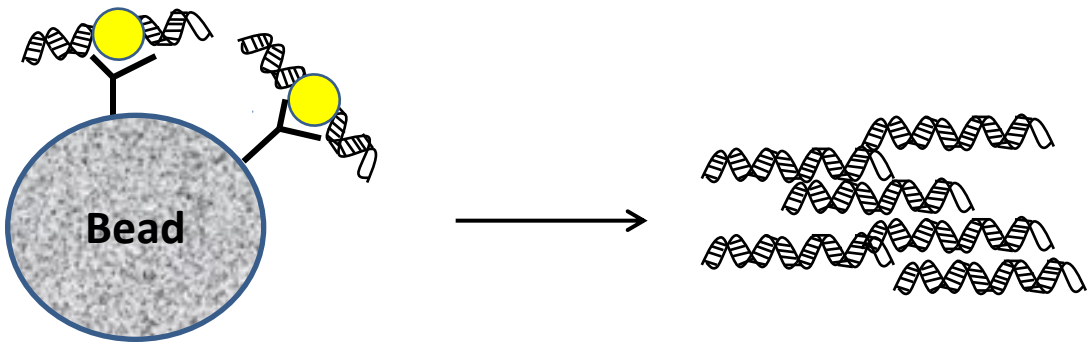
3. Immunoprecipitation (IP)



The protein of interest is immunoprecipitated together with the crosslinked DNA

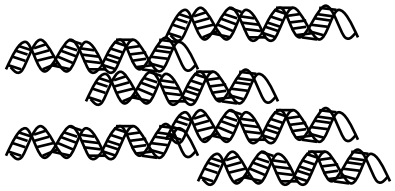
- **Specific antibody**
- **Epitope tagging of protein of interest (HA, myc, Flag, His)**

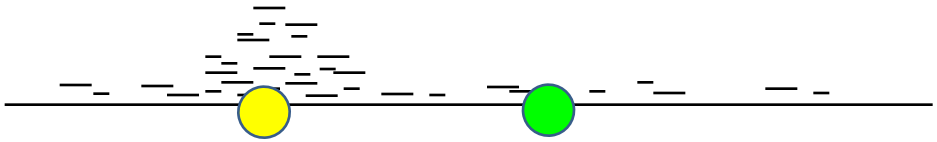
4. Decrosslinking and DNA purification of the DNA



5. Analysis

Representation of enrichment by ChIP

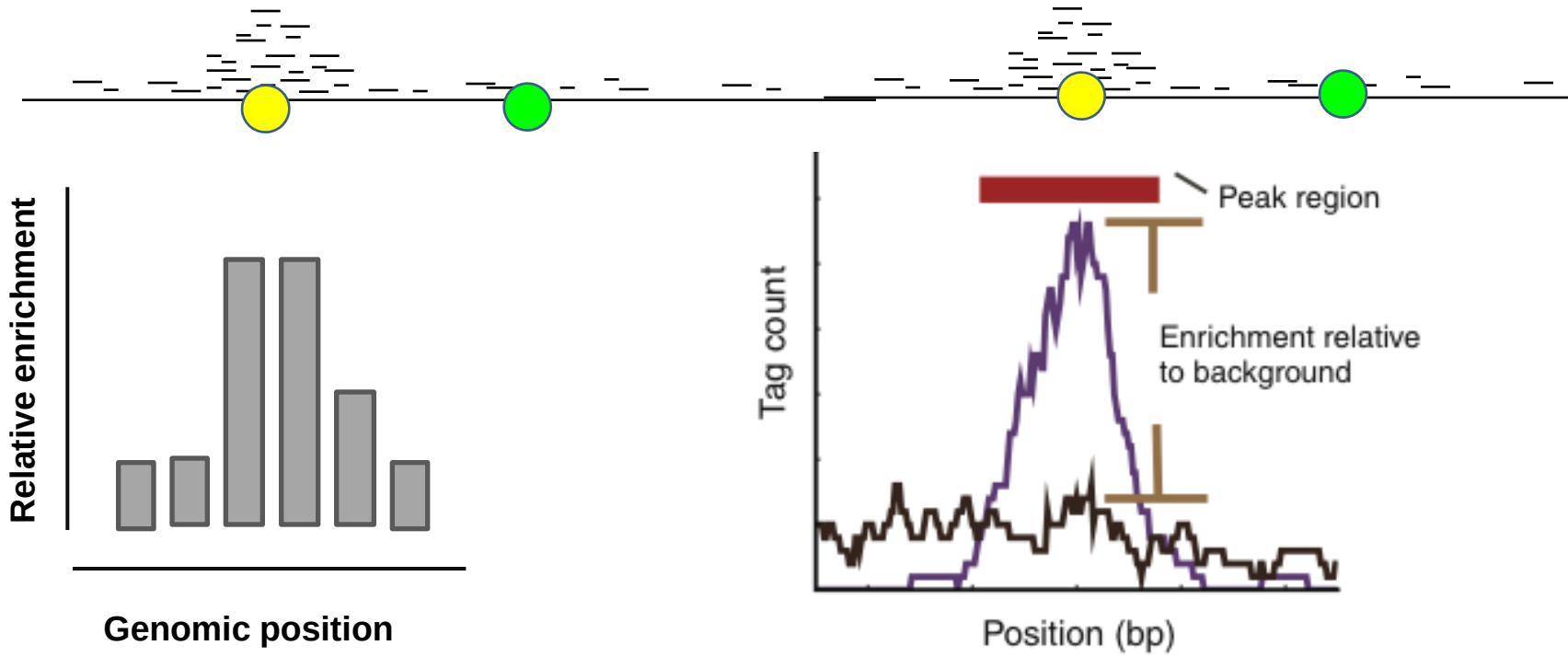




Genomic position

5. Analysis

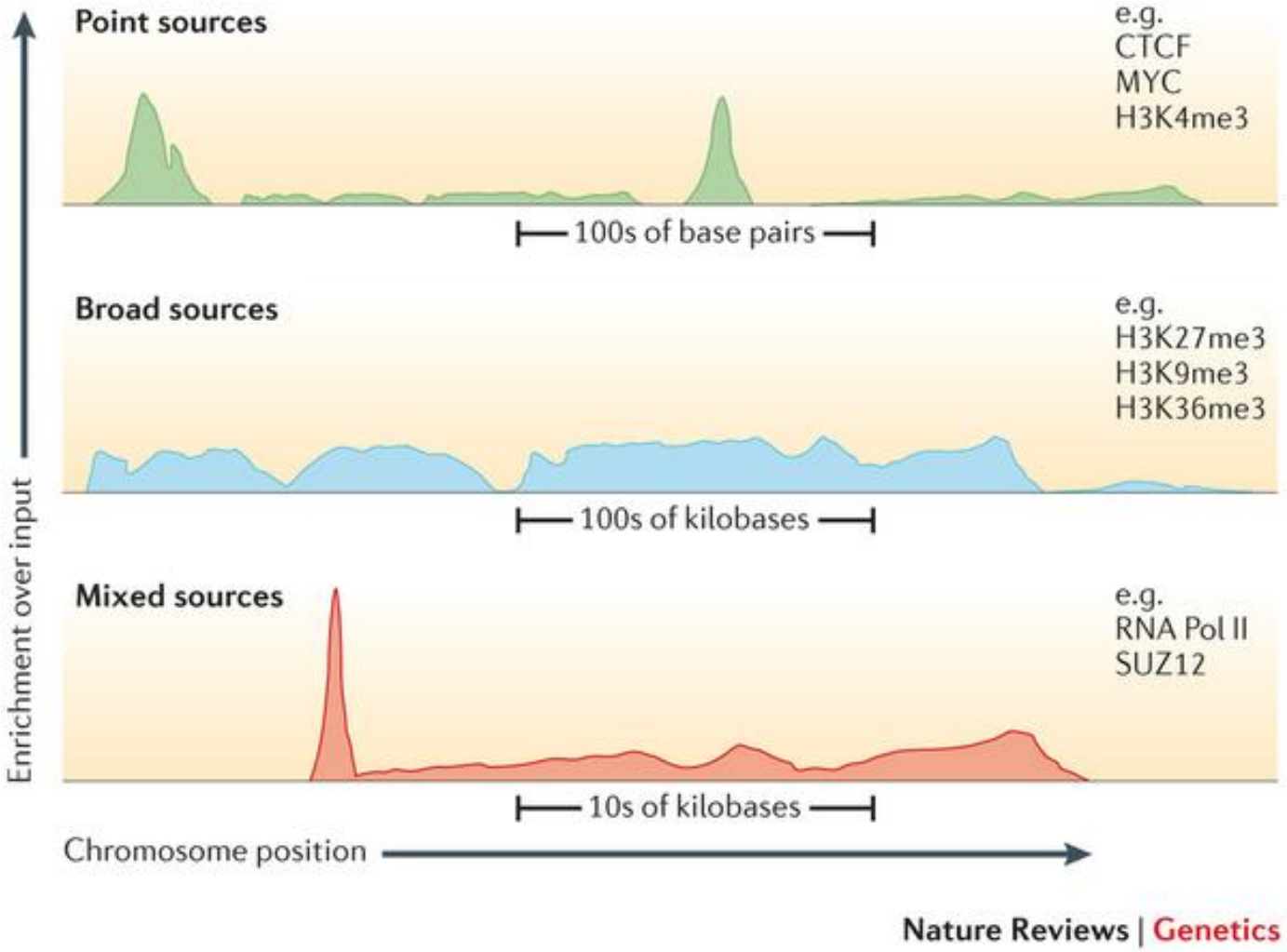
Identification of DNA regions associated with the protein/modification of interest



Real-time PCR

Sequencing

ChIP-seq Peak profiles are variable



Nature Reviews Genetics 15, 121–132 (2014)

Controls

- **Input DNA**

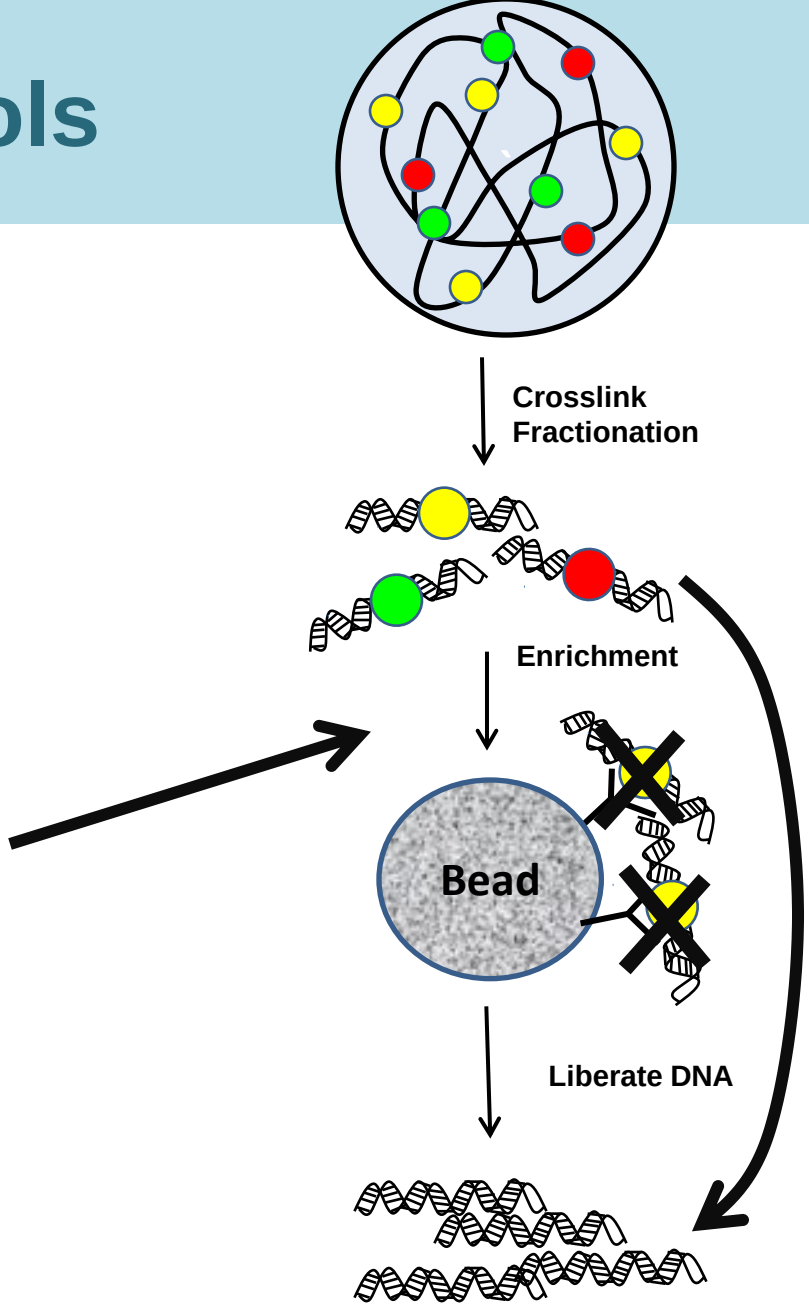
Chromatin sample processed without the immunoprecipitation step

- **No antibody control (IgG)**

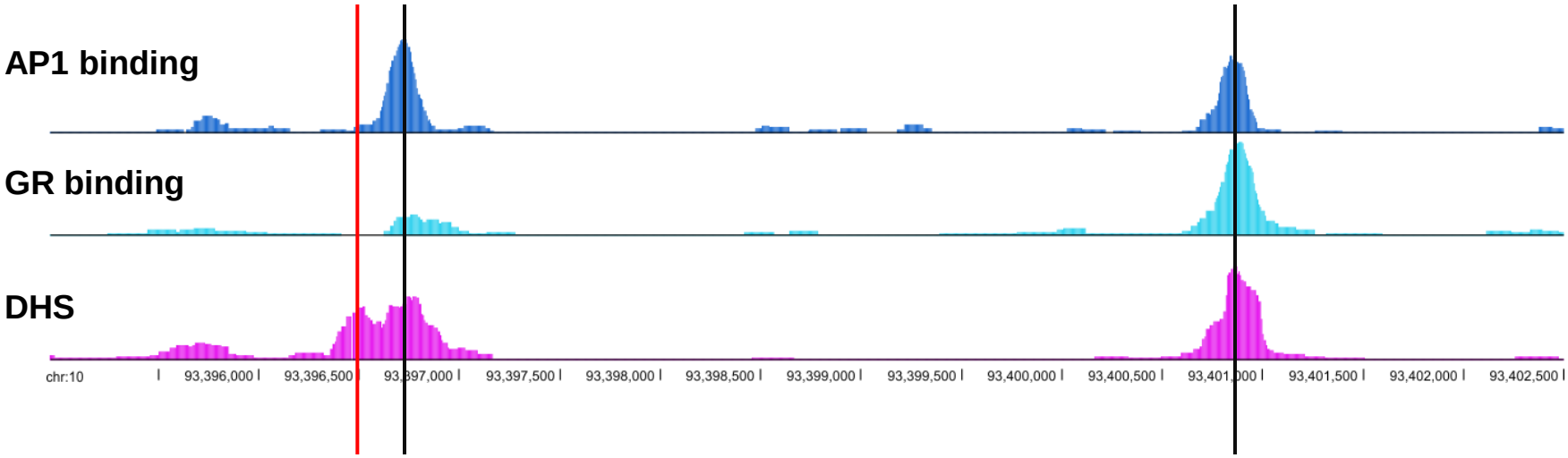
ChIP without specific antibody

- **No tag control**

ChIP in a cell not having a tag on the analyzed protein



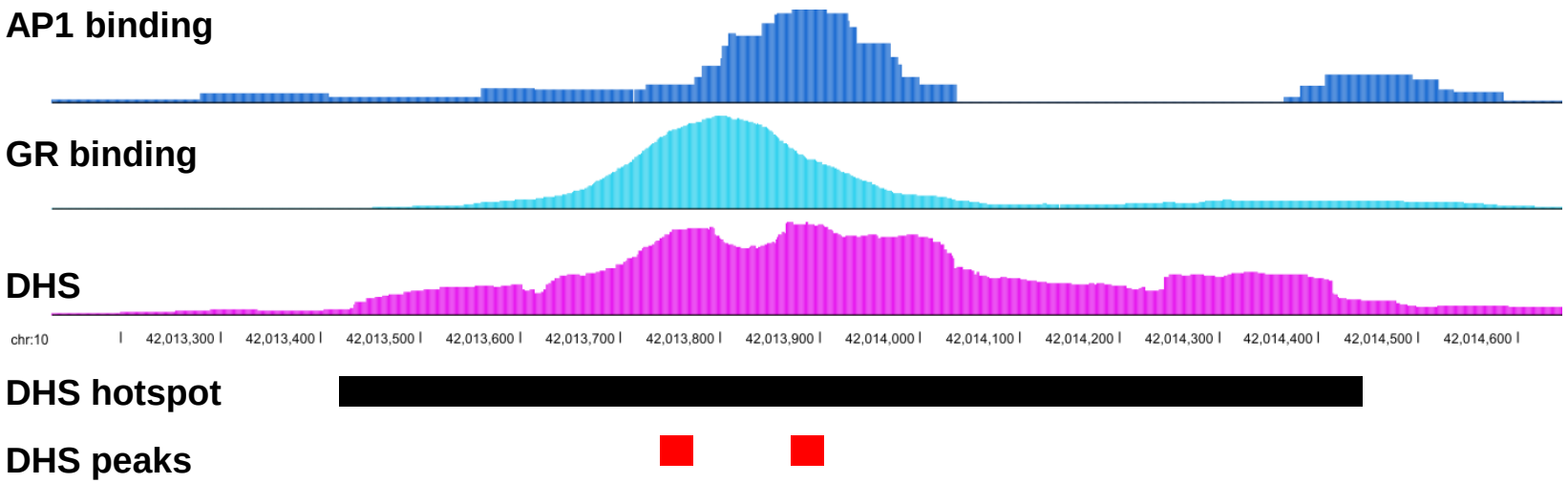
DHS and TF Binding



DHS coincide with multiple TF binding sites

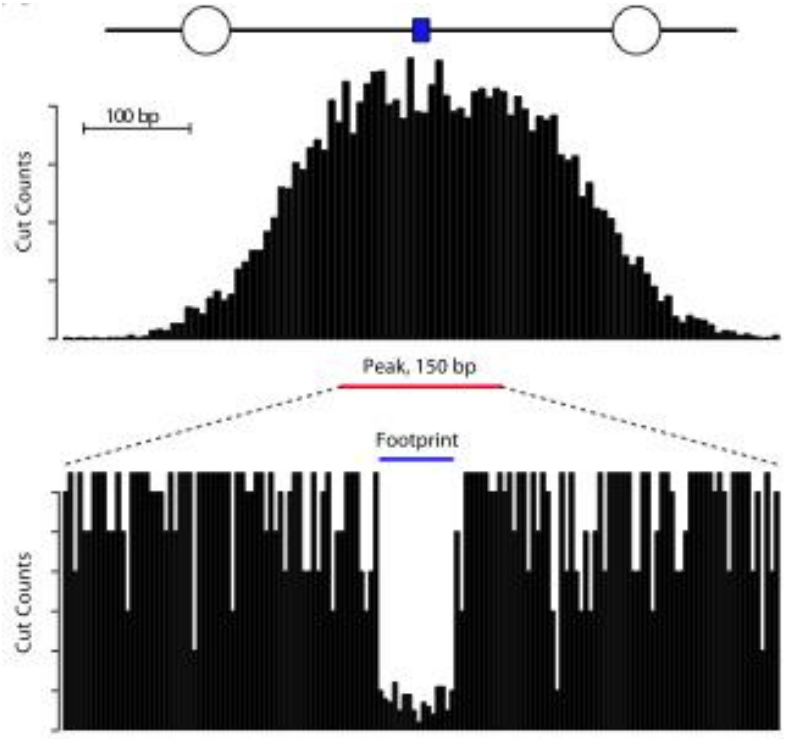
DHS may contain localized peaks of hypersensitivity

DHS and TF Binding



TF binding is correlated to localized enrichment of hypersensitivity within DHS

Localized Protection within DHS at TF Binding Motif



Vernon et al., Genome Res. 2012