Chromatin **I**mmuno**P**recipitation followed by **seq**uencing (ChIP-seq):

Probing for epigenetic marks

NGS analysis for gene regulation and epigenomics

Physalia 2021

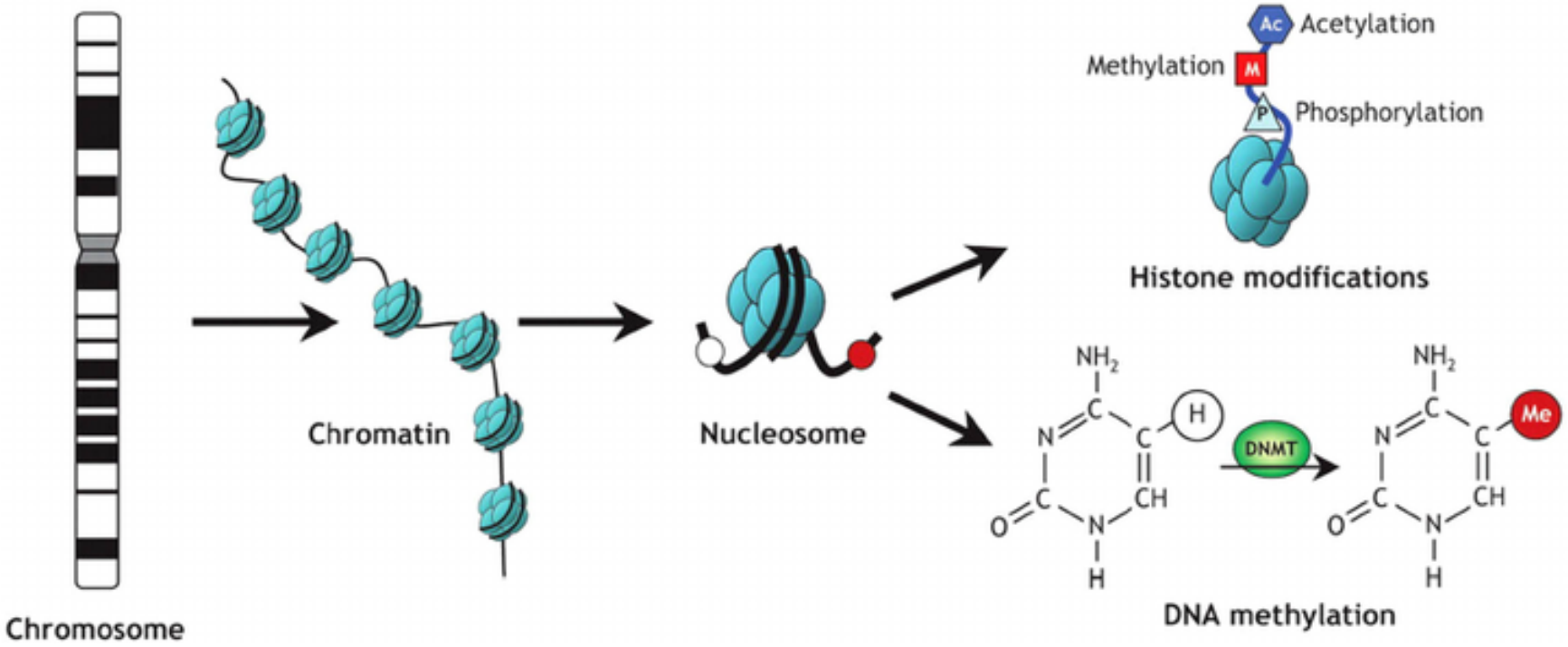
Jacques Serizay

Why probing binding of proteins on DNA is important

- Many different proteins bind on/interacts with DNA: this constitutes the chromatin

Why probing binding of proteins on DNA is important

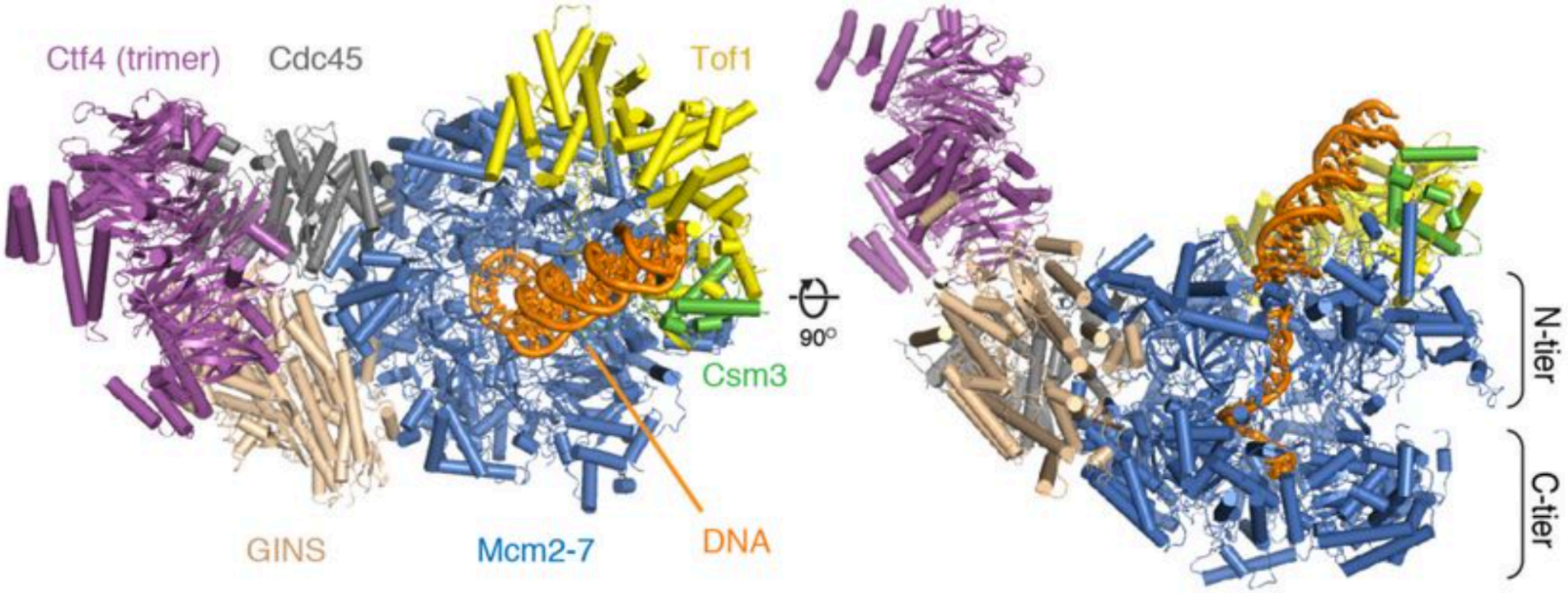
- Basic chromatin constituents



Zilbauer, Archives of Disease in Childhood: Education & Practice 2013

Why probing binding of proteins on DNA is important

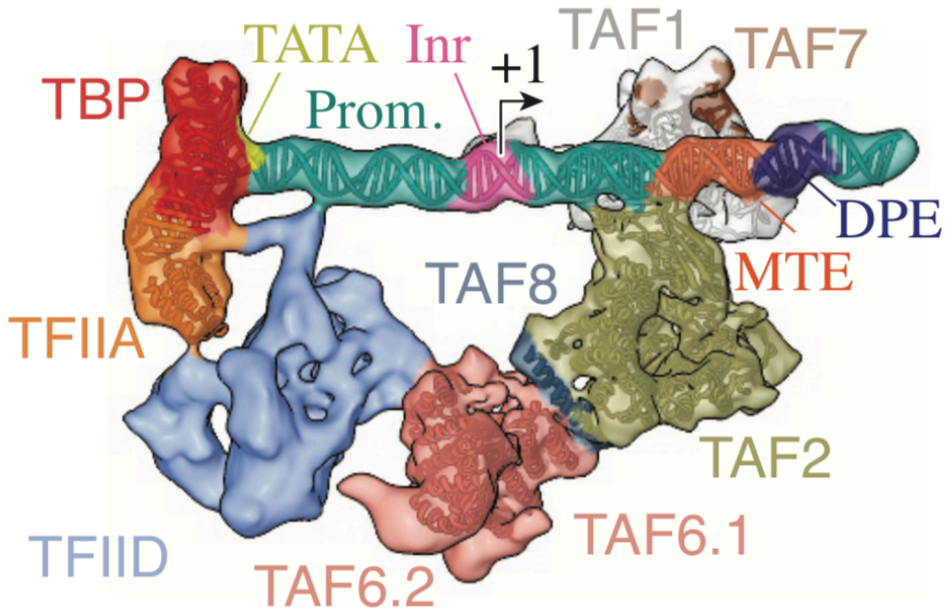
- Replication machinery

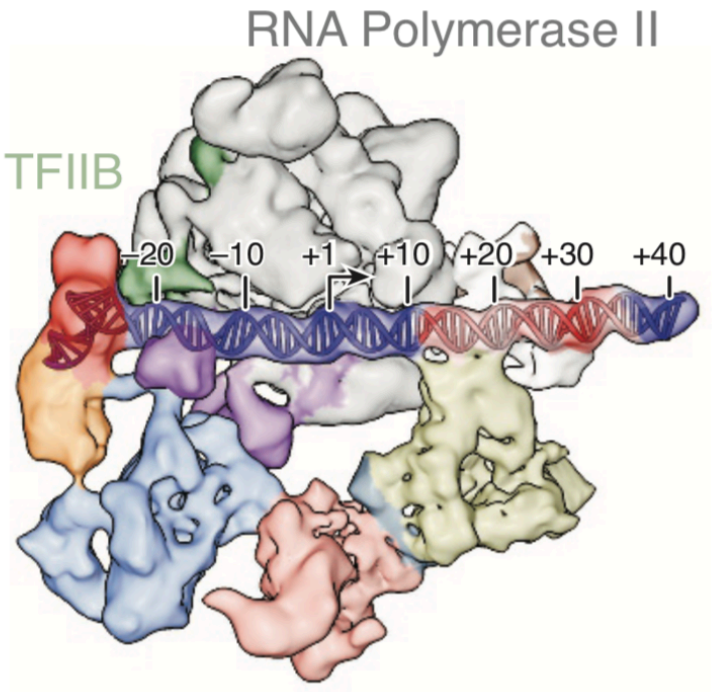


Baretić et al., Mol. Cell 2020

Why probing binding of proteins on DNA is important

- Transcription machinery

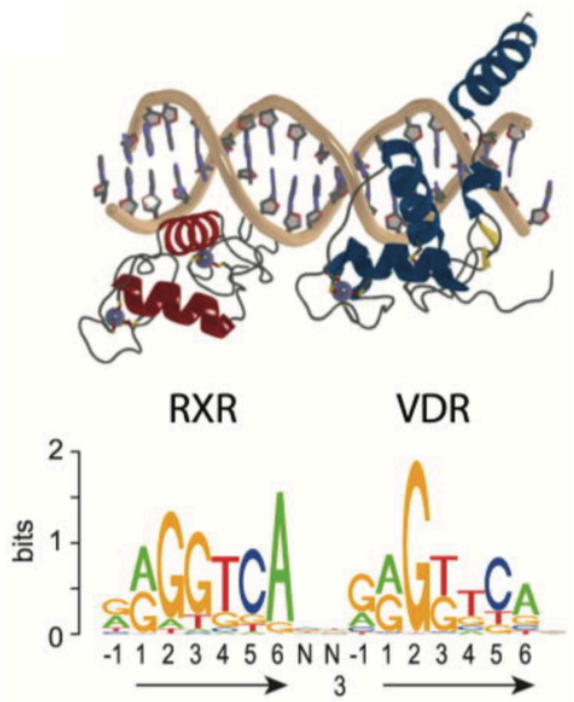


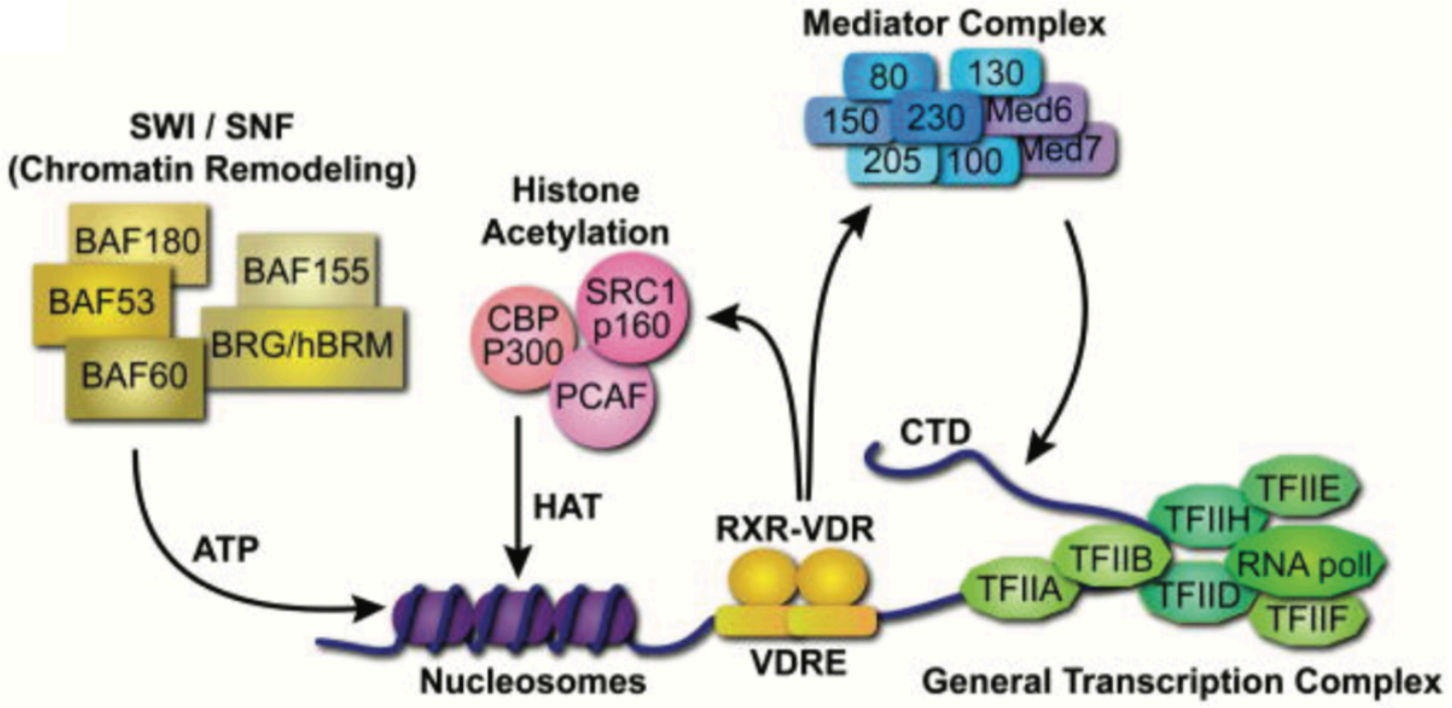


Louder et al., Nature 2016

Why probing binding of proteins on DNA is important

- Regulatory proteins: transcription factors & co





Carlberg & Campbell, Steroids 2013

Pike & Meyer, Endocrinol. Metab. Clin. North Am. 2010

Chromatin immunoprecipitation: an old tool rejuvenated by high-throughput sequencing

- Chromatin IP is not a new approach. It has been around for the past three decades

Cell

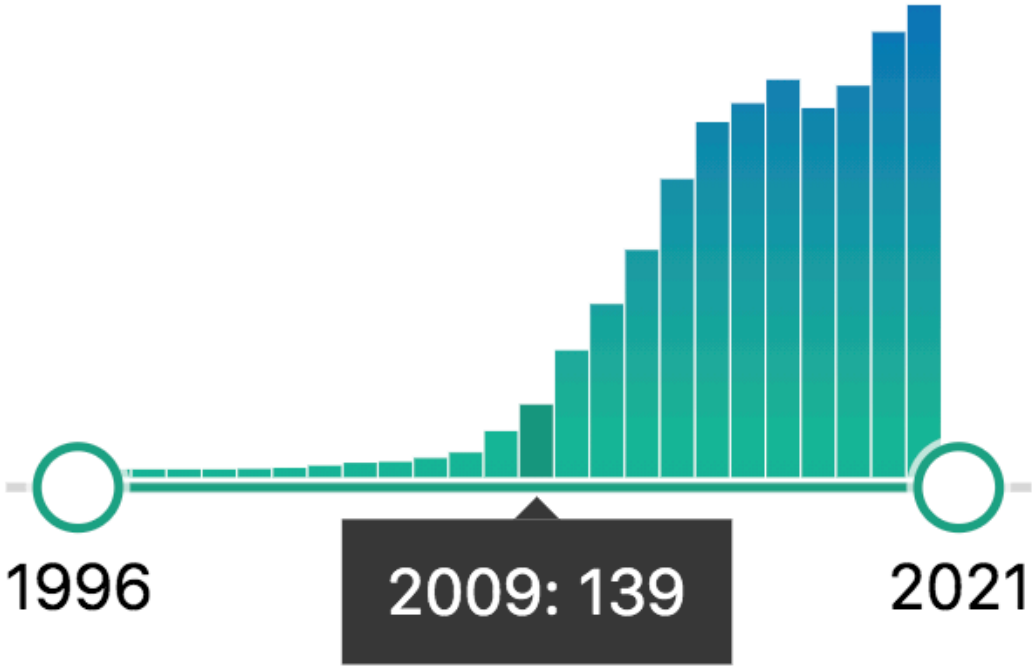
Summary

We have used formaldehyde-mediated proteinDNA crosslinking within intact cells to examine the in vivo chromatin structure of the D. melanogaster heat shock protein 70 (*hsp70*) genes. In agreement with previous in vitro studies, we find that the heat shockmediated transcriptional induction of the *hsp70* genes perturbs their chromatin structure, resulting in fewer proteinDNA contacts crosslinkable in vivo by formaldehyde. However, contrary to earlier in vitro evidence that histones may be absent from actively transcribed genes, we show directly, by immunoprecipitation of in vivocrosslinked chromatin fragments, that at least histone H4 remains bound to *hsp70* DNA in vivo, irrespective of its rate of transcription. The formaldehyde-based in vivo mapping techniques described in this work are generally applicable, and can be used both to probe proteinDNA interactions within specific genes and to determine the genomic location of specific chromosomal proteins.

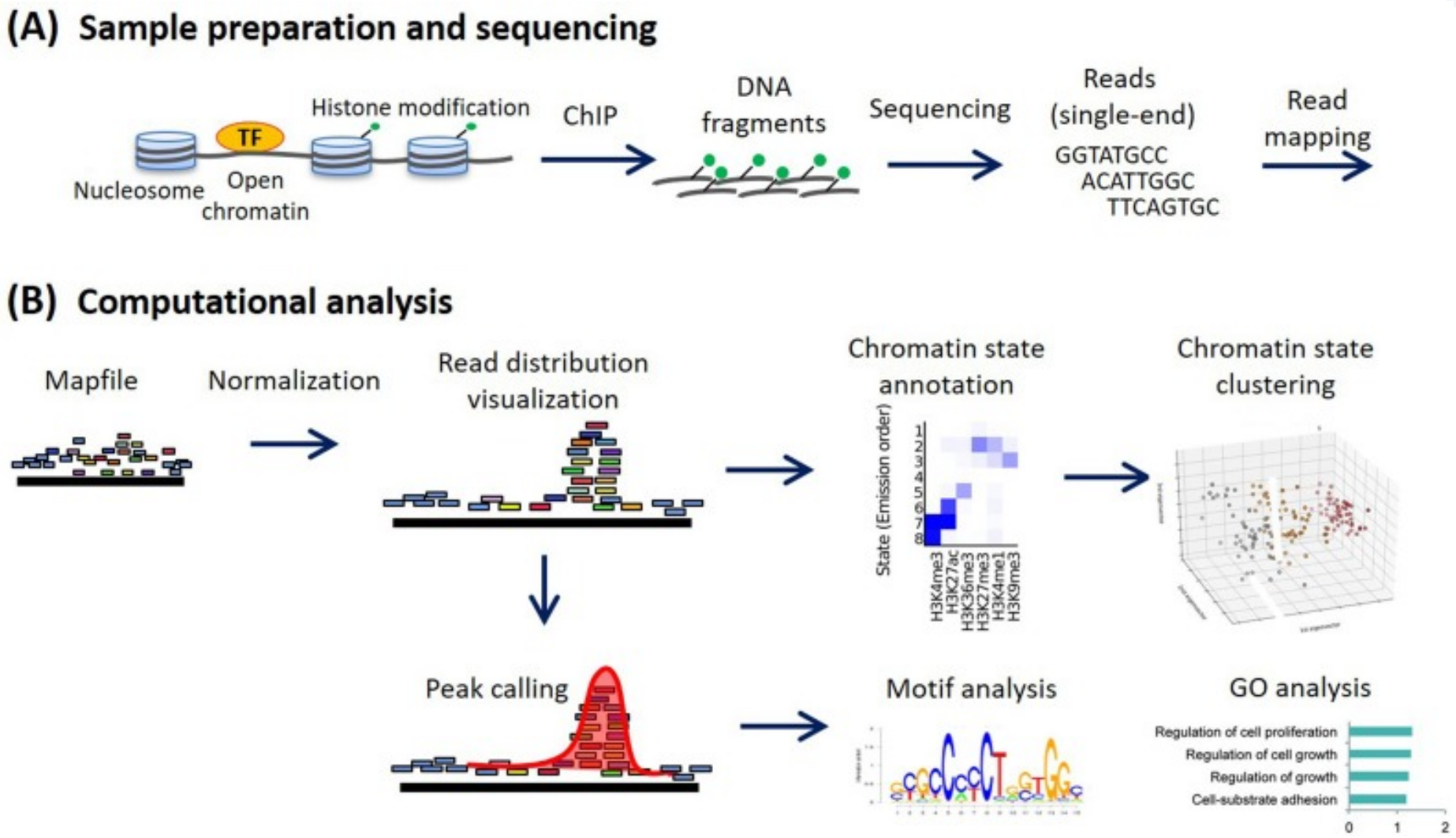
Solomon, Larsen & Varshavsky, Cell 1988

Chromatin immunoprecipitation: an old tool rejuvenated by high-throughput sequencing

- It gained a lot of traction when high-throughput sequencing emerged

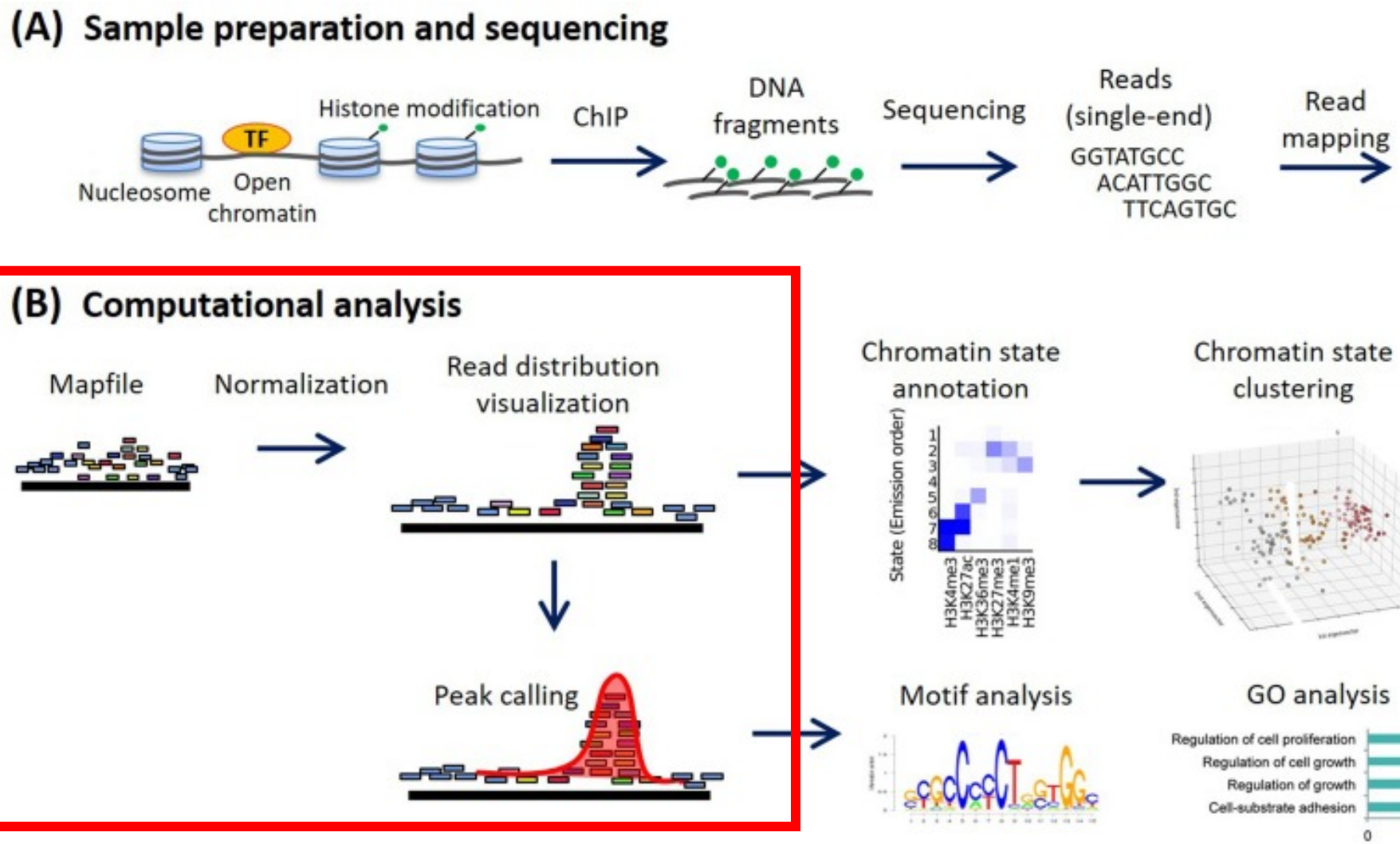


Classical workflow



Nakato et al., Methods 2020

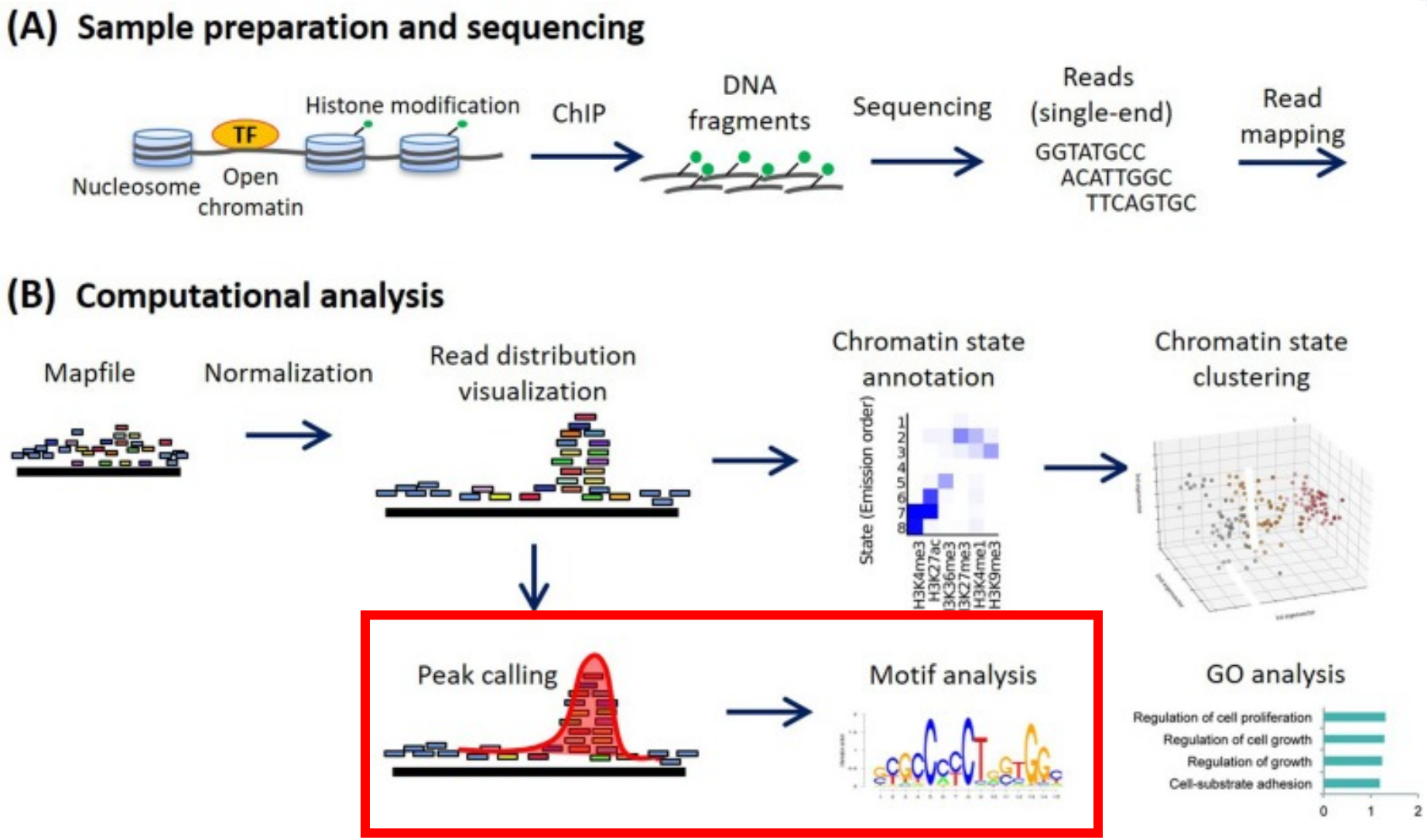
Classical workflow



Ex 03-1

Nakato et al., Methods 2020

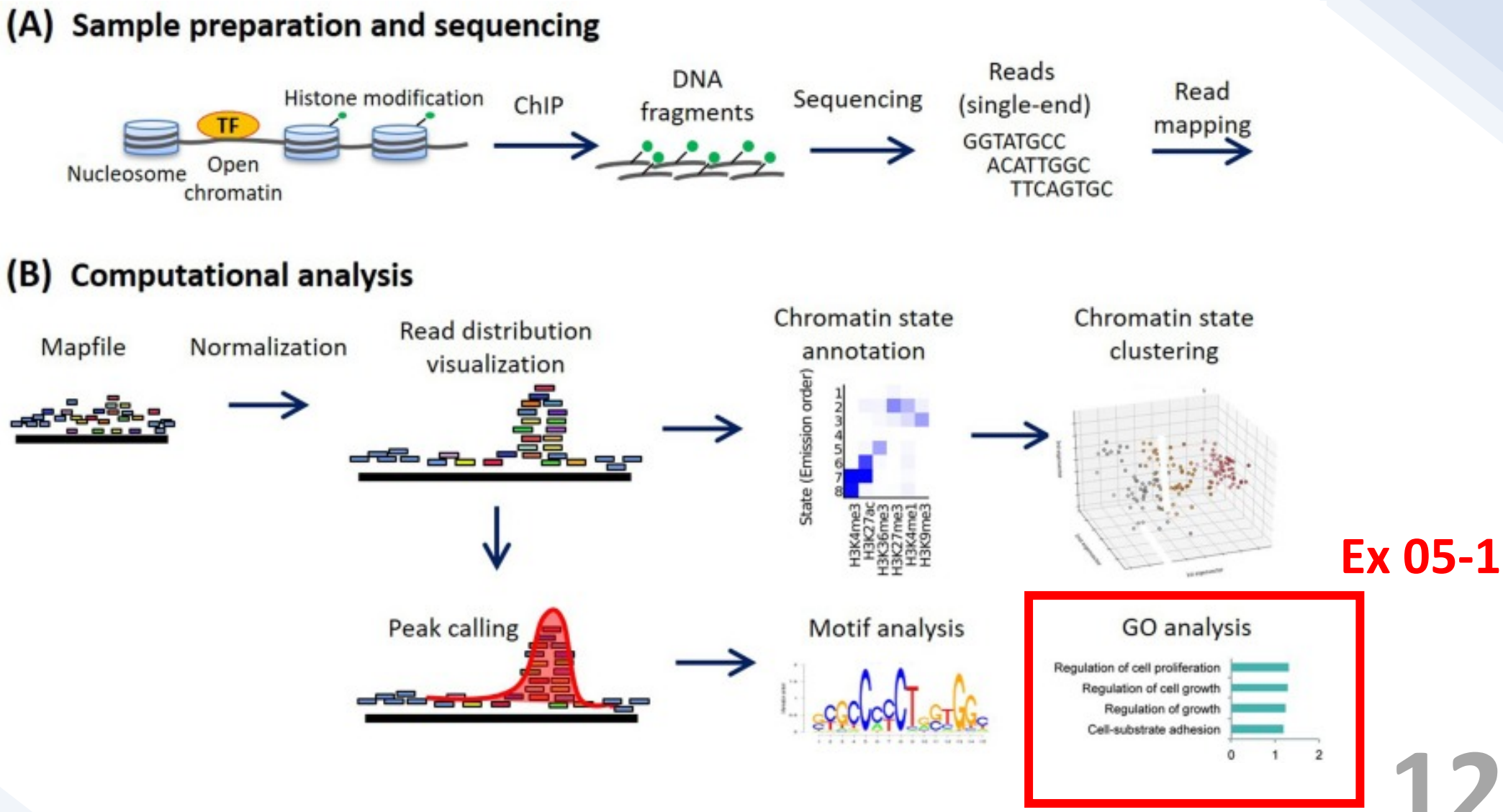
Classical workflow



Ex 03-2

Nakato et al., Methods 2020

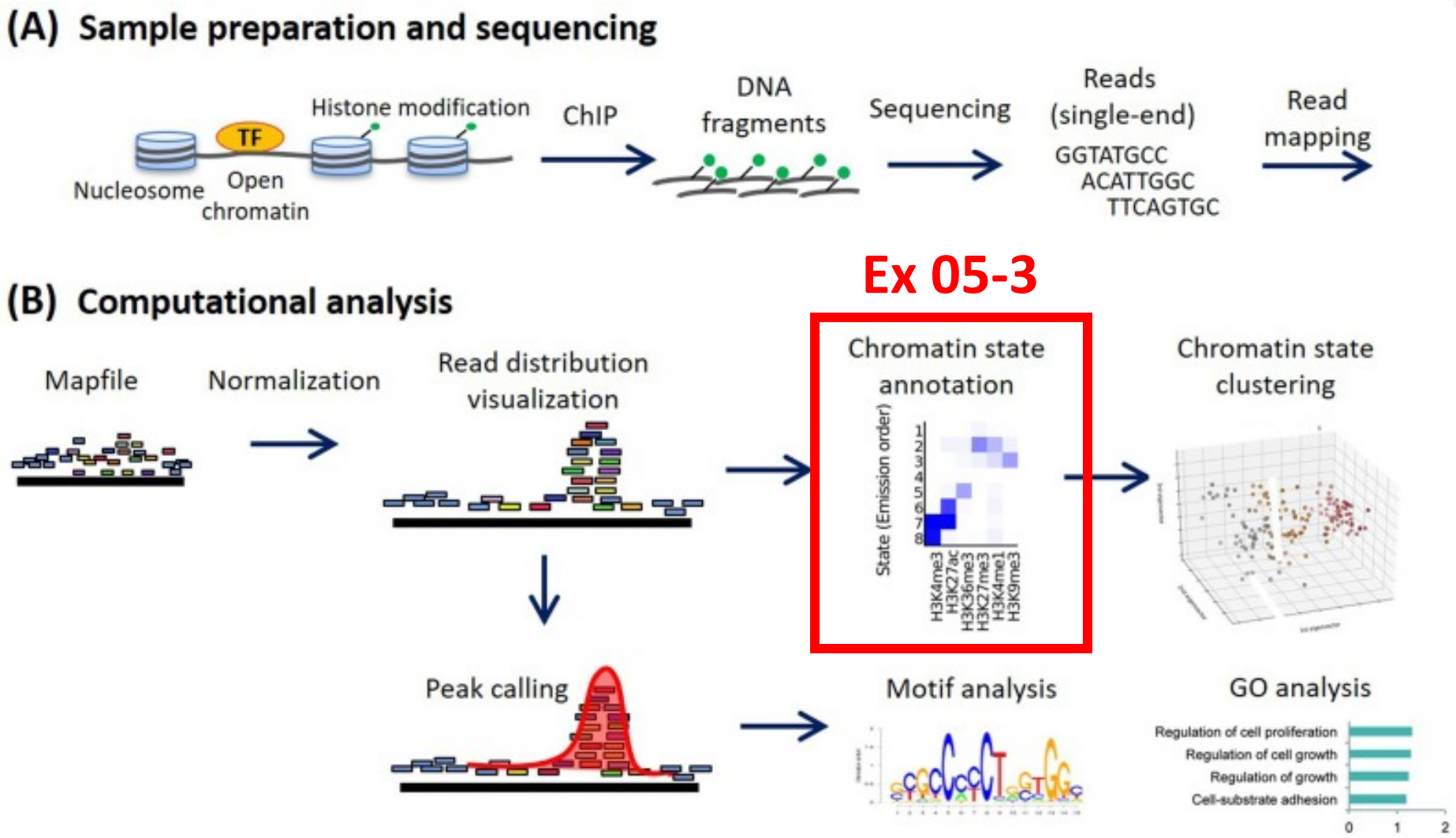
Classical workflow



Ex 05-1

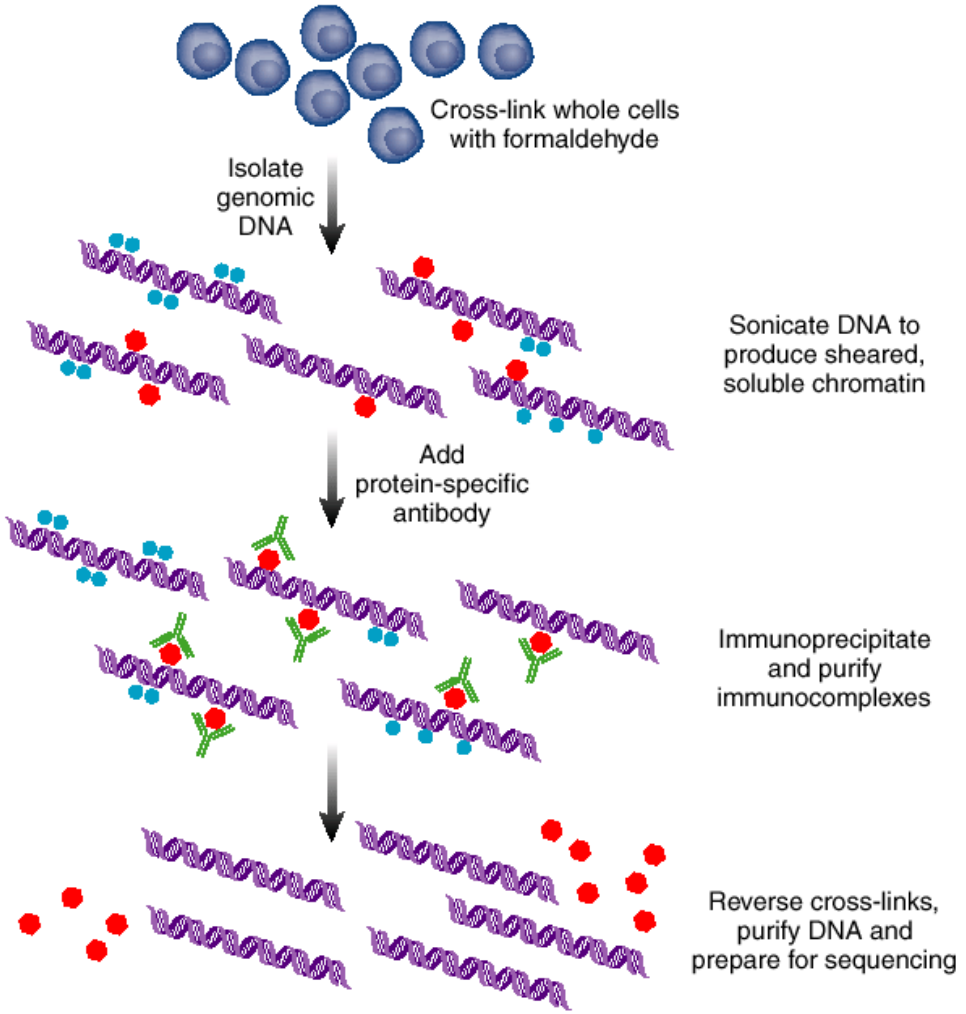
Nakato et al., Methods 2020

Classical workflow



Nakato et al., Methods 2020

Classical workflow: IP step



Mardis, Nature Methods 2007

Current ChIP-seq approaches

- ChIP-seq originally referred to a precise protocol, involving formaldehyde crosslinking, mechanical fragmentation and antibody-based chromatin pull-down.

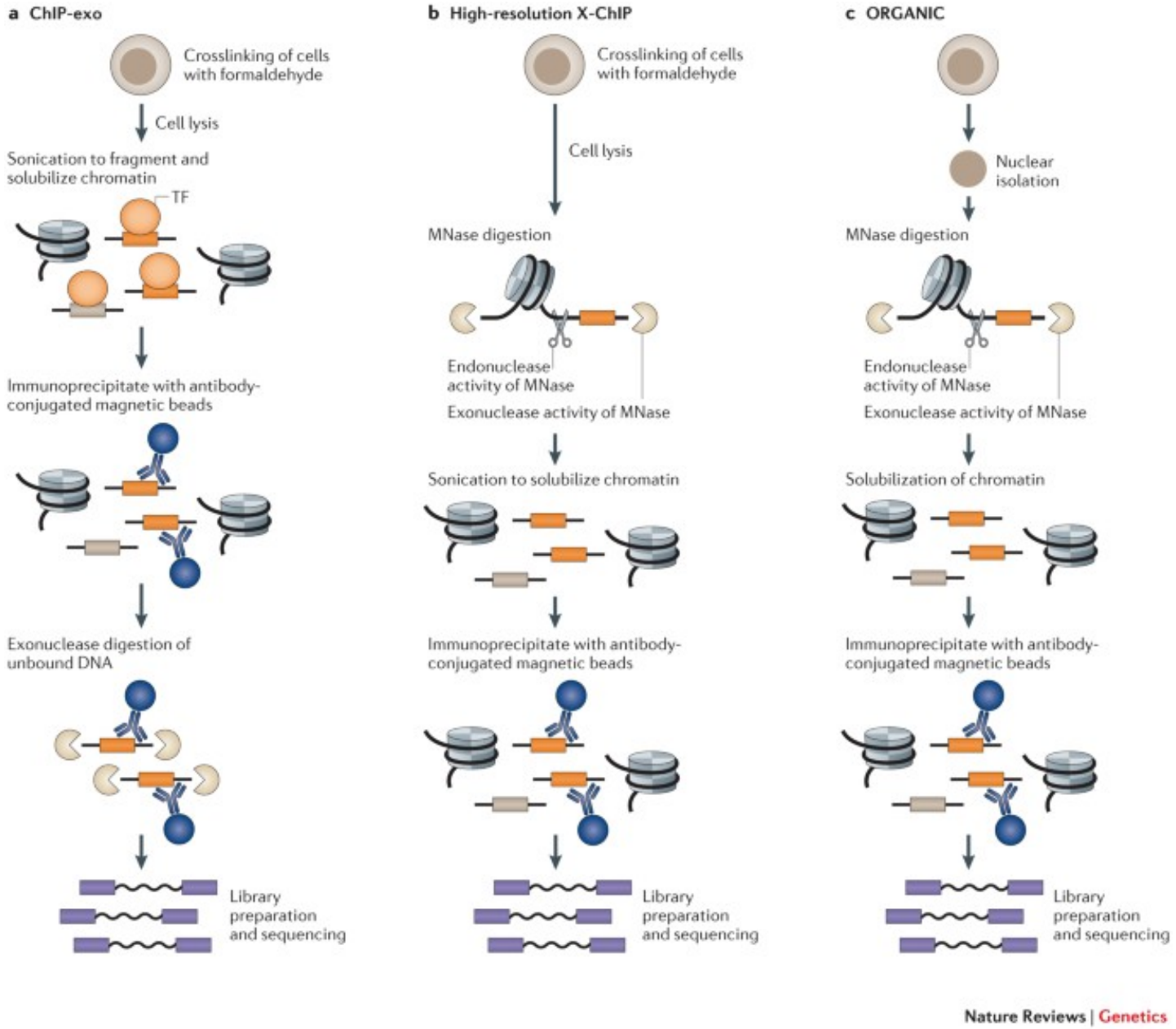
- It became more of a “generic” term to describe any chromatin immunoprecipitation experimental approach backed by high-throughput sequencing.

Current ChIP-seq approaches

- ChIP-seq
- Low input ChIP-seq
- Native ChIP-seq
- Indirect ChIP-seq with DamID
- ChIP-seq with chemical-based fragmentation
- Cut&Run, Cut&Tag
- Single-cell ChIP-seq
- …

Current ChIP-seq approaches

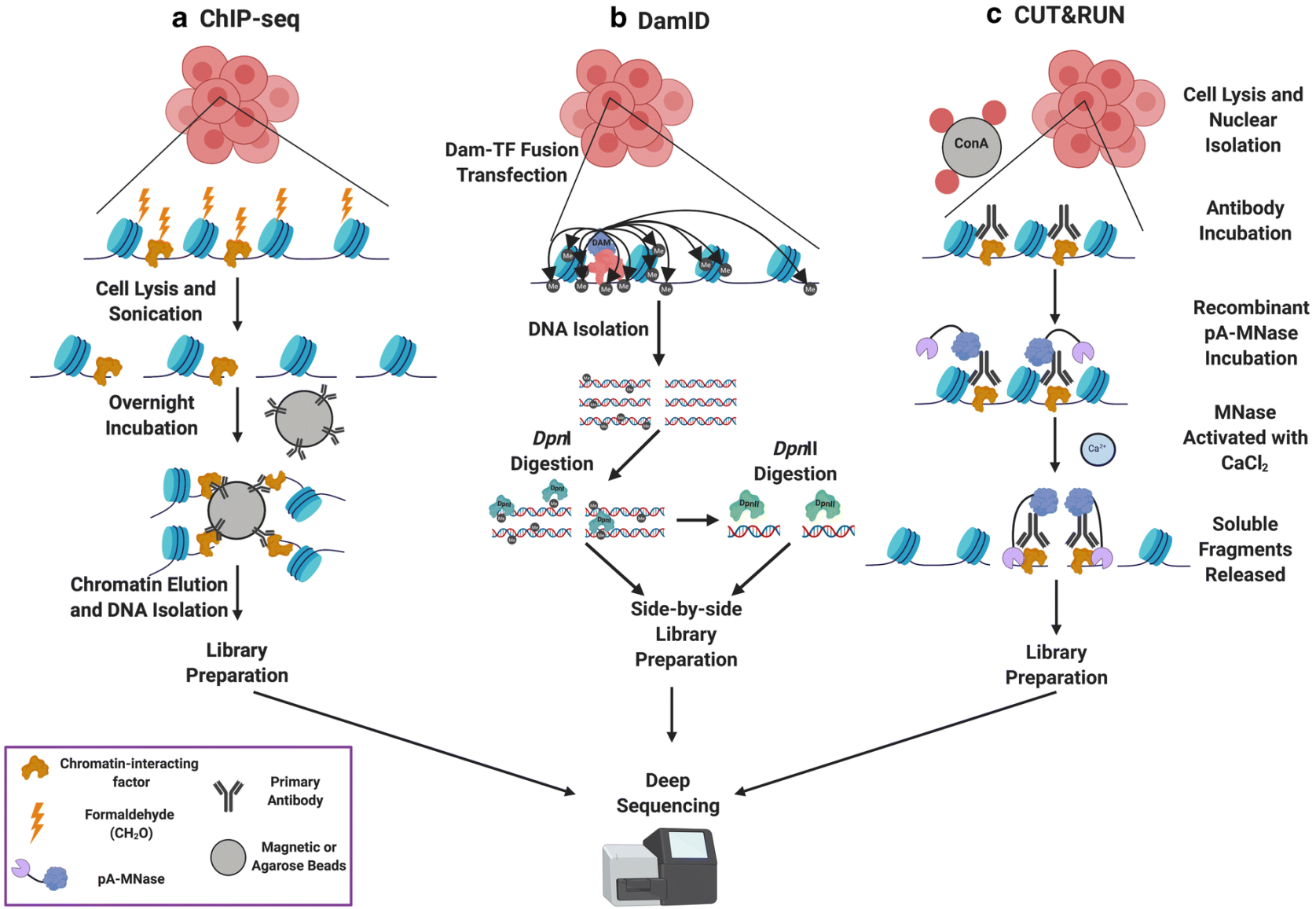
- Direct approaches



Zentner & Henikoff, Nat. Rev. Genet. 2014

Current ChIP-seq approaches

- Indirect approaches



Klein & Hainer, Chromosome Research 2020

A plethora of commercial kits to help with ChIP-seq

- Kits help to reduce expenses when trying out a new method.
- They also help to “gently” dive in the methodology of the experimental steps.
- They (usually) provide a streamlined, easy version of an originally complex approach.
- Sometimes include downstream analysis workflows

A plethora of commercial kits to help with ChIP-seq

- Diagenode
- ActiveMotif
- Abcam
- Illumina
- ThermoFisher
- Qiagen
- TakaraBio
- Novogene
- Epigentek

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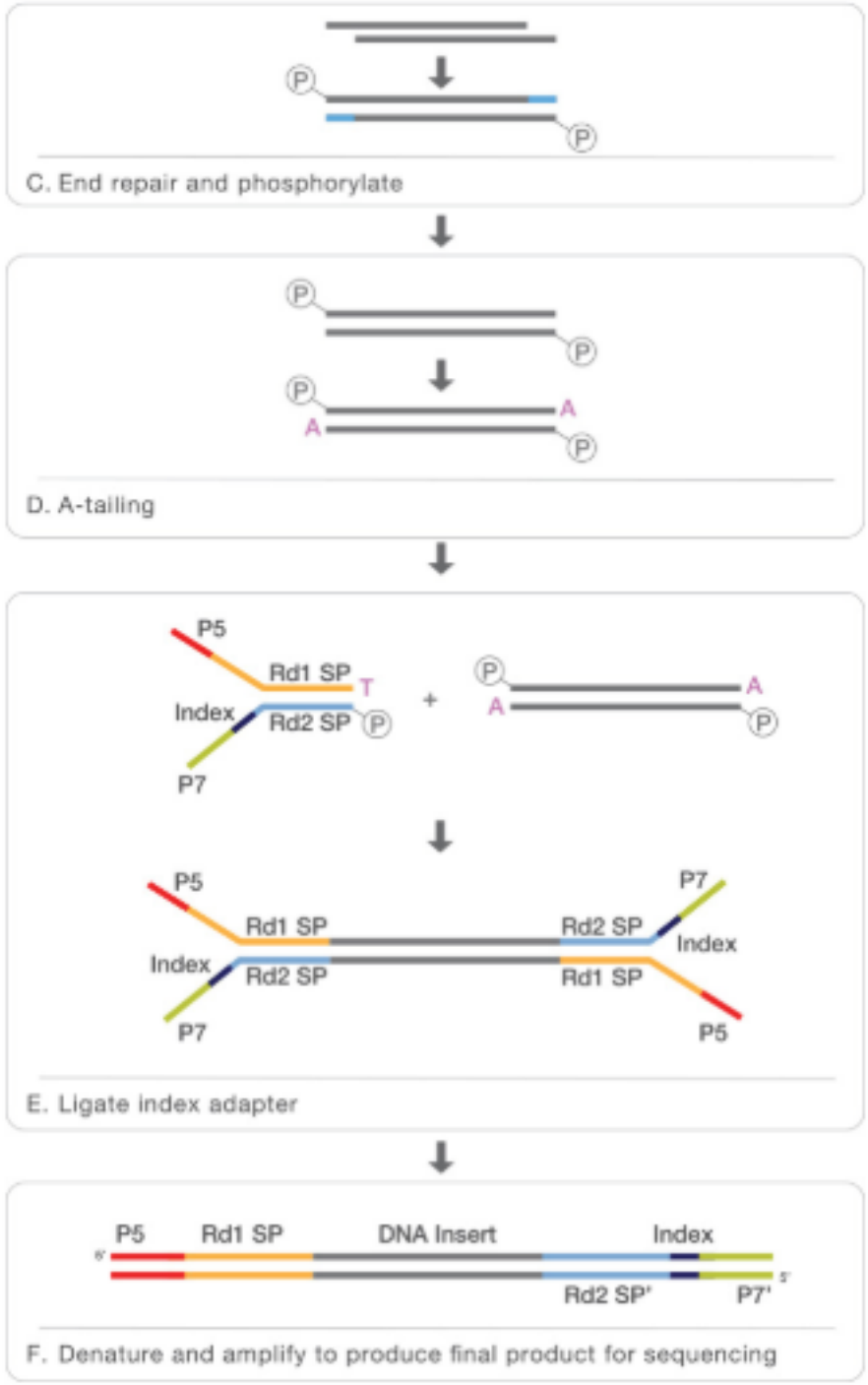
The required input, reagents, yield and/or quality will vary depending on the kit.

Once someone has experience in (1) molecular biology practice and (2) making high-throughput sequencing libraries, it becomes more straightforward / cost-effective to perform ChIP-seq using in-house protocols.

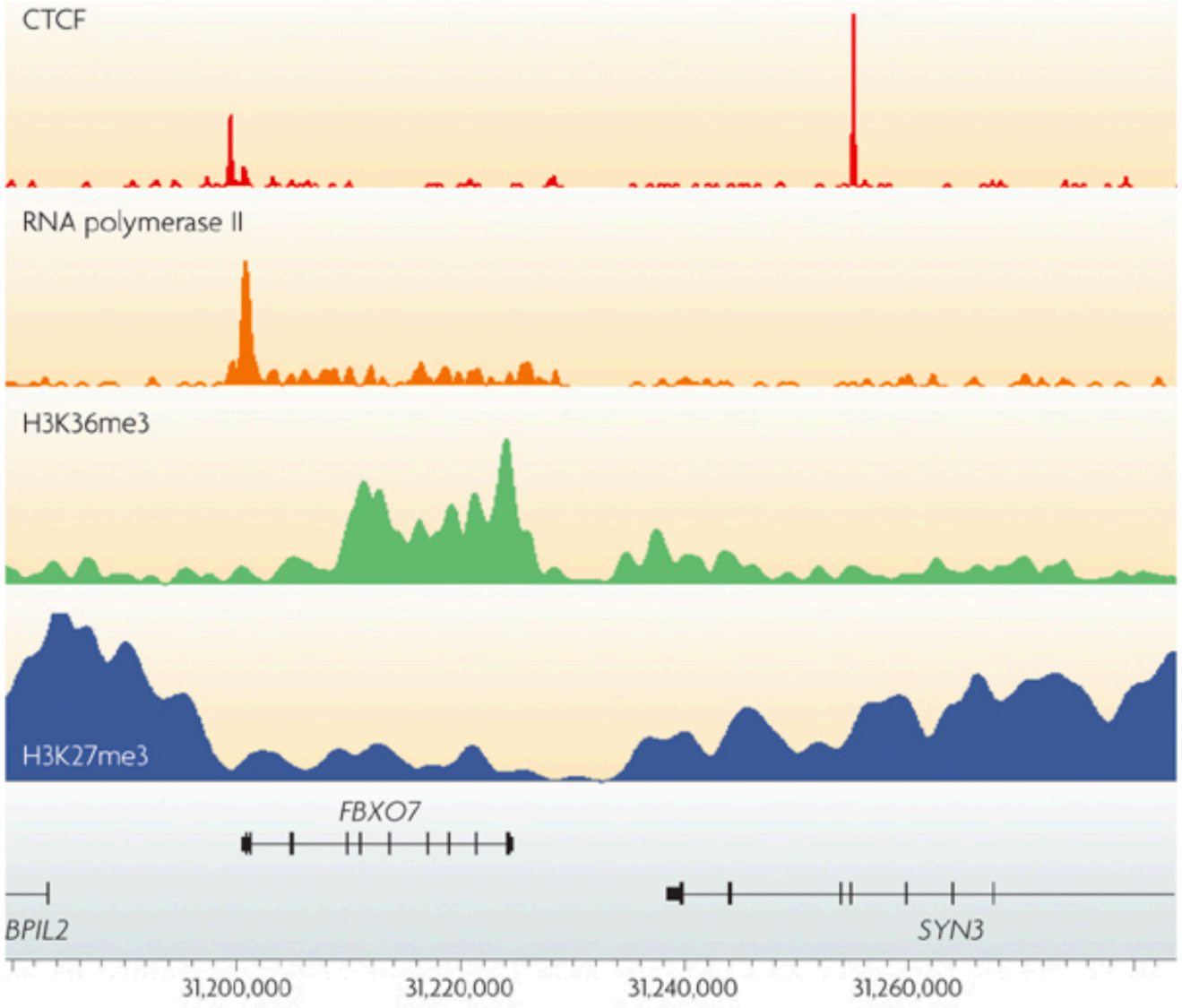
ChIP-seq outputs

- **[Biological material]** High-throughput sequencing libraries



ChIP-seq outputs

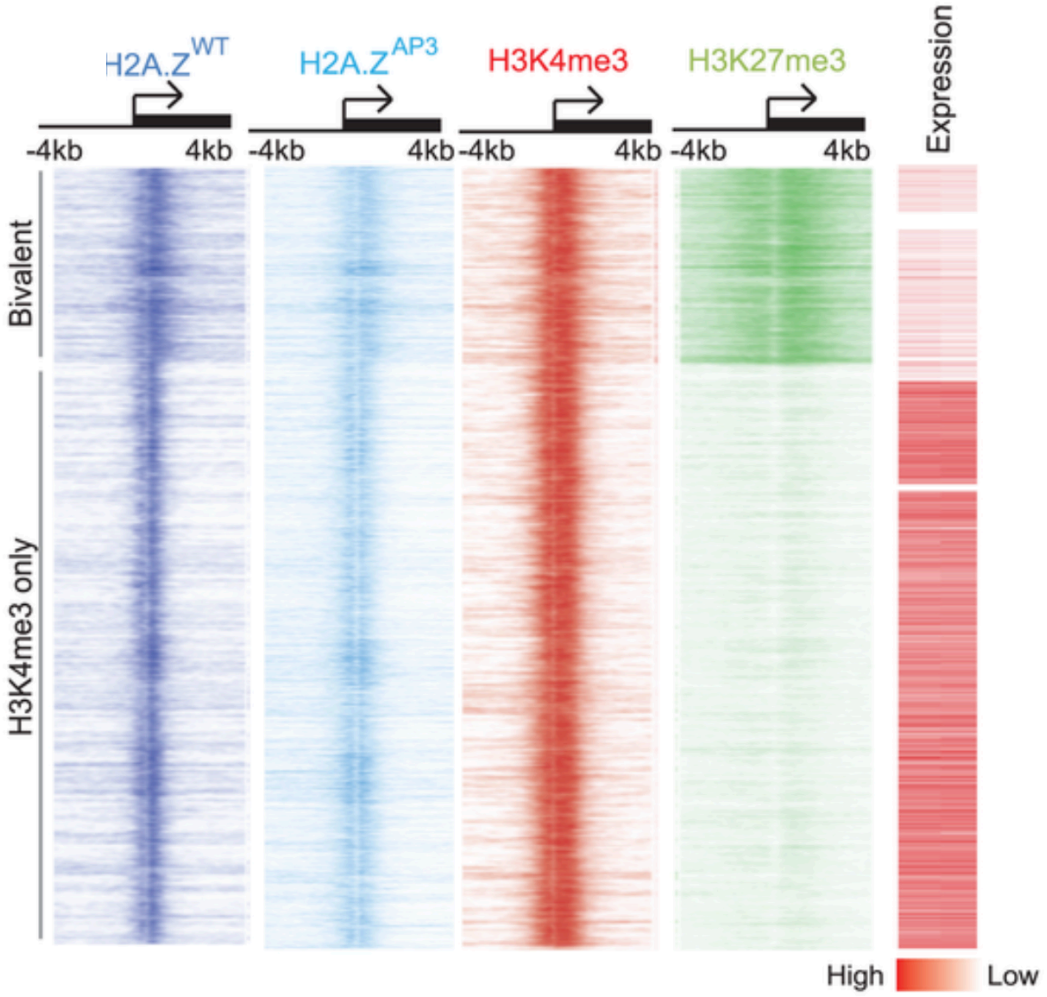
- Genome-wide coverage tracks
  - Can be directly viewed in a genome browser

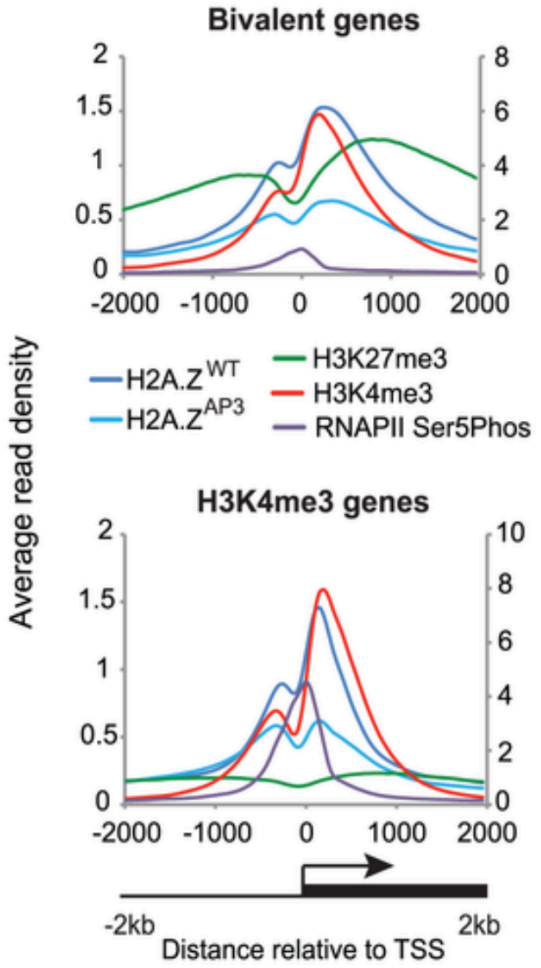


Park et al., Nat. Rev. Genet. 2009

ChIP-seq outputs

- Genome-wide coverage tracks
  - Can also be aligned at genomic features of interest (e.g. TSSs)

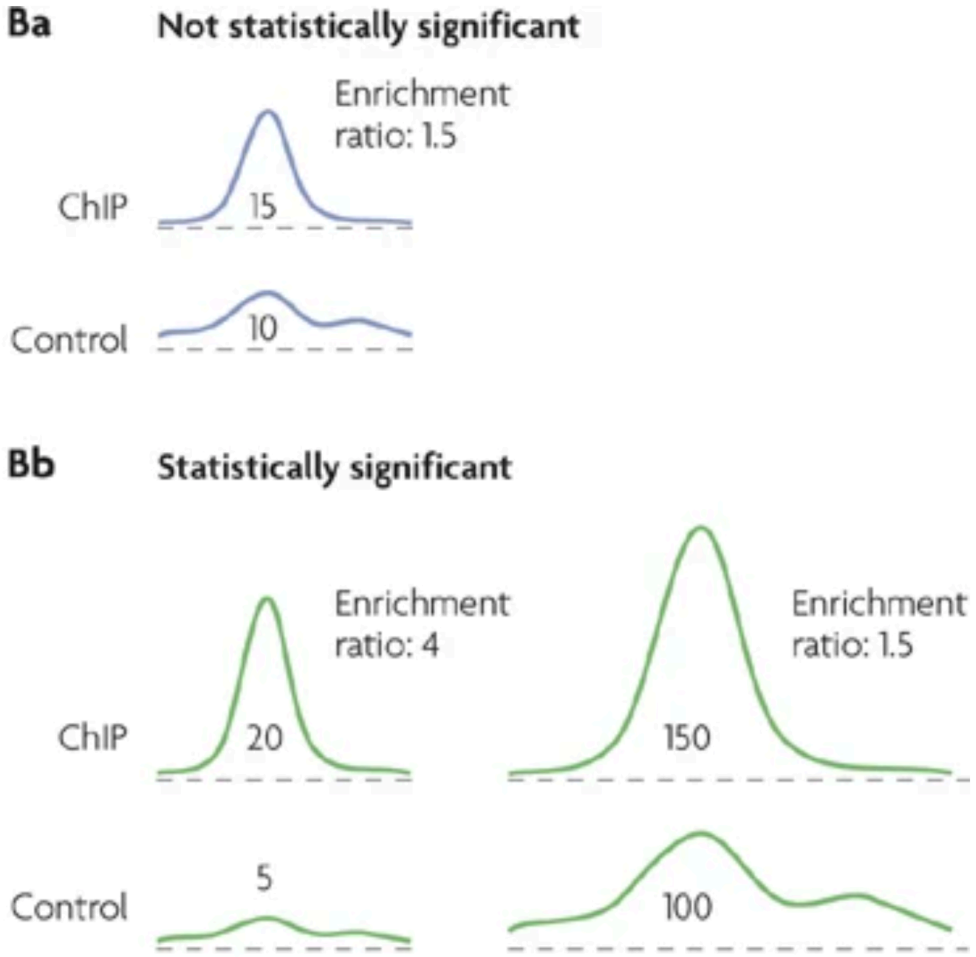


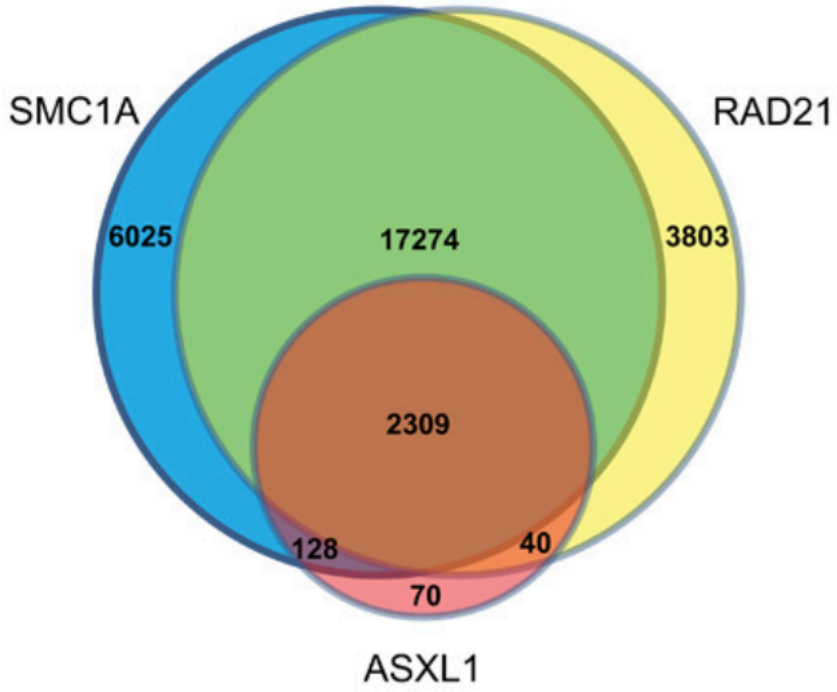


Subramanian et al., PLoS Genetics 2013

ChIP-seq outputs

- Peak sets

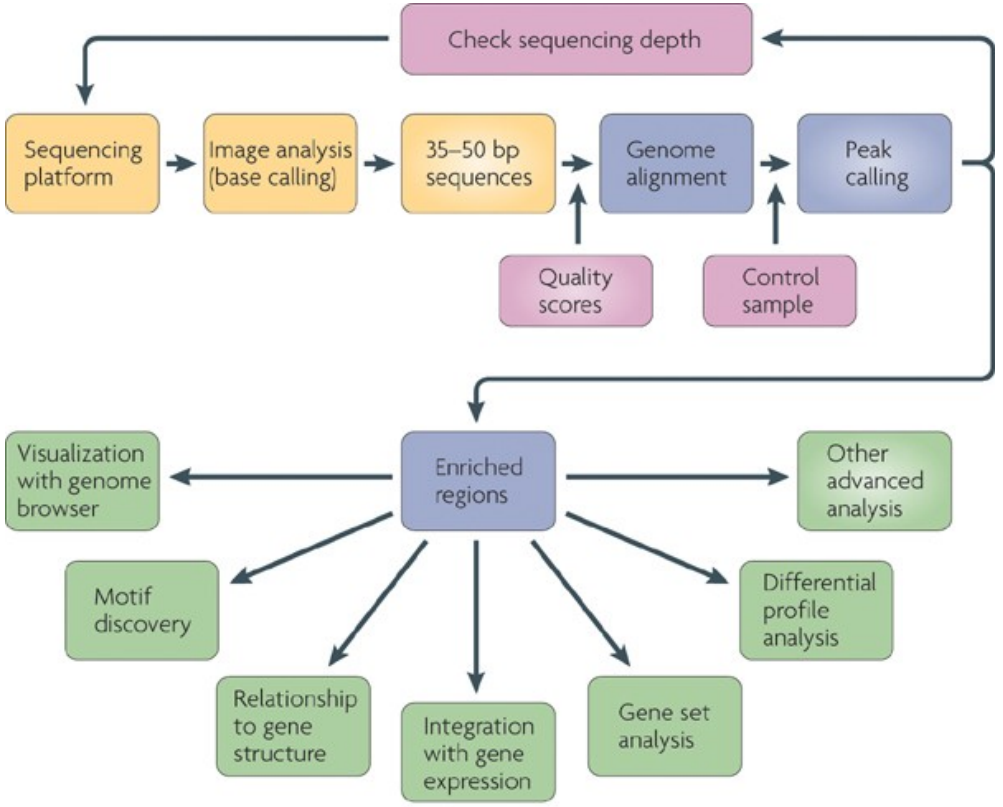




Park et al., Nat. Rev. Genet. 2009

ChIP-seq outputs

- And more from downstream analyses, according to the biological question:
  - DNA binding motifs
  - Differential binding of a factor
  - Cooperation between factors
  - Biological functions in which a factor is involved
  - Temporal dynamics of factor binding
  - Gene regulatory networks
  - …

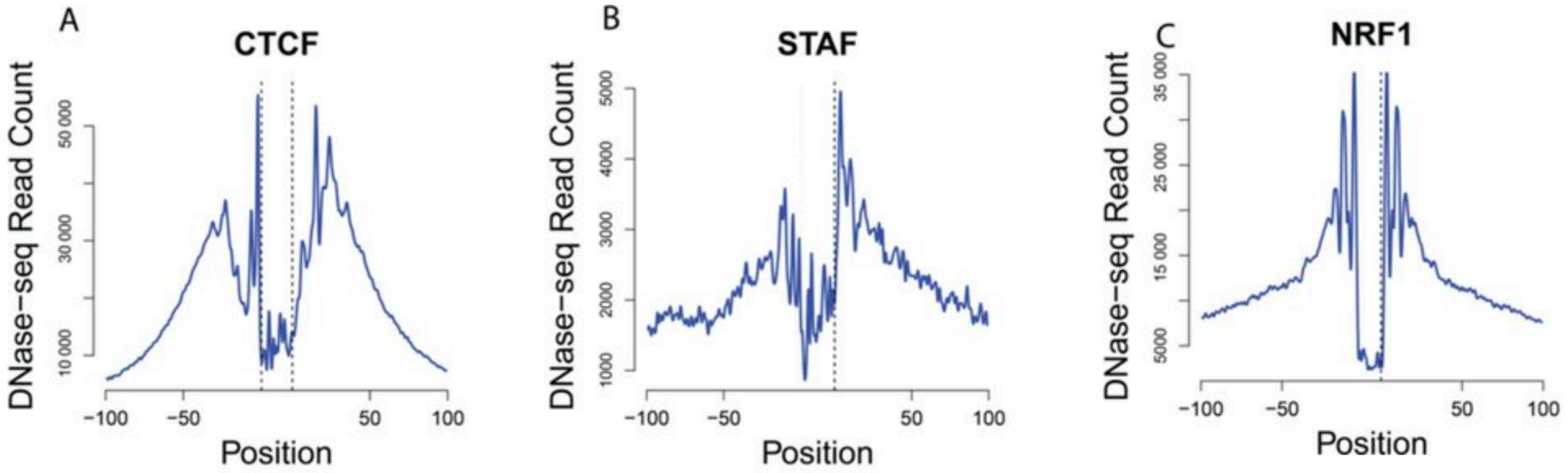


Park et al., Nat. Rev. Genet. 2009

Nature Reviews | Genetics

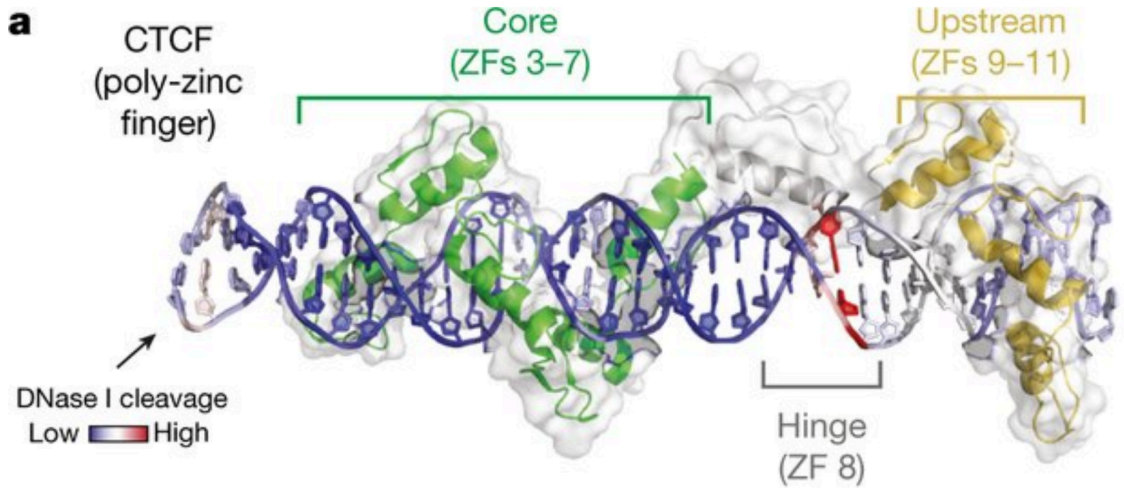
Alternative to chromatin immunoprecipitation approaches

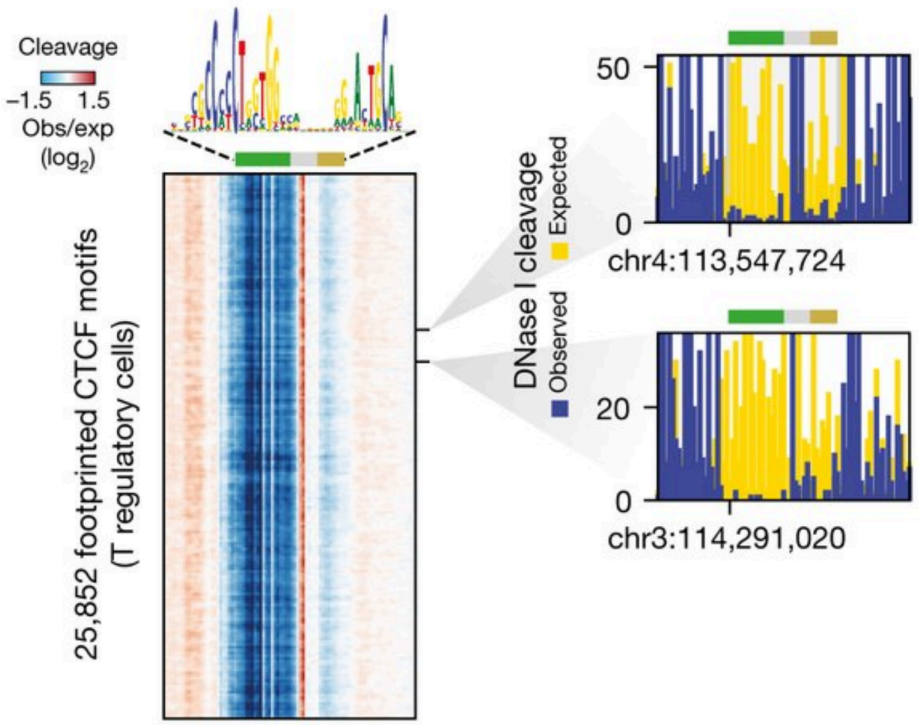
- DNAse footprinting methods



Alternative to chromatin immunoprecipitation approaches

- DNAse footprinting methods

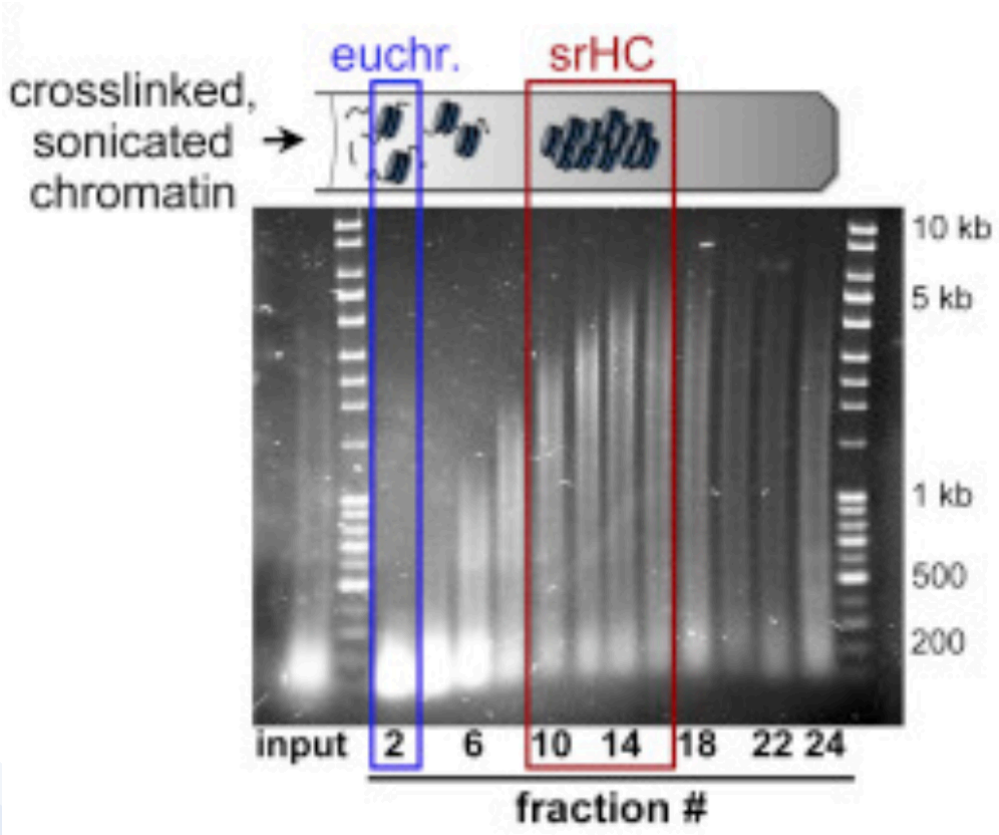


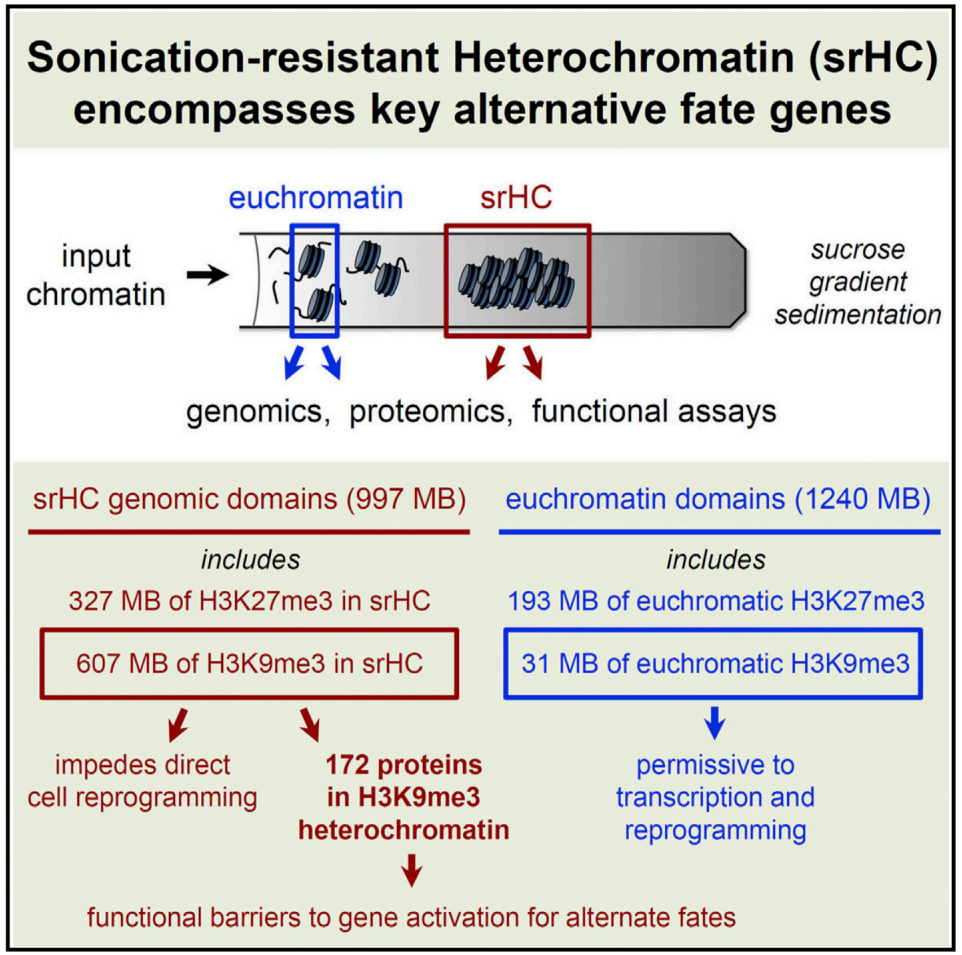


Vierstra et al., Nature 2020

Alternative to chromatin immunoprecipitation approaches

- Chromatin sedimentation methods



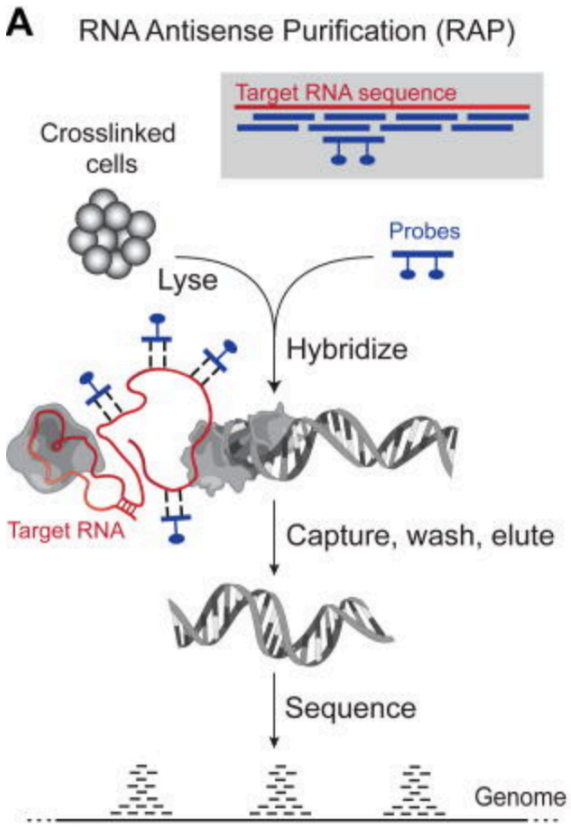


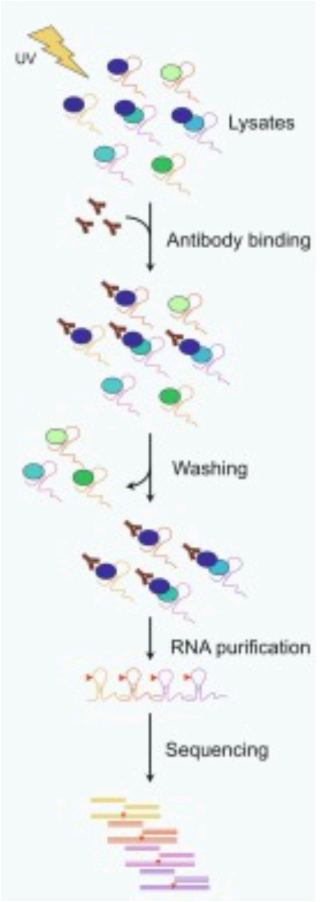
Becker et al., Mol. Cell 2017

Going further: probing RNA-DNA or protein-RNA interactions

- Methods also based on crosslinking, this time between RNA and DNA or proteins and RNA

Engreitz et al., Science 2013





Wang & Zie, Translational Epigenetics 2020

Some resources

- Github:
  - https://github.com/danielecook/Awesome-Bioinformatics#chip-seq
  - https://github.com/crazyhottommy/ChIP-seq-analysis

- Henikoff's lab methods website:
  - 20+ methods developed by the lab to profile chromatin
  - https://research.fredhutch.org/henikoff/en/methods.html

- Peter Park's review in Nature Review Genetics (2009)