

# Chromatin immunoprecipitation

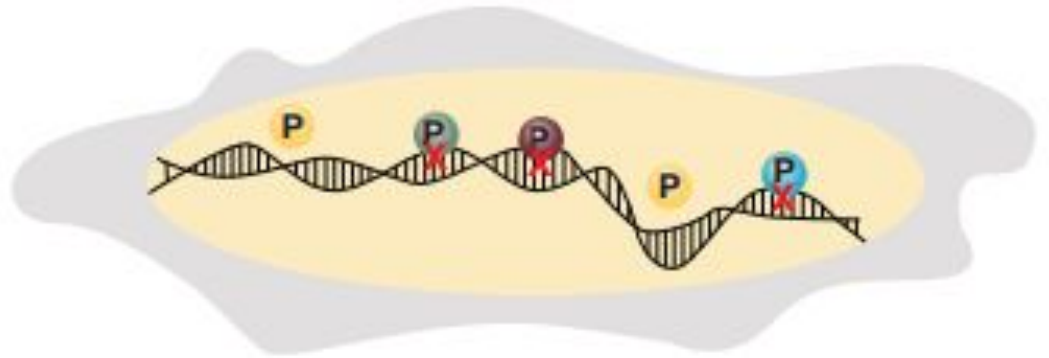


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Chromatin Immunoprecipitation (ChIP) is a type of immunoprecipitation experimental technique used to investigate the interaction between **proteins** and **DNA** in the cell. It aims to determine whether specific proteins are associated with specific genomic regions, such as transcription factors on promoters or other DNA binding sites. ChIP also aims to determine the specific location in the genome that various histone modifications are associated with, indicating the target of the histone modifiers.

# Procedure:

## Step 1: Crosslinking

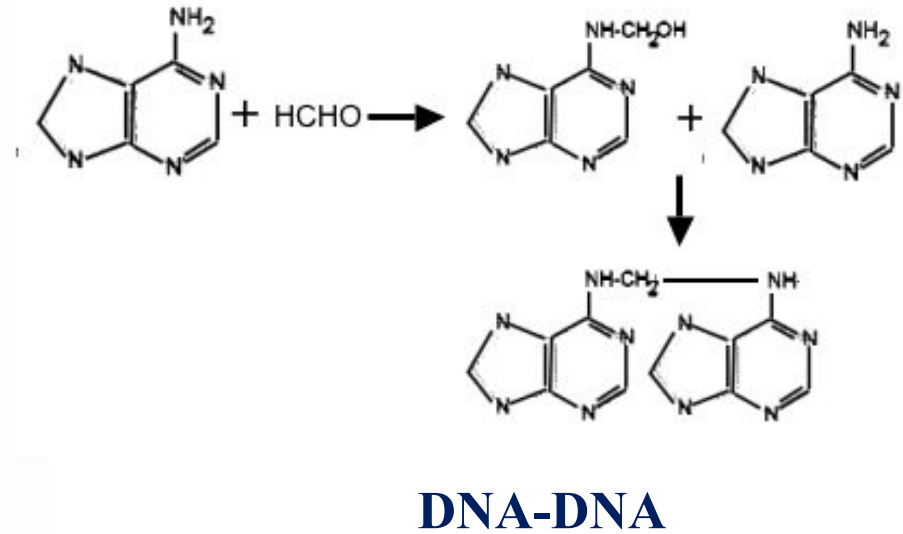
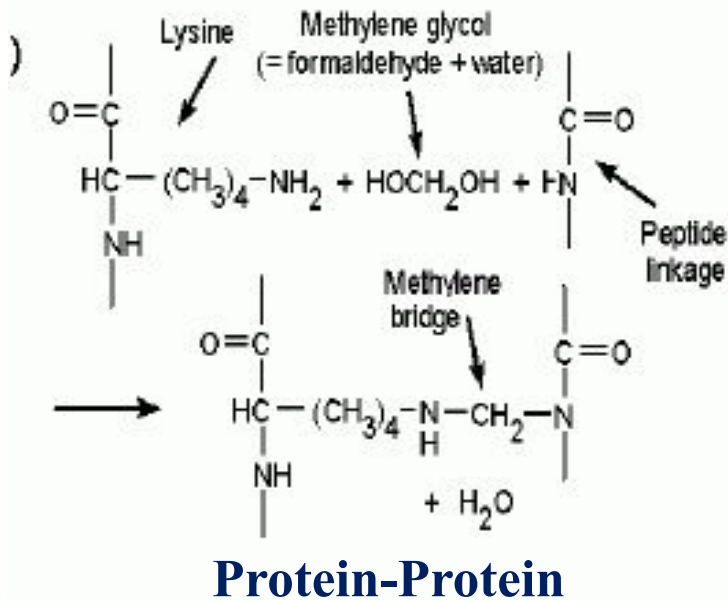


ChIP assays begin with covalent stabilization of the protein–DNA complexes. Many protein–DNA interactions are transient and involve multiprotein complexes to orchestrate biological functions. As there is constant movement of proteins and DNA, ChIP captures a snapshot of the protein–DNA complexes that exist at a specific time.

**For the fixation of cells or tissues immediately most frequently used - formaldehyde to keep protein-DNA interactions in place.**

**Note:** This is a point where ChIP can be stopped. After crosslinking, quenching, and washing the cell pellet, it can be stored at  $-80^{\circ}\text{C}$ .

# Formaldehyde Crosslinking



## Other Options

Table 1. Cross-Linking Agents

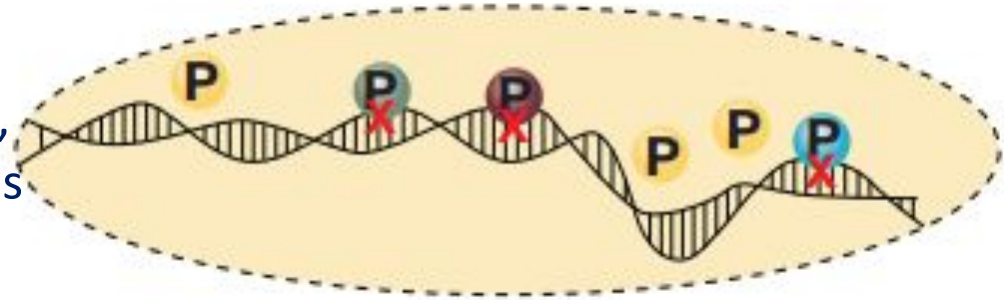
	Chemical Formula	Molecular Weight	Spacer Arm (Å)	Useful for Cross-Linking	Soluble In	Final Concentration
HCHO	CH <sub>2</sub> O	37% (conc.)	2.0	prot/DNA/RNA	—	1% in PBS
DMA	C <sub>8</sub> H <sub>18</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub>	245.5	8.6	prot/prot	water	5 mM in PBS
DSS	C <sub>16</sub> H <sub>20</sub> N <sub>2</sub> O <sub>8</sub>	368.34	11.4	prot/prot	DMSO	1.5 mM in PBS
DSP	C <sub>14</sub> H <sub>16</sub> N <sub>2</sub> O <sub>8</sub> S <sub>2</sub>	404.42	12.0	prot/prot	DMSO	1.5 mM in PBS
EGS	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>12</sub>	456.36	16.1	prot/prot	DMSO	1.5 mM in PBS

Molecular weights and spacer arm lengths are indicated. HCHO, formaldehyde; DMA, dimethyl adipimidate; DSS, disuccinimidyl suberate; DSP, dithiobis[succinimidyl propionate]; EGS, ethylene glycolbis[succinimidyl succinate]; prot, protein; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline.

# Procedure:

## Step 2: Cell lysis

In this step, cell membranes are dissolved with detergent (NP40, TX100, Tween and/or SDS) based lysis solutions to liberate cellular components, and crosslinked protein–DNA complexes are solubilized.

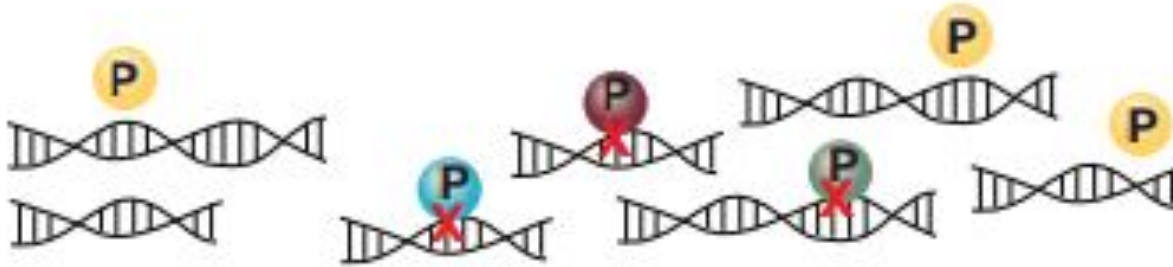


Also, Protease and phosphatase inhibitors are essential at this stage to maintain intact protein–DNA complexes. Because protein–DNA interactions occur primarily in the nuclear compartment, removing cytosolic proteins can help reduce background signal and increase sensitivity. The presence of detergents or salts will not affect the protein–DNA complexes, because the covalent crosslinking in step 1 will keep the complexes stable throughout the ChIP procedure.

- \*Although mechanical lysis of cells is not recommended
- \*Successful cell lysis can be visualized under a microscope
- \* If you use sonication, keep your chromatin on ice at all times and do not pulse for more than 30 seconds at a time to ensure that proteins are not denatured due to excessive heat.

# Procedure:

## Step 3: Chromatin preparation (shearing/digestion)



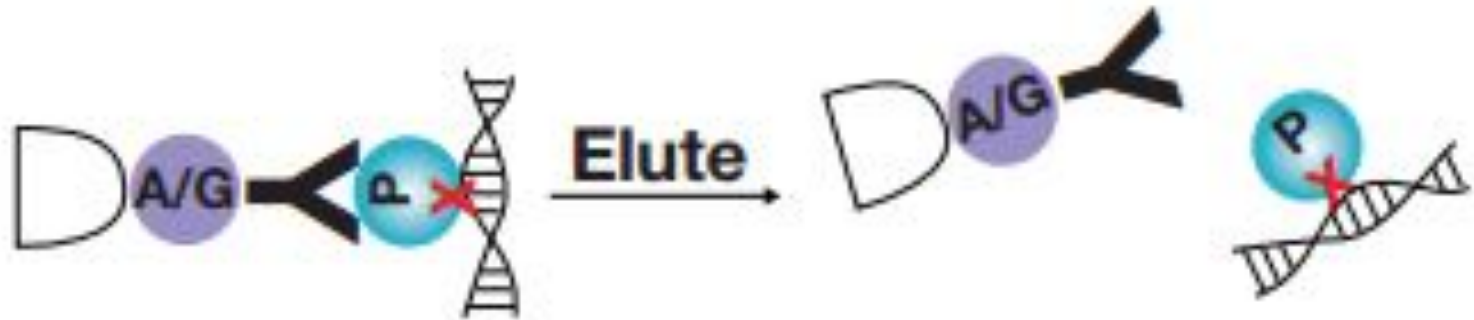
The extraction step yields all nuclear material, which includes unbound nuclear proteins, full-length chromatin, and the crosslinked protein–DNA complexes. DNA fragmentation is usually achieved either mechanically by sonication or enzymatically by digestion with nucleases. Ideal chromatin fragment sizes range from 200 to >700 bp;

\* Result of digestion could be checked by electrophoresis

**Note:** This is a point where ChIP can be stopped. After shearing/digestion of the chromatin, it can be stored at  $-80^{\circ}\text{C}$ .

# Procedure:

## Step 4: Immunoprecipitation



To isolate a specifically modified histone, transcription factor, or cofactor of interest, ChIP-validated antibodies are used to immunoprecipitate and isolate the target from other nuclear components. This step selectively enriches for the protein–DNA complex of interest and eliminates all other unrelated cellular material.

The antibody–protein–DNA complex is affinity purified using an antibody-binding resin such as immobilized protein A, protein G, or protein A/G. . Protein A/G combines the affinities of both protein A and G without reducing the affinity of either for the antibody, and considered to be suitable for the wide ranges application.

# Procedure:

## Step 5: Reversal of crosslinking, and DNA clean-up



Enrichment of DNA bound to the protein of interest is the goal for ChIP. Before the specific DNA products of a ChIP experiment can be quantified, the crosslinks between protein and DNA must be reversed. This is typically done through extensive heat incubations (**addition of  $\text{NaHCO}_3$  causes antibodies to release from their target proteins, and incubation at 65° for 6 hours**) and/or digestion of the protein component with Proteinase K. Treatment with RNase A is recommended as well to obtain a more pure DNA sample.

A final purification of the DNA from any remaining proteins should be performed using phenol-chloroform extraction or spin columns designed for DNA purification.

**Note:** This is a point where ChIP can be stopped. After reversal of crosslinks and/or DNA purification, samples can be stored at  $-20^\circ\text{C}$ .



# Procedure:

## Step 6: DNA quantitation

One of the hallmarks of ChIP is the ability to quantitate the purified DNA products by qPCR. qPCR enables analysis of target protein– DNA complex levels in different experimental conditions.

The purified DNA can be further processed to create an NGS library for ChIP-Seq.

# Advantages/disadvantages

**Table 1. Chromatin Immunoprecipitation (ChIP) and Its Variations**

Variation	Advantages	Disadvantages
X-ChIP	<p>Used for studying proteins that are tightly bound to DNA, thus preferred for analyzing histone-chromatin interactions.</p> <p>Can be performed on all cell types, tissues, and organisms.</p> <p>Under optimal conditions, formaldehyde cross-linking can produce relatively small DNA fragments by sonication.</p> <p>Enables DNA-protein, RNA-protein, and protein-protein cross-linking.</p> <p>Reduces the chances of chromatin rearrangement during processing.</p> <p>Requires fewer cells than N-ChIP.</p>	<p>Overfixation can prevent effective sonication.</p> <p>Enzymatic digestion not possible following formaldehyde treatment, probably due to the destruction of the nuclease reactive sites.</p> <p>Formaldehyde can alter the binding properties of the antigens and thus the immunoreactivity.</p> <p>Formaldehyde is a potential carcinogen and extra precautions are needed during handling.</p>
N-ChIP	<p>High antigen specificity for the analysis of histones and their isoforms.</p> <p>PCR amplification of the immunoprecipitated DNA is not needed because DNA recovery is higher.</p> <p>Specificity of the binding is more predictable because the antisera is raised against unfixed proteins.</p>	<p>High concentrations of nuclease may overdigest the chromatin, leading to subnucleosomal proteins, thus hindering the detection of protein-DNA interactions.</p> <p>Not all the nuclease-digested proteins are solubilized. Thus, a fraction of the chromatin is retained with the nuclear pellet and eliminated from the assay.</p> <p>Chances of chromatin rearrangement during processing are likely.</p>

**Thanks  
for  
attention**