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Chromatin immunoprecipitation: five steps to great results

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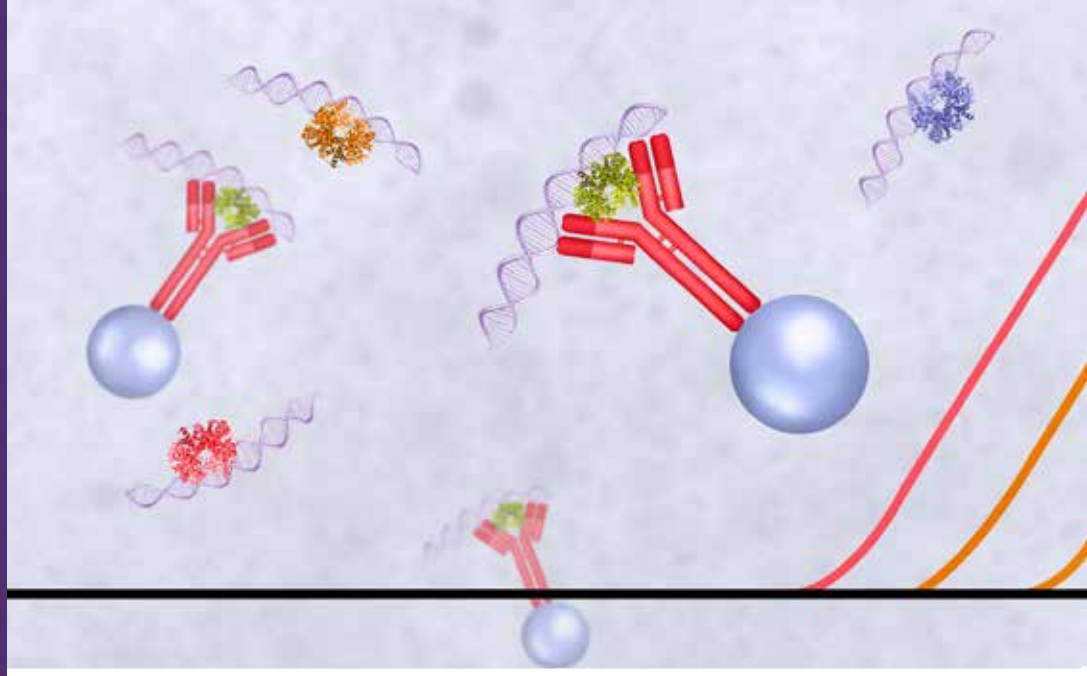
Introduction

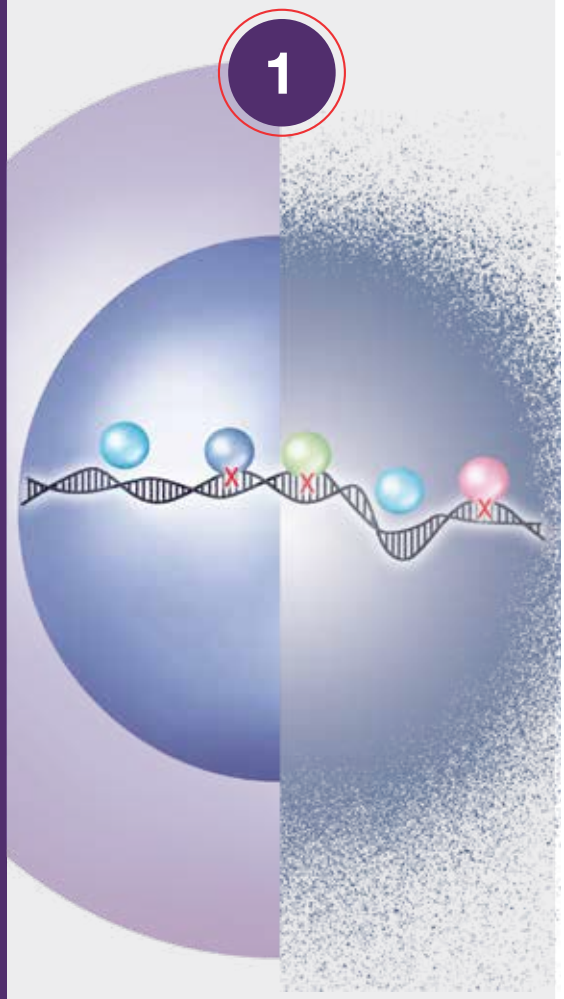
The discovery and use of antibodies in life science research has been critical to many advancements across applications, including chromatin immunoprecipitation (ChIP). The ChIP technique is a powerful tool in the investigation of protein–DNA interactions within a cell. Currently, ChIP is used to help determine the specific locations of histone modifications, transcription factors, and other proteins of interest in relation to genomic DNA, enabling scientists to elucidate gene function and regulation in the cell.

We support advancements in discovery research with a variety of tools that are developed, manufactured, and produced to a high standard of quality. We verify our procedures and provide documentation with our products so that researchers can spend their time obtaining answers to their experimental questions.

While using validated* antibodies to target a specific protein is crucial for optimal ChIP results, all steps in the ChIP workflow are critical to obtaining great results.

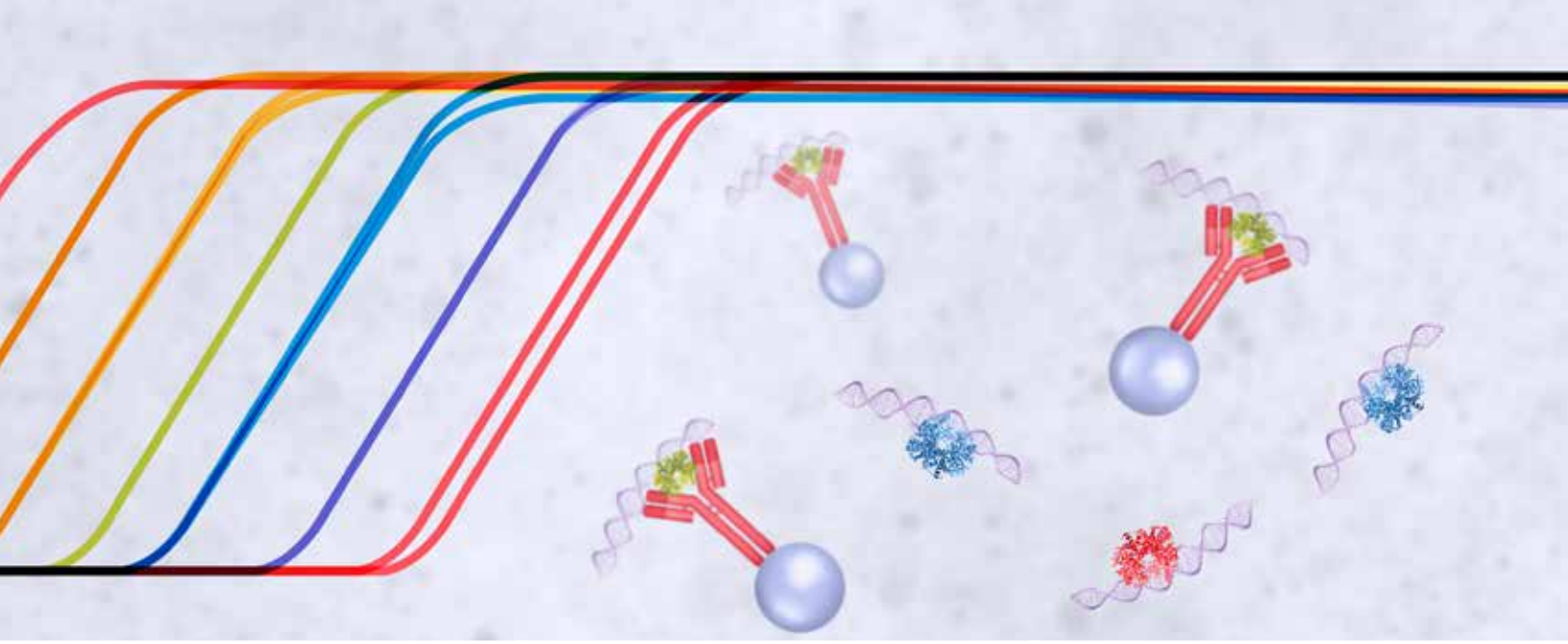
Consider these five steps to help achieve success in ChIP experiments.





Step 1: Crosslinking and lysis

Step 2: Chromatin fragmentation



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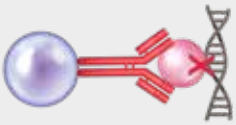

Step 3: Immunoprecipitation

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Step 4: DNA preparation

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Step 5: DNA quantitation

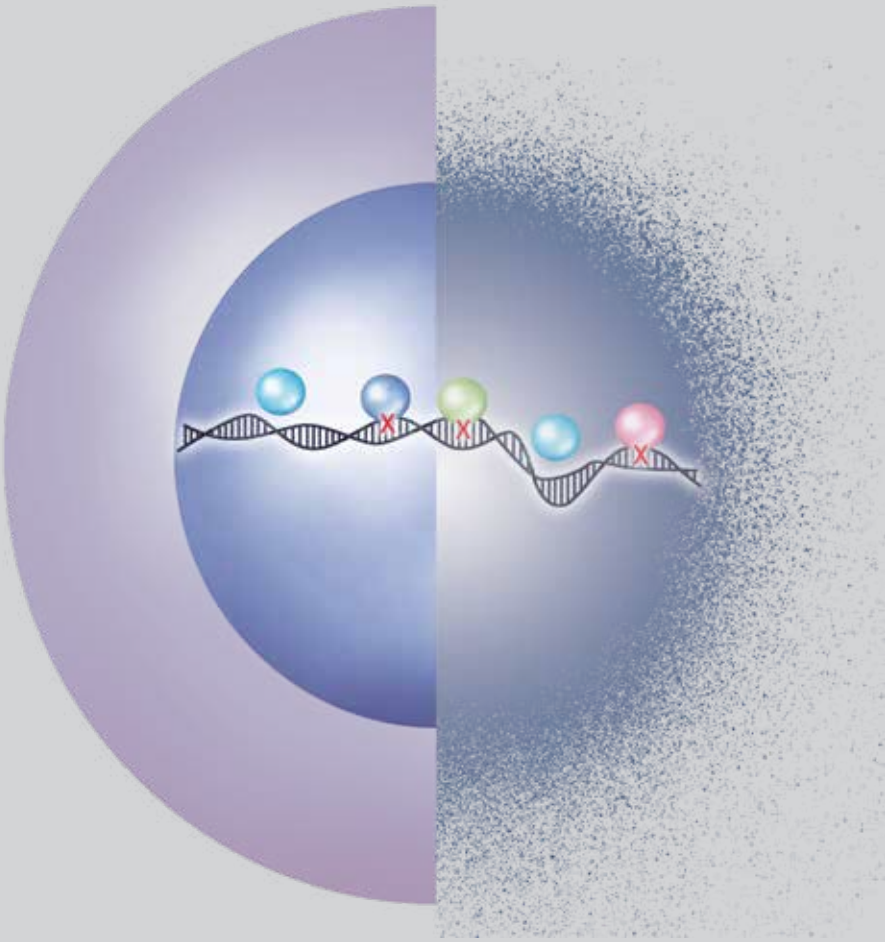
1 Step 1: Crosslinking and lysis

This step is very important and time-sensitive. DNA–protein and protein–protein interactions are locked into place by crosslinking, commonly with formaldehyde. This step ensures that chromatin is stabilized and preserved throughout the ChIP analysis. In the case of very strong DNA–protein interactions, native ChIP can be performed without crosslinking. Following crosslinking of the chromatin, lysis of cell membranes liberates the cellular components.

Find out more at **thermofisher.com/protein-DNA** and **thermofisher.com/cell-lysis**

Product highlights

- Thermo Scientific™ Pierce™ 16% Formaldehyde (w/v), methanol-free (Cat. No. 28906)
- Thermo Scientific™ Pierce™ Chromatin Prep Module (Cat. No. 26158)



Crosslink protein and DNA together to lock in place, and then lyse cell membranes to release the protein–DNA complexes.

2 Step 2: Chromatin fragmentation

The nuclear material derived from crosslinking and lysis contains a mixture of unbound nuclear proteins and crosslinked chromatin. The resulting crosslinked genomic DNA needs to be sheared into smaller fragment sizes, ideally between 200 and 800 bp, to enable good resolution. Whether fragmentation is performed by sonication or nuclease/enzymatic digestion, this step needs optimization for each cell line.

Product highlights

- Thermo Scientific™ Micrococcal Nuclease Solution (MNase, Cat. No. 88216)
- Invitrogen™ Proteinase K Solution, RNA grade (Cat. No. 25530049)
- Ion Torrent™ Covaris™ M220 Focused-ultrasonicator™ Instrument (Cat. No. 4482277)

Shearing or digestion of chromatin into smaller fragment sizes.

3 Step 3: Immunoprecipitation

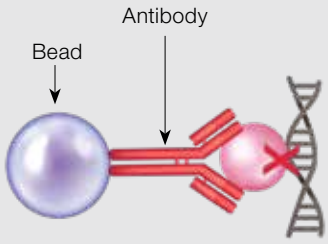
Antibodies are crucial for a successful ChIP experiment. Choosing the right antibody can be challenging but is necessary to ensure that your experiment produces accurate data. Ideally, choose an antibody that is already validated* for ChIP and proven to be specific. Advantages of monoclonal antibodies generally include being more specific and less cross-reactive, having low background, and performing consistently across batches compared to polyclonal antibodies. Polyclonal antibodies, however, can recognize multiple epitopes, enabling recognition of multiple conformations of the protein of interest in the cell. We also offer oligoclonal antibodies, which provide the benefits of minimal lot-to-lot variation and recognition of multiple epitopes, for a growing number of targets.

Magnetic or agarose beads are added to isolate the antibody–protein–DNA complex. The beads are incubated with the complex and washed extensively. The type and volume of beads for this step needs to be optimized, as high background or nonspecific binding can occur. Magnetic beads are easy to separate and offer reproducible results, while agarose beads have a higher binding capacity and binding surface.

Find out more at **thermofisher.com/ip**

Product highlights

- Thermo Scientific™ Pierce™ Agarose ChIP Kit (Cat. No. 26156)
- Thermo Scientific™ Pierce™ ChIP-Grade Protein A/G Magnetic Beads (Cat. No. 26162)
- Applied Biosystems™ MAGnify™ Chromatin Immunoprecipitation System (Cat. No. 492024)
- Invitrogen™ Dynabeads™ Protein G for Immunoprecipitation (Cat. No. 10004D)
- Invitrogen™ Dynabeads™ Protein A for Immunoprecipitation (Cat. No. 10002D)
- Antibodies for epigenetic research and tested for ChIP applications can be found at **thermofisher.com/epigeneticabs**
- Search our whole portfolio of primary antibodies at **thermofisher.com/primaryantibodies**



Capture and isolate protein–DNA complexes using protein-specific antibodies.

4 Step 4: DNA preparation

The isolated and purified protein–DNA complexes are now ready for reversal of the crosslink in preparation for DNA quantification. Crosslink reversal is typically done via extensive heat incubation and/or digestion of the protein with proteinase K. Proteinase K digestion also eliminates nucleases, preventing DNA degradation.

RNase A treatment is recommended as an additional step toward a purer DNA sample. A final DNA purification is performed using phenol/chloroform extraction or a DNA purification kit. The DNA is now ready for analysis.

Product highlights

- Invitrogen™ Proteinase K (fungal) (Cat. No. 25530015)
- Invitrogen™ PureLink™ RNase A (Cat. No. 12091021)
- Invitrogen™ PureLink™ PCR Purification Kit (Cat. No. K310002)

Crosslink reversal and DNA cleanup.

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Step 5: DNA quantitation

The purified DNA fragments are now ready to be quantitated. Normalizing samples for qPCR can be performed using the Thermo Scientific™ NanoDrop™ spectrophotometer—a simple way to quantitate DNA and assess for solvent contaminants—or using the Invitrogen™ Qubit™ 4 Fluorometer, which can accurately quantitate low sample levels to normalize for qPCR, ChIP-Seq, or ChIP-chip.

Invitrogen™ SYBR™ Green fluorescent dye is the most widely used DNA-based qPCR chemistry. SYBR Green dye fluoresces when bound to double-stranded DNA (dsDNA), and the fluorescence is proportional to the amount of dsDNA. Alternatively, you can design your own custom probes using the Applied Biosystems™ TaqMan® design tool to choose from our portfolio of proprietary dyes and quenchers. Using these dyes, a qPCR instrument can amplify and detect multiple samples for quantification of the target DNA molecules.

Find out more at **thermofisher.com/chipanalysis**

Product highlights

- Thermo Scientific™ NanoDrop™ One/OneC spectrophotometer (**thermofisher.com/nanodrop**)
- Applied Biosystems™ TaqMan® probes (**thermofisher.com/taqman**)
- Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix (Cat. No. A25742)
- Applied Biosystems™ QuantStudio™ qPCR instrument portfolio (**thermofisher.com/quantstudio**)
- Invitrogen™ Qubit™ 4 Fluorometer and dsDNA assays (**thermofisher.com/qubit**)

DNA quantitation using qPCR.

Conclusion

Whether you are new to ChIP or an experienced researcher, consider these five effective steps to help ensure that your experiment is successful and provides you with meaningful data on protein–DNA interactions.

Get additional information, or a list of troubleshooting tips, at **thermofisher.com/chip5steps**

* The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibodies can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic uses.

Find out more at **thermofisher.com/chip5steps**

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