Published in final edited form as:

Methods Mol Biol. 2018 ; 1767: 257–269. doi:10.1007/978-1-4939-7774-1_14.

Chromatin Immunoprecipitation in Human and Yeast Cells

Jessica B. Lee and Albert J. Keung

Chemical and Biomolecular Department, North Carolina State University, Raleigh, NC

Abstract

Chromatin Immunoprecipitation (ChIP) is an invaluable method to characterize interactions between proteins and genomic DNA, such as the genomic localization of transcription factors and post-translational modification of histones. DNA and proteins are reversibly and covalently crosslinked using formaldehyde. Then the cells are lysed to release the chromatin. The chromatin is fragmented into smaller sizes either by micrococcal nuclease (MNase) or sonication and then purified from other cellular components. The protein-DNA complexes are enriched by immunoprecipitation (IP) with antibodies that target the epitope of interest. The DNA is released from the proteins by heat and protease treatment, followed by degradation of contaminating RNAs with RNase. The resulting DNA is analyzed using various methods, including PCR, qPCR, or sequencing. This protocol outlines each of these steps for both yeast and human cells.

Keywords

Chromatin; immunoprecipitation; antibody; crosslinking; yeast; human

1. Introduction

ChIP is a powerful technique that has been widely used to study the association of specific proteins, or their modified isoforms, with defined genomic regions. Crosslinked ChIP (X-ChIP) was first described in 1984 by Gilmour and Lis as a technique to study the association of RNA polymerase II with transcribed and poised genes in live bacteria (1). This study used UV light to irreversibly bind proteins to DNA, followed by disrupting the cells in detergent. Specific DNA-bound proteins could then be immunoprecipitated from the lysate. A year later, Soloman and Varshavsky modified the technique by using formaldehyde to reversibly crosslink proteins, which is now the most widely used method. Reversible crosslinking allows DNA to be released from proteins and purified for use in various analysis methods (2–5). ChIP has since been expanded for use in cells from various origins including yeast (6), drosophila (7), tetrahymena (8), *Caenorhabditis elegans* (9), various mammalian cell lines, and whole mouse embryos (10) for the analysis of transcription factors, histone occupancy and histone post-translational modifications. The protocol given below outlines the procedure for X-ChIP in both yeast and human cells. A flowchart of the X-ChIP process is given in Figure 1.

The first step in X-ChIP is the covalent fixation of the protein-DNA complexes through reversible crosslinking. This is commonly performed with formaldehyde, which can crosslink proteins and DNA molecules within ~2 angstroms of each other. This is suitable for proteins that directly bind to DNA, but may not be for proteins that indirectly associate with DNA, such as those in larger complexes. In some cases, crosslinking between proteins of a complex may be able to link indirectly associated proteins to DNA. Alternatively, long-range bifunctional cross-linkers can be used along with formaldehyde to extend the distance of crosslinking (11). The crosslinking step is omitted in native ChIP (N-ChIP), which is sometimes used for analyzing histones, because of their high affinity for DNA, or for antibody targets that bind tightly to DNA but are sensitive to crosslinking. X-ChIP is more widely used across a broad range of targets including histones and transcription factors. Thus, here we will discuss X-ChIP and refer to other protocols for N-ChIP (11).

2. Materials

2.1 Crosslinking of cells

1. Phosphate-buffered saline (PBS, pH 7.4).
2. Eleven percent formaldehyde solution: 0.1 M NaCl, 1 mM EDTA (pH 8.0), 0.5 mM EGTA (pH 8.0), 50 mM HEPES (pH 8.0), and 11% formaldehyde. Use a chemical hood and take safety precautions.
3. 1.25 M glycine.
4. Spectrophotometer to check concentration of yeast.
5. Trypsin (optional) for adherent human cells.
6. Table-top shaker.

2.2 Cell lysis

2.2.1 Yeast cell lysis

1. Zymolyase buffer: Mix together 13.6 mL of 1.1 M Sorbitol, 0.75 mL Tris-HCl (pH 7.4), 0.64 mL of water. Right before use add 10.5 μ L 2-mercaptoethanol.
2. Zymolyase 20T.
3. NP-S buffer: 0.5 mM spermidine, 0.075 % NP-40, 10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 5 mM MgCl₂, 1 mM CaCl₂, 1 mM 2-mercaptoethanol. Store at 4°C. Add 200 μ L of protease inhibitor to 1800 μ L NP-S buffer immediately before using.
4. 1 M sorbitol.
5. Microscope to check for lysis.

2.2.2 Human cell lysis

1. Lysis buffer I: 50 mM HEPES (pH 7.5), 140 mM NaCl, 10 % glycerol, 0.5 % NP-40, 0.25 % Triton-X 100.

2. Lysis buffer II: 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 10 mM Tris (pH 8.0).
3. Lysis buffer III: 1 mM EDTA, 0.5 mM EGTA, 100 mM NaCl, 0.1 % Na-Deoxycholate, 0.5% N-Lauroylsarcosine.
4. Microscope to check for lysis.

2.3 Chromatin Fragmentation

2.3.1 Micrococcal nuclease (MNase) digestion

1. MNase: 1/500 dilution of Thermo Scientific product number PI88216.
2. 0.5 M EDTA
3. 1 % agarose gel

2.3.2 Sonication

1. Sonicator
2. 1 % agarose gel
3. Lysis buffer III: see section 2.2.2
4. 10 % Triton X

2.4 Immunoprecipitate chromatin and purify DNA

1. Antibodies (*see* Notes 1 and 2)
2. Magnetic stand
3. Magnetic beads: e.g. Fisher Scientific product number 26162.
4. Low salt buffer: 0.1 % Triton X-100, 2 mM EDTA, 0.1 % SDS, 150 mM NaCl, 20 mM HEPES (pH8.0).
5. High salt buffer: 0.1 % Triton X-100, 2 mM EDTA, 0.1 % SDS, 500 mM NaCl, 20 mM HEPES (pH 8.0).
6. LiCl buffer: 0.5 M LiCl, 1 % NP-40, 1 % Na-deoxycholate, 100 mM Tris-HCl (pH7.5).
7. TE
8. Elution buffer: 10 mM Tris (pH 8.0), 1 mM EDTA, 1 % SDS.
9. RNase A

¹Whether an antibody will work in ChIP as well as its specificity are two important factors to consider when choosing antibodies. It is best to use an antibody that has been already validated for use in ChIP or other IP applications. Vendors will usually indicate if a specific antibody is suitable for ChIP. If the target does not have an antibody that works with ChIP, it can be tagged with commonly used epitope tags such as Myc, His, human influenza hemagglutinin (HA), T7, GST, or V5. To test the specificity of an antibody to a target epitope prior to performing ChIP, a Western blot can be performed along with a positive control (13).

²Beads with an isotype matched control immunoglobulin (Ig) should be used as a negative control to provide a baseline for non-specific binding. This helps determine if a signal is real, since ChIP measurements are relative and not absolute. This control is very important and can save large amounts of time and resources.

10. Proteinase K
11. Thermal mixer
12. Silica-based DNA purification kit or reagents for phenol-chloroform extraction
13. qPCR supplies and equipment (optional)

3. Methods

3.1 Protein-DNA crosslinking

The protocols that follow are for two IP reactions, but can be linearly scaled to accommodate more.

3.1.1 Yeast cell crosslinking

1. For yeast, approximately $5\text{--}10 \times 10^9$ cells will be needed per IP reaction (*see* Note 3). They can be grown to the growth phase of interest.
2. Spin down cells at 2000 g for 5 minutes at room temperature and dissolve pellet with 9 mL of PBS.
3. Add 1/10 volume (1 mL) of fresh 11 % formaldehyde solution into cells (*see* Note 4).
4. Swirl gently at ~80 rpm on a tabletop shaker to fix cells for 10 minutes at room temperature. Time and temp may vary (*see* Note 5).
5. Quench the reaction by adding 1 mL of 1.25 M glycine (10 $\times$) for 5 minutes at room temperature (*see* Note 6).
6. Centrifuge cells at 2000 g for 5 minutes at 4°C.
7. Decant the media completely. Wash the cells once with 25 mL of 1M sorbitol at room temperature (*see* Note 7). Centrifuge at 2000 g for 5 minutes. Decant the supernatant.
8. Optional stopping point: Spin cells, remove supernatant, then flash freeze them in liquid nitrogen (or dry ice and EtOH) and store pellet at -80°C .

³Typically, because some material is lost during each step of the process, using more cells for each reaction results in better sample quality and data, but it may be difficult to generate a large number of cells. When determining the number of cells to use, other considerations include the abundance of the protein or histone modification and the quality of the antibody. The number of cells and volumes given in the protocol are suggestions. When possible, it is beneficial to empirically determine the minimum amount of cells that produces a higher signal-to-noise ratio through a titration of cell number and antibody concentration (5).

⁴For suspension cells, if timing is crucial or if a mechano-osmolarity related phenotype is being studied, step 2 can be skipped to avoid confounding effects of the sample due to handling prior to fixation. Formaldehyde would instead be added directly to the cell media to make the final concentration 11 % formaldehyde.

⁵The duration of the crosslinking step is very important and needs to be optimized for the epitope of interest. If the cells are crosslinked for too short of a time, there will be inefficient crosslinking, and for too long the epitope can be obscured, preventing the antibody from binding. Epigenetic changes and the dynamic binding of transcription factors can be very rapid, so it is important to minimize the time from when cells are in the state of interest to when they are fixed to avoid introducing artifacts from the handling of cells prior to fixation. In addition, it is important to consider that as fixation occurs over several minutes, proteins that transiently bind DNA for even a few seconds could be crosslinked by the formaldehyde.

⁶The glycine reacts with excess formaldehyde and helps prevent over-fixation of the epitope. This improves reproducibility of fixation since washing out the formaldehyde from cells would take time and vary depending on experiment and user.

⁷Sorbitol helps with balancing the osmolarity of the solution.

3.1.2 Human cell crosslinking

1. For transcription factors, use $20\text{--}50 \times 10^6$ cells for each IP reaction. For histones, use $5\text{--}10 \times 10^6$ for each IP reaction. This difference in amounts of cells is recommended because genome-bound transcription factor abundance is typically much lower than genome-bound histones (*see* Note 3).
2. For adherent cells, detach using trypsin. Spin down the cells at 300 g for 5 minutes and reconstitute with 9 mL of PBS. If cells are not adherent, spin them down and reconstitute with 9 mL of PBS. Do not centrifuge too fast since human cells are more fragile than yeast.
3. Add 1/10 volume (1 mL) of fresh 11 % formaldehyde solution into cells (*see* Note 4).
4. Swirl gently at ~80 rpm (for a standard shaker orbit of 1 inch) on a tabletop shaker to fix cells for 10 minutes at room temperature. Time and temp may vary (*see* Note 5).
5. Quench the reaction by adding 1 mL of 1.25 M glycine (10×) for 5 minutes at room temperature (*see* Note 6).
6. Centrifuge cells at 300 g for 5 min at 4°C.
7. Decant the media completely. Wash cells twice with cold PBS.
8. Optional stopping point: Spin cells, remove supernatant, then flash freeze them in liquid nitrogen (or dry ice and EtOH) and store pellet at -80°C .

3.2 Cell lysis

3.2.1 Yeast cells

1. Add 200μL of protease inhibitor to 1800μL NP-S buffer. This prevents degradation of the epitope.
2. Resuspend cell pellet in 14 mL zymolyase buffer with freshly added 2-mercaptoethanol (*see* Notes 8 and 9).
3. Add 1 mL of zymolyase buffer with 7 mg zymolyase 20T to the cell suspension. Incubate for 45 minutes at 30°C with gentle rotation.
4. Harvest the spheroplasts (centrifuge at 3000 g for 5 minutes) and wash with 1M sorbitol.
5. Resuspend spheroplasts in 1 mL NP-S buffer with protease inhibitors and spin at maximum speed for 10 minutes at 4°C (*see* Notes 10 and 11).

⁸With yeast, the cell wall must be first digested with zymolyase buffer to form spheroplasts. Alternatively, yeast cell walls can also be broken via bead beating (14). The protocol given contains directions for the use of zymolyase, which is gentler but more expensive than bead-beating (which generates heat).

⁹The purpose of 2-mercaptoethanol is to inhibit the oxidation of free sulfhydryl residues thereby minimizing protein crosslinking that could produce dimers and higher order oligomers.

¹⁰The amount of time for lysis may have to be adjusted and can be visualized by a microscope, using a sample of unlysed cells for comparison.

6. Remove supernatant and resuspend the pellet in 600 μ L NP-S buffer with protease inhibitors.
7. Check for cell lysis of spheroplasts using a microscope.
8. An optional short sonication step can be added if spheroplasts are not lysing in the NP-S buffer.

3.2.2 Human cells

1. Add protease inhibitors to ALL lysis buffers. This prevents degradation of the epitope. Resuspend cell pellet in 5 mL of lysis buffer I. Rock at 4°C for 10 minutes.
2. Spin down at 1000 g for 10 minutes at 4°C.
3. Aspirate and resuspend the pellet in 5 mL of lysis buffer II. Rock at 4°C for 5 minutes. Spin down at 1000 g for 10 minutes at 4°C.
4. Aspirate and resuspend pellet in 2 mL lysis buffer III (*see* Note 11).
5. Check for cell lysis using a microscope.

3.3 Chromatin fragmentation

DNA fragmentation is usually achieved using one of two methods: sonication or digestion with micrococcal nuclease (MNase). Sonication provides the most randomized fragmentation, but it is more labor intensive, heats the sample, and can cause foaming which most often ruins the sample. MNase has higher affinity for linker DNA (regions unprotected by nucleosomes) and thus fragments chromatin less randomly. However, MNase is more reproducible, more amenable to high throughput preparation since a sonicator is not required, and usually requires less hands-on time. Both methods are described below, and both need to be optimized for the cell line used. The ideal fragment size is typically 500–1000 bp, which can be checked using an agarose gel. MNase digestion creates distinct bands for mononucleosomes (~150 bp), dinucleosomes (~300 bp), trinucleosomes (~450 bp), etc. when run on a gel. Fragments run on a gel after sonication will produce a smear.

3.3.1 Micrococcal nuclease (MNase) digestion

1. Add 1 unit MNase (1 μ L of 1/500 dilution of Thermo Fisher MNase product number PI88216). This concentration should be titrated and tested.
2. Incubate at 37°C for 20 minutes.
3. Stop the digestion by adding 0.5 M EDTA to get a final concentration of 20 mM (24 μ L of 0.5 M EDTA stock solution).

¹¹Both yeast and human cells are lysed using detergents (in NP-S buffer or Lysis buffer 1) that break the membrane, and the nucleoprotein complexes are extracted (in NP-S buffer or Lysis buffer 2 and 3). The presence of detergents and/or salts in the lysis buffers will not affect the nucleoprotein complexes, so they will remain intact for the immunoprecipitation.

4. Run the digested DNA on a 1 % agarose gel to make sure most of the bands/smear is between 500 and 1,000 bp. Digest for a longer/shorter time or titrate the MNase concentration if needed (*see* Note 12).
5. Split into two 1.5 mL microfuge tubes (*see* Note 13).
6. Reserve 5–20 % of prepared DNA for input. Store at –20°C. The input will later be treated with proteinase, reverse crosslinked, and analyzed alongside the samples that undergo IP (*see* Note 14).

3.3.2 Sonication

1. Sonicate for 20 seconds at constant 30 % amplitude.
2. Rest for 40 seconds on ice.
3. Repeat steps 1 and 2 for 12–18 times (*see* Note 15).
4. Run a small sample of the sonicated DNA (or a spare extra sample) on a 1% agarose gel to make sure most of the smear is between 500 and 1,000 bp. Sonicate a few more times if needed (*see* Note 12).
5. Bring each sample up to 3 mL Lysis buffer III plus 1/10 volume of 10 % Triton X (1% final) and split into two 1.5 mL microfuge tubes. Spin out debris at 21,000 g for 10 minutes at 4°C. Keep the supernatant (*see* Note 13).
6. Reserve 5–20 % of prepared DNA for input. Store at –20°C. The input will later be treated with proteinase, reverse crosslinked, and analyzed alongside the samples that undergo IP (*see* Note 14).

3.4 Chromatin immunoprecipitation and purification of DNA

Immunoprecipitation (IP) uses magnetic beads (e.g. Fisher Scientific catalog number 26162) linked to an antibody that binds an epitope of interest (see Preparation of magnetic beads and antibody mixture). The prepared beads are added to the fragmented chromatin and bind to the DNA-protein complexes containing the target epitope, which can then be separated using a magnet. The remaining chromatin in the supernatant is removed. The beads, with the chromatin bound to them, are then washed with sequentially more stringent washes—low salt buffer, high salt buffer, then LiCl buffer—to remove non-specifically bound material. The separated chromatin is then heated to reverse the crosslinking. RNA and protein are degraded using RNase and proteinase, respectively. The remaining DNA can be purified using phenol-chloroform extraction followed by alcohol precipitation or a silica matrix spin column kit.

¹²Smaller fragments will give better spatial resolution, but could be harder to bioinformatically map back to the genome when doing whole genome sequencing following ChIP.

¹³Prior to fragmentation, chromatin is not soluble and will pellet during centrifugation. Post fragmentation, chromatin will become soluble and partition to the supernatant phase post centrifugation.

¹⁴The input is used to control for bias in chromatin fragmentation and is used to normalize immunoprecipitated samples. Each qPCR signal must be normalized to the input of the same genomic sequence to account for variation in PCR efficiency. Efficiency will vary for different sequences and primers. Additionally, there may be different amounts of each sequence depending on where the chromatin was fragmented.

¹⁵Avoid sample foaming and heating of sample by allowing it to rest on ice. Maintaining identical height and sample volumes is important for reproducibility.

1. Add antibody-bound magnetic beads (50 μ L for yeast cells or 100 μ L for human cells) to each IP (see section on Preparation of magnetic beads and antibody mixture). Keep samples on ice at all times.
2. Precipitate beads using magnetic stand by holding sample next to the magnet for ~30 seconds.
3. Wash the pellet 7 times with 1.5 mL as follows: 3 times with low salt buffer, once with high salt buffer, once with LiCl buffer, and twice with 1.5 mL TE (see Note 16 and 17). Between each wash, precipitate beads using a magnetic stand. Centrifuge at 1000 g for 3 minutes and remove residual TE. Do not spin at max speed as the beads will deform.
4. Resuspend the beads in 250 μ L elution buffer. Elute the DNA-protein complex from beads at 65°C for 30 minutes with 1000 rpm (for a standard orbit of 2 mm) shaking on a thermal mixer. Spin down the beads at 20,000 g for 1 minute and keep the supernatant (250 μ L).
5. Reverse the crosslinking by incubating the samples overnight at 65 °C on thermal mixer. Thaw input samples, add elution buffer up to a final volume of 250 μ L, and reverse crosslinks in the same manner. For the remaining steps, treat the input the same as the IP reactions.
6. Add 1 volume (250 μ L) of TE to each IP reaction and the input. Add RNase A to 0.2 μ g/ μ L final concentration. Incubate at 37 °C for 2 hours.
7. Add 5 μ L 20mg/mL proteinase K (final concentration: 0.2 μ g/ μ L) and incubate at 55 °C for 2 hours (see Note 18).
8. Extract DNA with silica spin columns (use PCR clean up protocol) and elute in 100 μ L ddH₂O, or use phenol-chloroform extraction (see Note 19).
9. If running qPCR analysis of IP samples, use 5 μ L per qPCR reaction. Run standard curves using serial diluted input samples (1:10, 1:100, 1:1000, 1:10000). For input samples, dilute 1:200 and use 5 μ L per qPCR reaction.

3.5 DNA analysis

The DNA collected from ChIP can be analyzed in a variety of downstream assays including quantitative polymerase chain reaction (ChIP-qPCR) and high-throughput sequencing (ChIP-seq). Table 1 gives an overview of each method.

¹⁶Efficient washing is critical to reduce background noise. If there is a lot of background noise, the stringency (controlled by salt concentration) and/or the number of washes can be increased (15).

¹⁷After each wash buffer is added, we recommend allowing it to mix gently using a rotatory paddle mixer for 5 minutes.

¹⁸RNase is added because high levels of RNA can interfere with DNA purification when using PCR purification kits. Competition with DNA binding to kit columns reduces yields. Proteinase K cleaves crosslinks between proteins and DNA to aid in DNA purification.

¹⁹The phenol-chloroform method can provide better yield of DNA, but may suffer from organic solvent contamination. DNA recovery can vary from sample to sample, so downstream data analysis cannot directly compare the amount of each chromatin fragment quantitatively. Rather, measurements of fragment abundance should be normalized to another genomic region using the same exact sample that acts as an internal control for variable DNA recovery. These genomic regions typically have known stable amounts of a transcription factor or histone modification and are thus used for normalization.

3.6 Preparation of magnetic beads and antibody mixture

Consult the manufacturers' instructions on how to bind the antibody to the magnetic beads. The following steps are an example protocol for a single IP reaction. It can be scaled for the number of reactions needed.

1. Resuspend the magnetic beads by gentle vortexing or by pipetting up and down.
2. Take an aliquot of resuspended beads (30 μ L per IP reaction for yeast or 60 μ L per IP reaction for human cells).
3. Place the aliquot on magnetic stand to separate beads from the storage solution. Remove the storage solution completely.
4. Wash magnetic beads 3 times with 1 mL fresh BSA/PBS (0.5% BSA, filtered).
5. Resuspend the beads in 250 μ L of BSA/PBS.
6. Add 2–10 μ g of antibody (see Note 20, 1, and 2) and incubate at RT for 20 minutes with gentle mixing.
7. Wash beads 3 times in 1.5 mL BSA/PBS, and then collect with a magnet.
8. Resuspend beads in 100 μ L BSA/PBS.

Acknowledgments

These protocols were developed with help from Aneeshkumar Arimbasseri and Kwan T. Chow. This work was supported by funds from North Carolina State University, NIH Grant R21E023377, the GI Bill, and the Simons Foundation Grant 495112.

References

1. Gilmour, DS., Lis, JT. Proc. Natl. Acad. Sci. Vol. 81. USA: 1984. Detecting protein-DNA interactions in vivo: Distribution of RNA polymerase on specific bacterial genes.
2. Kuo M-H, Allis CD. In Vivo Cross-Linking and Immunoprecipitation for Studying Dynamic Protein:DNA Associations in a Chromatin Environment. *Methods*. 1999; 19(3):425–433. doi:<http://dx.doi.org/10.1006/meth.1999.0879>. [PubMed: 10579938]
3. Bulyk ML. DNA microarray technologies for measuring protein–DNA interactions. *Current Opinion in Biotechnology*. 2006; (17):1–9. [PubMed: 16417997]
4. Mikkelsen TS, Ku MC, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, Lee W, Mendenhall E, O'Donovan A, Presser A, Russ C, Xie XH, Meissner A, Wernig M, Jaenisch R, Nusbaum C, Lander ES, Bernstein BE. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature*. 2007; 448(7153):553–U552. DOI: 10.1038/nature06008 [PubMed: 17603471]
5. Kidder BL, Hu G, Zhao K. ChIP-Seq: Technical Considerations for Obtaining High Quality Data. *Nature immunology*. 12:918–922. November 10. DOI: 10.1038/ni.2117
6. Kurdستاني SK, Grunstein M. In vivo protein-protein and protein-DNA crosslinking for genomewide binding microarray. *Methods*. 2003; 31(1):90–95. [PubMed: 12893178]

²⁰There are two different type of beads used for immunoprecipitation: magnetic or agarose beads. Magnetic beads are easier to separate and easier to visualize in the tube, which reduces the loss of material during the separation process. In contrast, porous agarose beads have a higher binding capacity because they have a higher surface area. The beads can be purchased with various antibody binding proteins linked to their surfaces: Proteins A, G, or A/G. Protein A has the better affinity for rabbit polyclonal antibodies, and protein G has better affinity for a range of antibodies. For more information, refer to charts from the manufacturer, e.g. ThermoFisher Scientific (13, 16).

7. Breiling A, Turner BM, Bianchi ME, Orlando V. General transcription factors bind promoters repressed by Polycomb group proteins. *Nature*. 2001; 412(6847):651–655. DOI: 10.1038/35088090 [PubMed: 11493924]
8. Dedon PC, Soultis JA, Allis CD, Gorovsky MA. A simplified formaldehyde fixation and immunoprecipitation technique for studying protein-DNA interactions. *Analytical biochemistry*. 1991; 197(1):83–90. [PubMed: 1952079]
9. Mukhopadhyay A, Deplancke B, Walhout AJ, Tissenbaum HA. Chromatin immunoprecipitation (ChIP) coupled to detection by quantitative real-time PCR to study transcription factor binding to DNA in *Caenorhabditis elegans*. *Nature protocols*. 2008; 3(4):698–709. DOI: 10.1038/nprot.2008.38 [PubMed: 18388953]
10. Botquin V, Hess H, Fuhrmann G, Anastassiadis C, Gross MK, Vriend G, Scholer HR. New POU dimer configuration mediates antagonistic control of an osteopontin preimplantation enhancer by Oct-4 and Sox-2. *Genes & development*. 1998; 12(13):2073–2090. [PubMed: 9649510]
11. Zeng PY, Vakoc CR, Chen ZC, Blobel GA, Berger SL. In vivo dual cross-linking for identification of indirect DNA-associated proteins by chromatin immunoprecipitation. *Biotechniques*. 2006; 41(6):694+.doi: 10.2144/000112297 [PubMed: 17191611]
12. Green, MR., Sambrook, J. *Molecular Cloning*. 4. Vol. 3. Cold Spring Harbor; New York: 2012.
13. A step-by-step guide to successful chromatin immunoprecipitation (ChIP) assays. [Accessed Mar. 8, 2017] ThermoFisher Scientific. 2016. <https://www.thermofisher.com/us/en/home/life-science/antibodies/antibodies-learning-center/antibodies-resource-library/antibody-application-notes/step-by-step-guide-successful-chip-assays.html>. 2017
14. Gibbons LE, Brangs HCG, Burden DW. *Bead Beating: A Primer*. Random Primers. 2014; (12)
15. Gade, P., Kalvakolanu, DV. Chromatin Immunoprecipitation Assay as a Tool for Analyzing Transcription Factor Activity. In: Vancura, A., editor. *Transcriptional Regulation: Methods and Protocols*. Springer New York; New York, NY: 2012. p. 85-104.
16. Immunoprecipitation. [Accessed Mar. 8, 2017] ThermoFisher Scientific. 2017. <https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-assays-analysis/immunoprecipitation.html>

Figure 1. Flowchart of ChIP protocol

In this example, ChIP targeting a histone post-translational modification, e.g. H3K9me2, is illustrated. **A)** Proteins such as histones are crosslinked to DNA, black lines, using formaldehyde. Crosslinking is shown as purple Xs. **B)** For yeast cells, the cell wall is digested using zymolase. **C)** Then, both the yeast and human cells are lysed. **D)** Next, the chromatin is broken into fragments about 500 bp in length using either sonication or digestion. **E)** The protein-DNA complexes, containing the histone modification of interest, are separated using magnetic beads coated with antibodies that bind the modification. **F)** The magnetic beads are then removed and the crosslinking is reversed by heating. **G)** Proteins and RNA are degraded using proteinase K and RNase, and the DNA is purified. **H)** The recovered DNA is analyzed by various methods, e.g. qPCR. An input is taken before the immunoprecipitation step and reserved until the step removing the magnetic beads (*see* Note 14).

Table 1

Overview of the ChIP-based methods taken from (12)

Method	Ease of use	Cost and time involved	Application
ChIP-qPCR	Easy	Cheap, 3–4 days	For analysis of a limited number of genomic regions
ChIP-seq	Medium	Expensive, 2–3 weeks, Additional data analysis and bioinformatics management required	To investigate the entire genome for distribution of proteins or histone PTMs