Protocol

Chromatin Immunoprecipitation (ChIP)

Michael F. Carey, Craig L. Peterson, and Stephen T. Smale

INTRODUCTION

Chromatin immunoprecipitation (ChIP) is an invaluable method for studying interactions between specific proteins or modified forms of proteins and a genomic DNA region. ChIP can be used to determine whether a transcription factor interacts with a candidate target gene and is used with equal frequency to monitor the presence of histones with post-translational modifications at specific genomic locations. In early ChIP studies, UV light from a transilluminator was used to cross-link proteins to DNA irreversibly. The cross-linked chromatin was then either sonicated or cleaved with restriction enzymes to generate smaller DNA fragments, followed by immunoprecipitation with the desired antibodies. The precipitated protein-DNA adducts were then purified, treated with a protease, and analyzed by dot blot or Southern blot using a radiolabeled probe derived from the cloned DNA fragment of interest. The use of formaldehyde as a reversible protein-DNA and protein-protein cross-linking agent for ChIP and the use of polymerase chain reaction (PCR) to detect precipitated DNA fragments were later added as components of the modern ChIP procedure. The protocol below represents a standard ChIP procedure for use in mammalian cells. Cross-linking is performed by adding formaldehyde to growing cells, and chromatin is prepared, sheared by sonication, and precleared to reduce nonspecific immunoprecipitation. Immunoprecipitation is performed with a specific antibody. After elution of the protein-DNA complexes from protein A- or protein G-agarose resin, the samples are heated to reverse the covalent cross-links. The DNA fragments are purified and analyzed by PCR or real-time PCR.

RELATED INFORMATION

The first chromatin immunoprecipitation (ChIP) assay was developed by Gilmour and Lis (1984, 1985, 1986) as a technique for monitoring the association of RNA polymerase II with transcribed and poised genes in *Escherichia coli* and *Drosophila*. The use of formaldehyde in the ChIP method was pioneered by Solomon and Varshavsky (Solomon et al. 1988). In their original studies, the association of histone H4 and RNA polymerase II with the *Drosophila hsp70* genes was monitored. Cells were treated with formaldehyde before and after heat shock, followed by shearing or restriction enzyme digestion of the DNA. Immunoprecipitation of cross-linked protein-DNA complexes containing histone H4 or RNA polymerase II was then carried out using specific antibodies. The covalent cross-links in the immunoprecipitates were reversed by heating, and the purified DNA fragments were analyzed by slot blot or Southern blot. The use of PCR as a detection method was first used by Hecht and Grunstein (Hecht et al. 1996) in their studies of SIR protein interactions in *Saccharomyces cerevisiae*. Soon thereafter, Rundlett et al. (1998) used ChIP with a PCR detection step to analyze histone modifications at specific loci. At approximately the same time, the ChIP procedure was adapted for use in mammalian cells, first with UV cross-linking and subsequently with formaldehyde cross-linking (Boyd and Farnham 1997; Wathelet et al. 1998; Parekh and Maniatis 1999). Although the emergence of ChIP as a common and robust technique coincided with the use of formaldehyde as a cross-linking reagent, ChIP can also be performed in the absence of cross-linking (referred to as native ChIP or N-ChIP) to examine proteins that remain stably associated with DNA during chromatin processing and immunoprecipitation (O'Neill and Turner 2003).

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Many variations on the basic protocol provided here have been used and can be found in the literature (Kuo and Allis 1999; Orlando 2000; Johnson and Bresnick 2002; O'Neill and Turner 2003; Ren and Dynlacht 2004) or on numerous websites, including the websites of companies that supply ChIP antibodies and reagents. In particular, the Abcam website contains a detailed ChIP protocol, along with excellent tips for designing experiments and troubleshooting. Methods papers describing the use of ChIP for genome-wide studies have also been published (Bernstein et al. 2004; Ciccone et al. 2004; Ren and Dynlacht 2004; Elnitski et al. 2006). Finally, specialized protocols have been published for ChIP when starting with unusually small numbers of cells (O'Neill et al. 2006; Acevedo et al. 2007; Attema et al. 2007). Additional technical information regarding the design and interpretation of ChIP experiments can be found in the published literature (Buck and Lieb 2004; Chaya and Zaret 2004; Das et al. 2004; Kim and Ren 2006; Southall and Brand 2007).

A protocol for **Purification of Nucleic Acids by Extraction with Phenol:Chloroform** (Sambrook and Russell 2006) is also available.

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <**!**>, and recipes for reagents marked with <**R**>.

Reagents

Agarose gel
Antibodies to native protein of interest (or a post-translationally modified form)
Antibody (control)
<**R**>Cell lysis buffer for ChIP (cold)
Cells (50-100 million per experiment)
<**R**>Dilution buffer
EDTA
<**R**>Elution buffer for ChIP
<**!**>Formaldehyde (37%)
Glycine (1.375 M)
Growth medium
<**R**>High-salt wash buffer for ChIP
Micrococcal nuclease (MNase)
<**R**>Micrococcal nuclease digestion buffer (MNase digestion buffer)
<**R**>Nuclei lysis buffer for ChIP
PCR or real-time PCR primers
<**!**>Phenol:chloroform (pH 8.0)
<**R**>Phosphate-buffered saline (PBS) (1X)
Protease inhibitors (Roche)
Protein A-agarose/salmon sperm DNA beads (Millipore) or Protein G-agarose/salmon sperm DNA beads (Millipore)
<**!**>Proteinase K (20 µg/uL)
<**R**>TE buffer (optional; see Step 30)

Equipment

Cell scraper
Centrifuge (benchtop)
Dishes or flasks (tissue culture)
Gel electrophoresis apparatus
Gel-loading tips (narrow, for aspiration; see Step 20)
Ice
Incubators preset to 55°C, 65°C
Microcentrifuge
Micropipettor with tips
PCR apparatus

Rotator
Sonicator
Spectrophotometer
Spin column (QIAGEN)
Tubes (conical, 15-mL)
Tubes (microcentrifuge, 1.5-mL)

METHOD

Cross-linking and Chromatin Preparation

Begin with ~50-100 million cells per experimental condition.
The time requirement for this portion of the method is ~2 h.

1. Add 27 µL of 37% formaldehyde per milliliter of cell culture (in growth medium on tissue culture plates or in tissue culture flasks) while slowly shaking the cells for 10 min at room temperature.
   The final concentration of formaldehyde is 1%.
2. Quench cross-linking by adding 100 µL of 1.375 M glycine per milliliter of culture.
3. Treat the cells as follows:

For adherent cells

   i. Remove the growth medium and dispose of it appropriately.
   ii. Wash cells twice with 10 mL of cold 1X PBS, and then scrape the cells into 1X PBS in a 15-mL conical tube.
   iii. Pellet the cells at 1500 rpm for 10 min at 4°C.

For nonadherent cells

   iv. Transfer the cells to a 15-mL conical tube.
   v. Pellet the cells at 1500 rpm for 10 min at 4°C.
   vi. Wash the cells twice with 10 mL of cold 1X PBS.

4. Resuspend the cell pellet in 10 mL of cell lysis buffer for ChIP (cold) and incubate for 10 min on ice.
5. Pellet the cell nuclei at 1000 rpm in a benchtop centrifuge for 10 min at 4°C. Aspirate the supernatant carefully.
6. (Optional) Resuspend the cell nuclei in 500 µL of MNase digestion buffer. Add MNase and incubate for 1 h on ice. Stop the digest by adding EDTA to a final concentration of 50 mM.
   The amount of MNase will need to be optimized according to final chromatin size in Step 8. A range of 50-300 U is a convenient starting point.
7. Resuspend the nuclear pellet in 1 mL of nuclei lysis buffer for ChIP supplemented with protease inhibitors and incubate for 10 min on ice.
8. Proceed with sonication. Ensure that the samples do not foam and are kept as cold as possible.
   Sonication conditions will need to be optimized. Typically, six 15-sec pulses followed by 45-sec rest periods at output 6.0 have been found to work. It is helpful to analyze each sample for proper sonication. For this analysis, reverse the cross-links and purify a small volume of chromatin as described below, and then analyze on an agarose gel. DNA should ideally be between 300 and 1000 bp in size.
9. Transfer chromatin to a 1.5-mL microcentrifuge tube and freeze at –80°C for storage.

Chromatin Preclearing and Immunoprecipitation

This portion of the method requires ~2 h, followed by an overnight incubation.

10. Thaw the chromatin on ice. Centrifuge samples at high speed in a microcentrifuge for 15 min at 4°C to pellet the precipitated SDS. Transfer the supernatant to a fresh microcentrifuge tube.
At this step, it is helpful to aliquot chromatin for storage at –80°C.

11. Measure the DNA concentration (A_{260}) of the chromatin using a spectrophotometer. Use nuclei lysis buffer for ChIP devoid of SDS as a blank.
Typically, the chromatin concentration should be >750 ng/µL. The A_{260}/A_{280} ratio should be ~1.4-1.6.

12. Aliquot 100 µg of chromatin per antibody to be used into microcentrifuge tubes.
It is important to have an irrelevant antibody such as α-glutathione S-transferase as a control.

13. Dilute the chromatin to a final volume of 300 µL with dilution buffer supplemented with protease inhibitors.

14. To preclear the chromatin, add 50 µL of protein A-agarose/salmon sperm DNA or protein G-agarose/salmon sperm DNA beads to the chromatin and rotate for 1-2 h at 4°C.
Use large-orifice tips or cut off the end of regular micropipettor tips for transfer of beads.

15. Centrifuge the chromatin samples at 3000 rpm for 5 min at 4°C. Transfer the supernatants to fresh microcentrifuge tubes.

16. Add relevant antibodies to the chromatin samples and rotate overnight at 4°C.
Consult supplier's information sheet for the amount of antibody to add. Typically, 5-10 µg is sufficient.

IP Washes and Elution

This portion of the method requires ~2 h, followed by an overnight incubation.

17. Add 50 µL of protein A-agarose/salmon sperm DNA or protein G-agarose/salmon sperm DNA (depending on antibody) beads to the chromatin samples on ice. Rotate for 2 h at 4°C.
Make sure bead slurry is completely resuspended before adding to samples.

18. Centrifuge the samples at 3000 rpm for 2 min at 4°C.

19. Collect 15 µL from the supernatant of the control antibody sample as a 5% input. Keep this sample on ice for later use.

20. Carefully aspirate the supernatants of all samples.
Be careful not to aspirate any beads. Narrow gel-loading tips are useful for careful aspiration.

21. Add 1 mL of high-salt wash buffer for ChIP to all samples and rotate for 10 min at room temperature.

22. Centrifuge the samples at 3000 rpm for 2 min at room temperature.

23. Carefully aspirate the supernatants and add 1 mL of high-salt wash buffer for ChIP. Rotate for 10 min at room temperature.

24. Repeat Steps 22-23 twice for a total of four high-salt washes.

25. Aspirate supernatants and wash twice with TE as above.

26. Resuspend beads and input samples in 300 µL of elution buffer for ChIP supplemented with 1 µL of proteinase K (20 µg/µL), and incubate samples for 2 h at 55°C.
Do not resuspend beads by pipetting. Instead, gently flick or vortex samples.

27. To reverse cross-links, incubate overnight at 65°C.

DNA Purification

The time required for DNA purification is ~1 h.

28. Centrifuge the samples at full speed for 5 min at room temperature.

29. Transfer supernatants to fresh microcentrifuge tubes.
*At this point, DNA can be purified by spin columns (QIAGEN) or standard phenol:chloroform extraction (see **Purification of Nucleic Acids by Extraction with Phenol:Chloroform** [Sambrook and Russell 2006]). If phenol:chloroform extraction is performed, yeast tRNA or glycogen carrier should be added.*

30. Resuspend DNA in 50 µL of H_2O or TE buffer and analyze by PCR or real-time PCR.
See Troubleshooting.

TROUBLESHOOTING

Problem: No specific signal is observed.
[Step 30]
Solution: There are several possible causes and solutions:

- *Antibodies do not efficiently precipitate protein from cross-linked or native chromatin samples.* Precipitation efficiencies can be monitored by Western blot. If immunoprecipitation is inefficient, even when incubated overnight, add more antibody. Alternatively, it may be necessary to identify or generate another antibody. A high percentage of commercially available antibodies were generated against peptides. Although anti-peptide antibodies sometimes bind their target antigens with high affinity, many do not.
- *The protein of interest does not cross-link efficiently to chromatin.* Cross-linking efficiency can sometimes be improved by adding protein-protein cross-linking reagents prior to formaldehyde cross-linking (Kurdistani and Grunstein 2003). However, some proteins will not cross-link efficiently because of the transient nature of their association with chromatin. Increasing the cross-linking time may be beneficial.
- *The protein epitope recognized by the antibody is masked in cross-linked chromatin.* Epitope masking is likely to be more problematic for monoclonal antibodies or peptide antibodies because these antibody preparations will usually recognize only a single epitope. Therefore, polyclonal antibodies generated against intact native proteins, fusion proteins, or large protein fragments are preferred.
- *The DNA fragments after sonication may be too large or too small.* Monitor the sizes of the DNA fragments on an agarose gel and adjust sonication time to yield fragments between 300 bp and 1000 bp.
- *The formaldehyde cross-linking may have been too short or too long.* Test various cross-linking times.
- *The protein A-antibody interaction may be disrupted during washing.* Reduce the stringency of washes or try protein G, which has a higher affinity for some antibody isotypes and for antibodies from some species.

Problem: High background is observed using the negative control antibodies.
[Step 30]
Solution: There are several possible causes and solutions:

- *The preclearing may have been inadequate.* Increase the time of preclearing with protein A-agarose resin or the quantity of beads for preclearing.
- *There is genomic or plasmid DNA contamination of buffers.* Perform control PCRs without chromatin template to monitor possible contamination.
- *The protein A resin may be of poor quality.* Test a different lot or supplier of resin.

DISCUSSION

ChIP-chip and ChIP-Seq

Chromatin immunoprecipitation is the most common method for examining the association of specific factors and modified histones with an endogenous DNA region of interest, as well as for studying protein-DNA interactions at a genome-wide scale. Since its initial development, the ChIP method has been expanded to include ChIP-chip and ChIP-Seq assays. In ChIP studies designed to identify the locations of transcription factors and histones at a genome-wide scale (Ren et al. 2000; Weinmann et al. 2002; Hanlon and Lieb 2004; Elnitski et al. 2006; Kim and Ren 2006), the precipitated DNA fragments were purified; amplified by whole-genome amplification, ligation-mediated (LM)-PCR, or other methods (see O'Geen et al. 2006); and then annealed to tiled DNA arrays

spanning an entire genome, a specific chromosome, or a large collection of putative promoter fragments (i.e., ChIP-chip). Detailed experimental strategies and procedures for these ChIP-chip (or ChIP-on-chip) methods can be found at the websites of companies that synthesize tiling arrays, including Agilent, NimbleGen, and Affymetrix. More recently, ChIP-sequencing (ChIP-Seq) methods, in which cross-linked, immunoprecipitated DNA is reverse cross-linked, fragmented, and analyzed by massively parallel DNA sequencing, have replaced ChIP-chip as the method of choice (Barski et al. 2007; Mardis 2007, 2008; Robertson et al. 2007; Jothi et al. 2008; Wang et al. 2008). The main advantage of ChIP-Seq is that it is thought to provide much more accurate and quantitative information because, unlike ChIP-chip, ChIP-Seq is not influenced by the highly variable and often weak annealing efficiencies of genomic DNA fragments to the immobilized oligonucleotides used on tiled ChIP-chip microarrays. ChIP-Seq is also thought to yield higher-resolution data than ChIP-chip because it provides the actual DNA sequences of precipitated fragments.

ChIP, ChIP-chip, and ChIP-Seq methods can provide invaluable information about the proteins associated with an individual control region of interest or the collection of genomic sites associated with a transcription factor, chromatin protein, or a histone possessing specific post-translational modifications. The results are often extremely useful for developing hypotheses regarding the functions of specific transcription factors or histone modifications. Conversely, these methods can be used to test hypotheses generated using other strategies, such as the hypothesis that a given transcription factor associates directly and consistently with control regions for genes expressed at specific developmental stages or in response to a specific stimulus. Furthermore, ChIP-chip and ChIP-Seq results are being used with increasing frequency to build transcription networks (Blais and Dynlacht 2005).

Limitations of the ChIP Methods

Despite the tremendous value of ChIP methods, it is important to be aware of their limitations. (1) The ChIP assay often yields low signals in comparison to negative controls, which can lead to inconclusive results; (2) it is difficult to determine the precise binding site for a factor because of the limited resolution of the assay; and (3) ChIP is not a functional assay and cannot by itself demonstrate the functional significance of a protein or modified histone found to be located at a genomic region of interest. This final limitation is especially problematic because many studies have now provided strong evidence that proteins associate with genomic sites at which they have no obvious function (Walter et al. 1994; Li and Johnston 2001; Martone et al. 2003; Phuc Le et al. 2005; Beima et al. 2006; Hollenhorst et al. 2007; Dong et al. 2008; Li et al. 2008). It has been difficult to design experiments to rigorously test the possibility that specific histone modifications, as opposed to transcription factor-DNA interactions, are not functionally important at specific genomic locations at which they are found. Nevertheless, given the nonfunctional interactions observed with transcription factors, it is important to keep in mind the possibility that modified histones exist at genomic locations at which the modification has no functional significance.

One additional limitation of the ChIP assay is that antibodies often have the potential to cross-react with other nuclear proteins, even when the antibodies appear to be highly specific in immunoprecipitation and Western blot experiments. An experiment that can reveal cross-reactivity, but that rarely is performed, involves a parallel ChIP analysis in cells lacking the specific factor targeted by the antibodies. An analysis of targets of the T-bet (T-box expressed in T-cells) transcription factor by Beima et al. (2006) provides one example of this control experiment. This ChIP-chip study identified novel T-bet target genes, which were carefully evaluated using a number of experimental strategies. One experiment was to monitor the ChIP signals obtained at candidate target genes when using the T-bet antibodies in T-bet-deficient cells. Surprisingly, ~10% of the candidate sites that exhibited strong signals in the original ChIP-chip analysis, as well as in follow-up ChIP experiments performed with individual PCR primer pairs, yielded positive results of similar magnitude in cells lacking T-bet. This result revealed that the T-bet antibodies, although highly specific on the basis of standard criteria, cross-reacted with other nuclear proteins. Thus, even the strongest ChIP signals must be interpreted with caution.

For ChIP-chip and ChIP-Seq studies, the difficulties associated with low signals and low signal-to-background ratios are further amplified. Whenever possible, it is important to use conventional ChIP assays to test the validity of results obtained in genome-wide studies. Another major issue for ChIP-chip and ChIP-Seq results is that the massive amount of data, combined with the complexity of gene regulation strategies, can make it difficult to draw meaningful conclusions. There has been a tendency in the literature to draw firm conclusions on the basis of trends that are observed,

even though there may be as many genes that deviate from the trend as those that conform to the trend.

Additional Considerations

Several additional points should be considered for this protocol:

1. Real-time PCR is usually the preferred method for analyzing specific DNA fragments in the immunoprecipitated samples. Results are often presented as "percent input" values, calculated by using real-time PCR to quantify the abundance of the DNA fragment of interest added to the ChIP reaction, with respect to the abundance of the DNA fragment found in the final immunoprecipitate.
2. As a critical negative control for ChIP experiments, real-time PCR can be used to monitor other genomic DNA fragments that are not expected to precipitate with the specific antibody used. Preferably, a series of real-time PCR primer pairs spanning the putative interaction site should be examined. A peak "percent input" signal at the putative interaction site would strengthen the evidence that the interaction is truly specific for the site. As a second critical negative control, parallel ChIP experiments should be performed with an antibody directed against a protein that should not be associated with the chromatin at the site of interest.
3. As mentioned in the Introduction to this protocol, ChIP can also be performed in the absence of cross-linking. ChIP performed in the presence and absence of cross-linking is sometimes referred to as X-ChIP and N-ChIP. For an excellent discussion of the advantages and disadvantages of X-ChIP and N-ChIP, as well as a detailed N-ChIP protocol, see O'Neill and Turner (2003). A similar protocol is also available on the Abcam website. The advantages of N-ChIP include a higher resolution, the ability to test the antibody's immunoprecipitation efficiency more effectively, and elimination of the concern that the antibody's ability to recognize its target protein will be compromised by cross-linking. Disadvantages of N-ChIP include the fact that the method is limited to proteins such as histones that associate with DNA with unusually high stability and the possibility that nucleosomes may rearrange during processing of the chromatin.
4. Chromatin can be prepared for ChIP solely by MNase digestion rather than sonication (O'Neill and Turner 2003). MNase digestion is the preferred method for fragmenting chromatin for N-ChIP, whereas chromatin for X-ChIP is usually sonicated, sometimes in conjunction with MNase digestion.
5. One of the major limitations of ChIP is the need for antibodies that recognize the target protein with a high degree of specificity and that efficiently precipitate the protein even after cross-linking. It is important to be aware that antibodies can cross-react with other chromatin-associated proteins in ChIP experiments, even if the antibodies appear to be highly specific on Western blots. An ideal control to examine this possibility is to perform parallel ChIP experiments in cells that lack the target protein (preferably cells containing a null mutation in the gene encoding the target protein). This issue is discussed by Beima et al. (2006).

REFERENCES

Acevedo LG, Iniguez AL, Holster HL, Zhang X, Green R, Farnham PJ. 2007. Genome-scale ChIP-chip analysis using 10,000 human cells. *BioTechniques* **43:** 791–797.

Attema JL, Papathanasiou P, Forsberg EC, Xu J, Smale ST, Weissman IL. 2007. Epigenetic characterization of hematopoietic stem cell differentiation using miniChIP and bisulfite sequencing analysis. *Proc Natl Acad Sci* **104:** 12371–12376.

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. 2007. High-resolution profiling of histone methylations in the human genome. *Cell* **129:** 823–837.

Beima KM, Miazgowicz MM, Lewis MD, Yan PS, Huang TH, Weinmann AS. 2006. T-bet binding to newly identified target gene promoters is cell type-independent but results in variable context-dependent functional effects. *J Biol Chem* **281:** 11992–12000.

Bernstein BE, Humphrey EL, Liu CL, Schreiber SL. 2004. The use of chromatin immunoprecipitation assays in genome-wide analyses of histone modifications. *Methods Enzymol* **376:** 349–360.

Blais A, Dynlacht BD. 2005. Constructing transcriptional regulatory networks. *Genes & Dev* **19:** 1499–1511.

Boyd KE, Farnham PJ. 1997. Myc versus USF: Discrimination at the *cad* gene is determined by core promoter elements. *Mol Cell Biol* **17:** 2529–2537.

Buck MJ, Lieb JD. 2004. ChIP-chip: Considerations for the design, analysis, and application of genome-wide chromatin immunoprecipitation experiments. *Genomics* **83:** 349–360.

Chaya D, Zaret KS. 2004. Sequential chromatin immunoprecipitation from animal tissues. *Methods Enzymol* **376:** 361–372.

Ciccone DN, Morshead KB, Oettinger MA. 2004. Chromatin immunoprecipitation in the analysis of large chromatin domains across murine antigen receptor loci. *Methods Enzymol* **376:** 334–348.

Das PM, Ramachandran K, vanWert J, Singal R. 2004. Chromatin immunoprecipitation assay. *BioTechniques* **37:** 961–969.

Dong J, Jimi E, Zhong H, Hayden MS, Ghosh S. 2008. Repression of gene expression by unphosphorylated NF-κB p65 through epigenetic mechanisms. *Genes & Dev* **22:** 1159–1173.

Elnitski L, Jin VX, Farnham PJ, Jones SJ. 2006. Locating mammalian transcription factor binding sites: A survey of computational and experimental techniques. *Genome Res* **16:** 1455–1464.

Gilmour DS, Lis JT. 1984. Detecting protein-DNA interactions in vivo: Distribution of RNA polymerase on specific bacterial genes. *Proc Natl Acad Sci* **81:** 4275–4279.

Gilmour DS, Lis JT. 1985. In vivo interactions of RNA polymerase II with genes of *Drosophila melanogaster*. *Mol Cell Biol* **5:** 2009–2018.

Gilmour DS, Lis JT. 1986. RNA polymerase II interacts with the promoter region of the noninduced *hsp70* gene in *Drosophila melanogaster* cells. *Mol Cell Biol* **6:** 3984–3989.

Hanlon SE, Lieb JD. 2004. Progress and challenges in profiling the dynamics of chromatin and transcription factor binding with DNA microarrays. *Curr Opin Genet Dev* **14:** 697–705.

Hecht A, Strahl-Bolsinger S, Grunstein M. 1996. Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. *Nature* **383:** 92–96.

Hollenhorst PC, Shah AA, Hopkins C, Graves BJ. 2007. Genome-wide analyses reveal properties of redundant and specific promoter occupancy within the ETS gene family. *Genes & Dev* **21:** 1882–1894.

Johnson KD, Bresnick EH. 2002. Dissecting long-range transcriptional mechanisms by chromatin immunoprecipitation. *Methods* **26:** 27–36.

Jothi R, Cuddapah S, Barski A, Cui K, Zhao K. 2008. Genome-wide identification of in vivo protein-DNA binding sites from ChIP-Seq data. *Nucleic Acids Res* **36:** 5221–5231.

Kim TH, Ren B. 2006. Genome-wide analysis of protein-DNA interactions. *Annu Rev Genomics Hum Genet* **7:** 81–102.

Kuo MH, Allis CD. 1999. In vivo cross-linking and immunoprecipitation for studying dynamic protein:DNA associations in a chromatin environment. *Methods* **19:** 425–433.

Kurdistani SK, Grunstein M. 2003. In vivo protein-protein and protein-DNA crosslinking for genome-wide binding microarray. *Methods* **31:** 90–95.

Li Q, Johnston SA. 2001. Are all DNA binding and transcription regulation by an activator physiologically relevant? *Mol Cell Biol* **21:** 2467–2474.

Li XY, MacArthur S, Bourgon R, Nix D, Pollard DA, Iyer VN, Hechmer A, Simirenko L, Stapleton M, Luengo Hendriks CL, et al. 2008. Transcription factors bind thousands of active and inactive regions in the *Drosophila* blastoderm. *PLoS Biol* **6:** e27. doi: 10.1371/journal.pbio.0060027.

Mardis ER. 2007. ChIP-seq: Welcome to the new frontier. *Nat Methods* **4:** 613–614.

Mardis ER. 2008. Next-generation DNA sequencing methods. *Annu. Rev Genomics Hum Genet* **9:** 387–402.

Martone R, Euskirchen G, Bertone P, Hartman S, Royce TE, Luscombe NM, Rinn JL, Nelson FK, Miller P, Gerstein M, et al. 2003. Distribution of NF-κB-binding sites across human chromosome 22. *Proc Natl Acad Sci* **100:** 12247–12252.

O'Geen H, Nicolet CM, Blahnik K, Green R, Farnham PJ. 2006. Comparison of sample preparation methods for ChIP-chip assays. *BioTechniques* **41:** 577–580.

O'Neill LP, Turner BM. 2003. Immunoprecipitation of native chromatin: NChIP. *Methods* **31:** 76–82.

O'Neill LP, VerMilyea MD, Turner BM. 2006. Epigenetic characterization of the early embryo with a chromatin immunoprecipitation protocol applicable to small cell populations. *Nat Genet* **38:** 835–841.

Orlando V. 2000. Mapping chromosomal proteins in vivo by formaldehyde-crosslinked-chromatin immunoprecipitation. *Trends Biochem Sci* **25:** 99–104.

Parekh BS, Maniatis T. 1999. Virus infection leads to localized hyperacetylation of histones H3 and H4 at the IFN-β promoter. *Mol Cell* **3:** 125–129.

Phuc Le P, Friedman JR, Schug J, Brestelli JE, Parker JB, Bochkis IM, Kaestner KH. 2005. Glucocorticoid receptor-dependent gene regulatory networks. *PLoS Genet* **1:** e16. doi: 10.1371/journal.pgen.0010016.

Ren B, Dynlacht BD. 2004. Use of chromatin immunoprecipitation assays in genome-wide location analysis of mammalian transcription factors. *Methods Enzymol* **376:** 304–315.

Ren B, Robert F, Wyrick JJ, Aparicio O, Jennings EG, Simon I, Zeitlinger J, Schreiber J, Hannett N, Kanin E, et al. 2000. Genome-wide location and function of DNA binding proteins. *Science* **290:** 2306–2309.

Robertson G, Hirst M, Bainbridge M, Bilenky M, Zhao Y, Zeng T, Euskirchen G, Bernier B, Varhol R, Delaney A, et al. 2007. Genome-wide profiles of STAT1 DNA association using chromatin immunoprecipitation and massively parallel sequencing. *Nat Methods* **4:** 651–657.

Rundlett SE, Carmen AA, Suka N, Turner BM, Grunstein M. 1998. Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3. *Nature* **392:** 831–835.

Sambrook J, Russell DW. 2006. Purification of nucleic acids by extraction with phenol:chloroform. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot4455.

Solomon MJ, Larsen PL, Varshavsky A. 1988. Mapping protein-DNA interactions in vivo with formaldehyde: Evidence that histone H4 is retained on a highly transcribed gene. *Cell* **53:** 937–947.

Southall TD, Brand AH. 2007. Chromatin profiling in model organisms. *Brief Funct Genomic Proteomic* **6:** 133–140.

Walter J, Dever CA, Biggin MD. 1994. Two homeo domain proteins bind with similar specificity to a wide range of DNA sites in *Drosophila* embryos. *Genes & Dev* **8:** 1678–1692.

Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ, et al. 2008. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet* **40:** 897–903.

Wathelet MG, Lin CH, Parekh BS, Ronco LV, Howley PM, Maniatis T. 1998. Virus infection induces the assembly of coordinately activated transcription factors on the IFN-β enhancer in vivo. *Mol Cell* **1:** 507–518.

Weinmann AS, Yan PS, Oberley MJ, Huang TH, Farnham PJ. 2002. Isolating human transcription factor targets by coupling chromatin immunoprecipitation and CpG island microarray analysis. *Genes & Dev* **16:** 235–244.

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