# Yeast Transcription Factor Chromatin Immunoprecipitation

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**[Abstract]** This ChIP protocol was developed and improved over the years by various researchers in the Snyder lab, Stanford University, especially Anthony Borneman and Christopher Yellman. I have used this method to successfully map the genome-wide binding of transcription factors Ste12. The ChIPed DNA is suitable for downstream analysis using PCR, microarray or sequencing.

## Materials and Reagents

1. 500 ml of log-phase yeast cell culture per ChIP (at ~0.9 x $10^7$ cells/ml, ~4.5 x $10^9$ cells per sample)
2. 37% formaldehyde
3. 2.5 M glycine in $H_2O$ (heat sterilized)
4. Liquid nitrogen, dry ice/ethanol bath or -70 °C freezer
5. 0.5 mm Zirconia/Silica Beads (Bio Spec Products, catalog number: 11079105z)
6. Commercial protease inhibitor cocktails, for example:
   Roche Complete protease inhibitor cocktail tablets (F. Hoffmann-La Roche, catalog number: 11697498001)
   Roche Complete Mini protease inhibitor cocktail tablets (F. Hoffmann-La Roche, catalog number: 11836153001)
7. EZview anti-Myc affinity gel (red colored beads) (Sigma-Aldrich, catalog number: E6654)
8. Minelute kit for final DNA purification (QIAGEN, catalog number: 28004)
9. LiCl
10. NaOAc
11. Ethanol
12. Triton X-100
13. TE
14. NaCl
15. EDTA
16. Isopropanol
17. NP-40

18. Na-deoxycholate
19. SDS
20. Tris-buffered saline (TBS) (10x stock) (see Recipes)
21. Lysis/IP buffer (see Recipes)
22. Lysis buffer/500 mM NaCl (see Recipes)
23. IP wash solution (see Recipes)
24. TE/1% SDS (100 ml) (see Recipes)
25. TE/0.67% SDS (100 ml) (see Recipes)
26. TE (100 ml) (PH 8.0) (see Recipes)
27. 1 mM PMSF (Fluka, catalog number: 93482) (see Recipes)

## Equipment

1. Millipore stericup sterile vacuum filter units, 500 ml funnel, 0.22 μm or 0.45 μm pore size (EMD Millipore, catalog number: SCGVU05RE, SCHVU11RE)
2. Syringe needle (BD Biosciences, catalog number: 305155 or 305156)
3. 5 ml snap-cap tubes, polypropylene (preferred) or polystyrene (BD Biosciences, Falcon®)
4. 15 and 50 ml conical polypropylene screw-top tubes (BD Biosciences, Falcon®)
5. Branson Sonifier 250 with microtip or Digital Sonifier S-450D (BD Biosciences)
6. Refrigerated tabletop centrifuges, *e.g.* Beckman GS-6R, GS-15R (Beckman Coulter), or Eppendorf refrigerated multipurpose centrifuges (Eppendorf™, model: 5810R and 5804R) (or simply put an ordinary tabletop centrifuge in the cold room)
7. Fume hood
8. FastPrep machine (FastPrep, catalog number: 6004500)
9. Hemacytometer
10. Spectrophotometer

## Procedure

Day 0

1. Set up the experiment.

   Each IP is from 500 ml of cells in mid-log phase at $OD_{600}$ of ~0.6, a density of ~0.9 x $10^7$ cells/ml. The total number of cells per IP is ~4.5 x $10^9$, and the total cell weight per sample should be 0.2-0.25 g.

   *Note: Other ChIP protocols specify 100 ml of cells at $10^7$ cells/ml, or $10^9$ total cells. If in doubt about cell number (for example when dealing with clumpy yeast strains), use a hemacytometer to count cells instead of using the spectrophotometer.*

2. Grow cells under the desired conditions

   Growth conditions for inducing pheromone response transcription are described in Zheng *et al.* (2010).

3. Treat the cells with formaldehyde to crosslink proteins and DNA

   In a fume hood, add 37% formaldehyde to the cells to a final concentration of 1% (use 14 ml formaldehyde). Maintain the cells at room temperature (RT) for 15 min, swirling occasionally to mix. Effective fixation conditions vary according to the protein that will be immunoprecipitated. The simplest way to optimize this variable is to change (in most cases increase) the fixation time.

4. Quench the crosslinking reaction with glycine

   Add 2.5 M glycine to a final concentration of 125 mM (a 20x dilution, so add 27 ml). Incubate the samples for 5 min at RT with occasional mixing.

5. Collect and wash the cells

   Collect the cells by filtration using a 0.45 or 0.22 µm filter. Wash the cells twice on the filter with 100 ml of water at RT. Rinse the cells from the filter using 20 ml of water and transfer them to a 50 ml polypropylene tube. Repeat the rinse to collect residual cells.

   Spin at 4,000 rpm for 5-25 min to pellet the cells and discard the supernatant. Resuspend the cells in 1 ml of water and transfer them to a 2 ml screw cap tube (for later lysis). Spin the cells down in a microcentrifuge (3 min at max rpm) and thoroughly remove the supernatant. Weigh the samples at this point. Each 500 ml culture should yield 0.2-0.25 g of cells. Add 1 ml of zirconium beads to each sample to prepare it for the lysis step. Keep the samples on ice or freeze them for storage. Process the experimental replicates separately from here forward.

   *Notes:*

   a. *It is a good idea to check the total weight of cells recovered. Cell weight can be strain dependent and differs significantly between haploids and diploids.*

   b. *The cells can be kept on ice for several hours at this point or frozen for storage. Putting the samples directly into a -70 °C freezer works well.*

Day 1

1. Prepare lysis/IP buffer with protease inhibitors

   Prepare 6 ml of lysis/IP buffer for each sample of cells and some extra for equilibrating the antibody beads (50 ml for 6 ChIPs works). Use Roche complete protease inhibitor pellets, which will treat 50 ml of buffer. When using the tablets, it is still necessary to add PMSF. Add 0.75 ml of lysis/IP buffer with protease inhibitors to each sample tube.

   *Note: It is best to add PMSF last, just before using the buffer, since it is unstable in aqueous solutions, with a half-life of ~35 min at pH 8.*

 

2. Lyse the cells with cubic zirconium beads

   Perform all manipulations in an ice/water bath. Disrupt the cells with the FastPrep machine, using a total of five 1 min bursts at speed 6.0 (additional rounds only if needed). After each burst, immerse the samples in ice water for a minute or so to keep them cold. *Note: Examine the cells under the microscope to check for effective lysis. The number of lysed cells should approach 100%.*

3. Recover the crude lysate

   Prepare a Falcon Sml snap-cap tube for each sample. Place the 2 ml lysis tube top-down on the benchtop. Heat a syringe needle to red hot in a flame and use it to pierce the bottom of each sample tube. Put the 2 ml tube about 1 cm into a 5 ml Falcon tube, where it should rest snugly. Centrifuge in a tabletop (preferably chilled) for 1 min at 1,500 rpm, bringing the lysate down into the Falcon tube. Add 0.75 ml of cold lysis buffer to each tube to wash the beads and spin again. Transfer the lysate to a 15 ml conical tube (good for sonication), and add 2.4 ml of lysis buffer. Total lysate volume should be ~4 ml.

4. Sonicate the lysate to shear chromatin

   Shear the chromatin by sonicating the suspension with a Branson 2S0 Sonifier fitted with a microtip. Use the sonifier at amplitude 6 and 100% duty cycle. Sonicate each sample 5 times for 30 sec each time. Hold the tube in a small beaker of ice/water while sonicating. Between sonications chill the samples in ice/water for at least 2 min. Also chill the sonifier tip in ice water periodically (after 18x sonications) to keep it from getting too hot. If using the Digital Sonifier S-450, use 15 times for 10 sec each to avoid overheating. Set total run time as 2 min 30 sec, amplitude 50%, pulse on 10 sec, pulse off 1 min. Hold the tube in a small beaker of ice/water. Preferably the whole procedure is done in a cold room. The average length of DNA post-sonication should be 500 bp, with a range of 100-1,000 bp.

   *Notes:*

   a. *Sonication should be monitored and adjusted to yield the desired average DNA length as described in Notes section.*
   b. *Clean the sonicator tip after use.*
   c. *A suggested routine is to dip the probe in 0.1 % SDS, then water, spray it with ethanol and dry it with a kimwipe tissue.*

5. Remove cell debris from the lysate by centrifugation

   Centrifuge the lysates at 3,000 rpm in a refrigerated tabletop centrifuge for 5 min at 4 °C, remove the supernatant and divide it into two 2 ml microcentrifuge tubes. Spin in a cooled microcentrifuge at 14,000 rpm for 10 min, remove the supernatants, and pool the two lysates into a fresh 15 ml conical tube. The lysates are now ready to use for IP, and one

 

can save aliquots at this point for analysis of total chromatin and protein. This is often used as input control.

*Note: Avoid carrying over any aggregated debris by staying away from the pellet, sacrificing ~50 µl of lysate. Improper performance of this step is a likely source of contamination.*

6. Immunoprecipitate the protein of choice

Wash the antibody-coupled beads carefully to eliminate any free antibody (see note). Add 400 µl of Myc-coupled beads (20% suspension, so ~80 µl bead volume) to each IP sample using a 1 ml pipette tip that has been cut off to increase the bore. Bring the total volume of each IP up to 5 ml with buffer. Incubate overnight (12-20 h) on a rocker at 4 °C.

*Note: When using any antibody-coupled bead, follow the supplier's recommended bead prewash procedure to avoid bringing along free antibody. To wash an entire bottle of 50% Sigma anti-Myc bead suspension, remove the beads from the supplier's bottle with a 1 ml pipette and follow with 2 washes of 2 ml of lysis buffer to transfer all of the beads into a 15 ml conical tube. Vortex the suspension briefly (or just mix vigorously by hand) and centrifuge for 2 min at 2,000 rpm in a tabletop centrifuge to bring the beads down. Wash the beads 3 times with 4-5 ml fresh lysis buffer each time. Finally, add lysis/IP buffer to the beads to reach a total volume of 5 ml. This amount of beads is sufficient for 12 IP's using ~400 µl of 20% suspension for each IP.*

Day 2

1. Remove the IP supernatant

Pellet the beads in a tabletop centrifuge (3,000 rpm for S min) and remove the supernatant. Add 600 µl of lysis buffer and transfer the beads to a fresh 1.5 ml microcentrifuge tube using a 1 ml pipette tip. Repeat with 600 µl of lysis buffer to collect any residual beads.

2. Wash the IP beads

Between washes, spin the beads down for 1 min at 1,000 *x g* (3,000 rpm in an Eppendorf S417 microcentrifuge) and remove the supernatant with a small pipette tip attached to an aspirator, taking care to avoid the pellet. Perform the washes on a rocker (at RT or in the cold room) with 1 ml of the indicated solution for 5 min. Twice with lysis buffer (the first wash was done with the transfer of beads). Once with lysis buffer/500 mM NaCI. Twice with IP wash solution. Once with TE. When aspirating away the last wash, thoroughly remove the small amount of remaining TE from the beads.

*Notes: It may be useful to save the IP supernatant fraction to analyze protein content and IP efficiency.*

3. Elute the immunoprecipitate with TES (TE/1% SDS)

 

Elute the immunoprecipitate from the beads with 100 µl of TE/1% SDS (PH 8.0), incubating at 65 °C for 15 min. Mix the samples briefly after 10 min. Pellet the beads for a few seconds at full speed (14,000 rpm) and transfer the eluate to a 1.5 ml tube. Add 150 µl of TE/0.67% SDS to the beads, heat for a few minutes and pellet again. Remove the supernatant and add it to the first eluate fraction. Spin the pooled eluate once more to pellet residual beads, and transfer it to a screw-cap tube, avoiding the ~10 µl left with the beads at the bottom of the tube.

4. Reverse crosslinking

   Incubate the eluates at 65 °C over night to reverse the crosslinking.

   *Notes: Screw-cap microcentrifuge tubes eliminate evaporation during the heating.*

Day 3

1. Cool the samples down at RT. Briefly spin down to collect condensation. Purify the samples using a spin column designed for small DNA fragments. Qiagen MinElute kit or PCR purification kit can be used for this step. Follow the manufacturer's instructions for using the kit.

   *Note: One can vary the volume of EB as needed, keeping in mind that the DNA sequencing library construction protocol is set up for a 34 µl sample.*

**Notes**

1. DNA quantification

   The precipitated DNA can be quantified with NanoDrop and assayed for emichment of transcription factor bound sequences by PCR or microarray (see below). If too little DNA is purified or the DNA is not emiched for a subset of sequences, some parameters of the chromatin IP procedure can be altered, as described below.

2. Quantitative PCR

   If you know of some sites where the protein of interest will be bound, you can use them as positive controls to assay the ChIP. This is the best way to quantitatively determine the success or failure of an experiment. See the protocol for qPCR of ChIP DNA samples.

3. Optimizing crosslinking

   Extent of crosslinking can be adjusted by changing the time of incubation with the cross linking agent, the concentration of formaldehyde, or the temperature of crosslinking. The extent of crosslinking is critical and can depend on the individual protein. Too much crosslinking may mask epitopes, while too little will cause failure to co-IP chromatin.

4. Assaying sonication

 

Sonication should be monitored since chromatin fragments that are too large will pellet with the lysate debris. The settings described in the protocol were empirically tested by experimenters in Snyder lab. Since different sonifiers and tips may perform differently, it is strongly recommended that users adjust sonication parameters to different levels and monitor the resulting chromatin fragment size. To check DNA fragment size, take a 250 µl aliquot of the total chromatin (lysate just before the IP step) and add 250 µl of TE/1% SDS. Incubate for 6-8 h at 65 °C to reverse crosslinking, then add 20 µl of 20 mg/ml protease K and incubate for 2 h at 37 °C. Add 50 µl of 5 M LiCl, extract (3x with Phenol-Chloroform-Isopropanol, 1x with chloroform) and ethanol-precipitate the DNA (add 1 ml of ethanol, chill at -20 °C for 1 h). Resuspend the DNA in 50 µl of TE and add 2 µl of DNase-free RNase A. Incubate for 30 min at 37 °C. To resolve the DNA fragments, add DNA loading buffer to the sample. Use a loading buffer with only xylene cyanol as a marker, since it runs just above 3 kb. Pour a 1.5% agarose gel and run the gel until the marker is very well separated. The range of fragment sizes should be 100-1,000 bp, averaging 400-500.

Input control DNA can be purified using the same procedure.

5. Optimizing antibody amount

   The amount of antibody used for IP is another critical parameter. Preliminary IP experiments should be performed to determine the appropriate amount of antibody to be used for purification of the specific protein of interest. The amount used in this protocol is specifically tested for the myc-tagged Ste12 protein. To ensure that the crosslinking is not rendering the protein refractory to immunoprecipitation, the IP supernatant from step 11 can be analyzed by SDS-PAGE and immunoblotting. The material should be boiled in sample buffer for 20 min before running a protein gel.

6. Cell lysis

   If necessary, it is possible to increase the efficiency of cell lysis by either increasing the number of cycles in the FastPrep machine or using rnore beads. The new FastPrep machine should give >95% lysis if used as described in the protocol.

7. Two-step IP

   This is an alternative to use when no bead-coupled antibody is available.

   primary IP: Add the appropriate amount of free primary antibody against the protein of interest (or epitope tag) to the lysate (see Notes for determination of antibody amount to use). Incubate overnight on a rocker at 4 °C.

   secondary IP: Add 50 µl (of ~50% suspension) of protein A or G sepharose beads. Incubate on a rocker at 4 °C for 1-2 h.

   *Note: This is a high-affinity binding step, and extending time is not likely to improve the IP.*

 

**Recipes**

1. TBS (1 L 10x stock)
   200 ml 1 M Tris/HCl (pH 7.6)
   300 ml 5 M NaCl
   $H_2O$ to reach 1 L
   *Note: Dilute to working concentration and store in the cold room, as it is to be used cold.*
2. Lysis/IP buffer (1 L)
   50 ml 1 M Hepes/KOH (pH 7.5)
   28 ml 5 M NaCl
   2 ml 500 mM EDTA
   100 ml 10% Triton X-100
   1 g Na-deoxycholate
3. Lysis buffer/500 mM NaCl (250 ml)
   Add NaCl to Lysis/IP buffer to bring the NaCl concentration up to 500 mM. For 250 ml final volume of Lysis buffer/500 mM NaCl, this requires 18 ml of 5 M NaCl.
4. IP wash solution (250 ml)
   2.5 ml 1 M Tris/HCl (pH 8.0)
   12.5 ml 5 M LiCl
   6.25 ml 20% NP-40
   1.25 g Na-deoxycholate
   0.5 ml 500 mM EDTA
5. TE/1% SDS (100 ml)
   5 ml 1 M Tris/HCl (pH 8.0)
   2 ml 500 mM EDTA
   5 ml 20% SDS
6. TE/0.67% SDS (100 ml)
   5 ml 1 M Tris/HCl (pH 8.0)
   2 ml 500 mM EDT A
   3.35 ml 20% SDS
7. TE (PH 8.0) (100 ml)
   5 ml 1 M Tris/HCl (pH 8.0)
   2 ml 500 mM EDTA
8. 1 mM PMSF
   Prepare 100 mM PMSF stock solution (17.4 mg/ml) in isopropanol, and store small aliquots (0.5-1 ml) at -20 °C. Alternatively, use 100 mM PMSF.

## References

1. Aparicio, O., Geisberg, J. V. and Struhl, K. (2004). Chromatin immunoprecipitation for determining the association of proteins with specific genomic sequences *in vivo*. *Curr Protoc Cell Biol* Chapter 17: Unit 17 17.
2. Zheng, W., Zhao, H., Mancera, E., Steinmetz, L. M. and Snyder, M. (2010). Genetic analysis of variation in transcription factor binding in yeast. *Nature* 464(7292): 1187-1191.

 