Immunoprecipitation of Chromatin from Fixed Yeast Protocol

This protocol was modified (June 2001) by Jason Lieb from published methods, including:

Hecht, A., Strahl-Bolsinger, S. & Grunstein, M. Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. *Nature* **383**, 92-6 (1996).

Chromatin-IP (or ChIP) has been a useful method for the localization of proteins to specific regions of DNA. ChIP applied to microarrays (ChIP on Chip) is becoming an increasingly efficient method to localize protein across entire genomes.

Materials

1 M HEPES-KOH, pH 7.5 (119.55g/500 ml, pH w/ KOH),
37% Formaldehyde
2.5 M Glycine
3M KCl
0.5M EDTA
20% NP-40
1M $MgCl_2$
Glycerol
17.4 mg/mL PMSF in Ethanol (or isopropanol)
100 mM Benzamidine (Sigma B6506) in water
1mg/ mL Pepstatin in Ethanol
100 mM Sodium Metabisulfite in Water
0.5 mm Glass Beads

Protocol

1. Grow a 200 ml culture of yeast under the desired conditions to the desired density.

If grown to an OD of 1, this will produce enough extract for 10 IPs.

2. Add formaldehyde directly to the culture, to a final concentration of 1% (2.7 ml of 37% formaldehyde). Continue incubation for 30 mins at 30 °C .

Others incubate for 15-20 mins at room temperature, and claim that for some IPs, overnight incubation at 4 °C is helpful.

3. Add Glycine to 125 mM (from a 2.5 M stock, add 5 ml for 100 ml of culture). Incubate for 5 minutes.

4. Pellet the cells in 50 mL conical tubes at 1500 G for 5 mins.

5. Wash each pellet twice with 50 mL PBS

6. Combine all pellets into 1 new 15 mL tube.

7. Wash the combined pellet with 15 mL Beadbeater lysis buffer.

Beadbeater lysis buffer is:

50 mM Hepes-KOH, pH 7.5
10 mM $MgCl_2$
150 mM KCl
0.1 mM EDTA
10% Glycerol
0.1% NP-40

Add just before use:
1mM DTT
1 mM Sodium Metabisulfate
0.2 mM PMSF
1 mM Benzamidine (Sigma B6506)
1 µg/mL Pepstatin

8. Weigh the pellet (1g≈1mL of cell volume).

A 200 ml culture grown to an OD of 1 generally yields a ~1g pellet. If other cultures are lagging, I let them catch up by holding the faster-growing cultures on ice for up to an hour after this step. This does not seem affect results, presumably because the yeast are dead and fixed, but your proteins may behave differently.

9. Resuspend cells such that 0.4g of cells are resuspended in a total volume of 1 mL of Beadbeater lysis buffer (for a 1g pellet the total resuspension volume is 2.5 mL). If your pellet is less than 0.4g, resuspend it in 1 mL as well, but the pellet should weigh at least 0.1g.

6. Pipet 1 mL of the resuspended cells into a 2 mL screw-top tube. Add 1 mL of 0.5 mm glass beads.

Normal 1.5 mL Eppendorf tubes may be used, but the rubber-gasket screw-tops prevent leaking.

7. Lyse cells in the mini-beadbeater-8 with four 1 minute sessions at the highest setting. Place tubes on ice for two minutes between each session.

8. Recover the extract by pouring the bead/extract slurry into a 6 mL syringe fitted with a 25 gauge, 5/8^{ths} inch-long needle. Allow the extract to drip into a clean 15 mL tube on ice. The plunger may be used to speed up the flow. After the last liquid has dripped out of the syringe, add 0.75 mL of beadbeater lysis buffer to wash out the remaining extract.

If the needle becomes clogged, insert the plunger, invert, and flick near the needle. The needle may need to be replaced if it remains clogged.

9. Keep the extract in the 15mL tube and sonicate for three 30-second sessions with a Branson 250 microtip sonicator at 50% duty cycle, power of 5. Ice for 2 mins between sessions.

Sonication further breaks the nuclei and should shear the DNA to a length of 300-1000 base pairs. Higher powers tend to cause too much frothing. The extract should be at about room temperature after each sonication session. If it becomes hot, decrease the time of each session and increase the number of sessions.

10. Spin the extract at full speed in the microfuge at 4°C for 5 min to clear the extract of debris and unlysed cells. Transfer the supernatant to a new tube and spin again to clear any remaining debris.

Bradfords done on the extract at this point usually report a concentration of 5-20 ug of protein/mL, depending on the size of the pellet, and the volume of the bead-beading solution.

11. Run the extract on an agarose gel to be sure that you have a bunch of DNA and that the smear ranges from 500-2000 bp, with a center near 1 kb.

10 µL should be enough to see a bright streak of DNA. After this step, salt or detergent concentrations may be increased for more stringent IPs.

12. **OPTIONAL- I have not found that any of these steps make a big difference, but they may in individual cases.**

1.) Pre-clear the extract by incubating it with 1/10th volume of pre-swelled, washed protein G beads for 1 hour at 4°C. Spin down the beads at full speed in the microfuge, and transfer the supernatant to a new tube.

2.) Block the protein G beads to be used in the IP with BSA (IgG-free, Sigma, 2mg/mL).

Some block with Salmon Sperm DNA, but I think this is a bad idea because of downstream DNA amplification.

13. Add the antibodies to the extract and incubate at 4°C for 4 hours to overnight with nutation or rocking.

For each IP, use extract equivalent to ~20 OD_{600} units. For example, use extract equivalent to 20 mL of an OD 1 culture. The final volume of extract in one IP is generally 500 µL. Alternatively, use about 1 mg of total protein per IP. Very rich extract may be diluted with Beadbeater lysis buffer to bring the volume up to 500 uL. I generally use 1-5 µg of antibodies per IP.

Variation: Add the antibodies to ~25µL of packed, swollen (wet) beads per IP. Incubate for 45 mins at 4°C in a total volume of 200 uL lysis buffer. After incubation, discard unbound antibodies, and add Ab-bead conjugates to extract.

14. Recover the Antibody/Protein/DNA complexes with Protein G beads that have been washed/equilibrated in IP buffer. Incubate at 4 degrees for 1-2 hours with nutation.

I use ~25µL of packed, swollen (wet) beads per IP. This is done by making a 50% v/v slurry of Protein-G Agarose Beads in IP buffer, and adding 50 uL of the slurry. For pipetting, cut off the end of a pipet tip to make the opening wider. During washes, do not spin the beads at full speed because they can be crushed. 4000 rpm in the microfuge is OK.

IP buffer is:

25 mM Hepes-KOH, pH 7.5
150 mM KCl
1 mM EDTA
12.5 mM $MgCl_2$
0.1% NP-40

Add just before use:
1 mM Sodium Metabisulfate
0.2 mM PMSF
1 mM Benzamidine (Sigma B6506)
1 µg/mL Pepstatin

15. Wash the beads 4 X 15 mins with 1 mL of IP buffer.
Don't take shortcuts here, and more washes are probably even better. Increase salt/detergent, or reducing agent concentrations if more stringent conditions are desired.
16. Elute the IP'd material from the beads by adding 100µl IP elution buffer (50 mM Tris/HCl pH 8.0, 10 mM EDTA, 1% SDS) to the washed beads. Mix and incubate at 65°C for 30 minutes.
16. Spin down the beads for a few seconds at full speed, transfer 80 µL of the supernatant to a new tube.
17. Repeat step 15 with 50 µl IP elution buffer. Take 50 µl of the supernatant and pool the eluates.

For DNA Analysis:
1. Incubate the eluate at 65°C for 6 hours-overnight to reverse the crosslinks.
2. Add an equal volume of TE pH 7.4, 1 µL of 20 mg/ml Glycogen, and Proteinase K to 100 µg/ml. Incubate at 37°C for two hours.
3. Phenol/Chloroform extract, re-extract the organic phase with 100 µL of TE.
Variation: Purify DNA over QiaQuick PCR column.
4. Add Sodium Acetate to 0.3 M. Add 2 volumes of 100% ethanol. Place at -20°C for 1 hour.
5. Spin down pellet, wash with 70% ethanol and speed-vac dry.
6. Resuspend in 25 uL of TE, 100 µg/mL RNAse A (Boiled, to preclude DNAse activity). Incubate at 37°C for 30 minutes.
7. For array analysis, use the Round A/B/C amplification protocol.