Chromatin Immunoprecipitation

Make Whole Cell Extract:

1. Grow 100ml of cells in YPD at 30 C to OD_{600} of ~1.0. Add 1.35 ml 37% formaldehyde and incubate cells at 30 C for fifteen minutes. (For heat shock, add 100ml pre-warmed 42C YPD to culture and transfer cells to 35C water bath for 45 minutes. After 30 minutes, add 2.7 ml 37% formaldehyde per 100ml of media and continue incubation for 15 minutes more.)
2. Spin cells down in SLA-1500 at 6K for 5', discard supernatent, and wash with 80ml cold TBS (30ml 5M NaCl, 20ml 1M Tris pH 7.5/per 500ml).
3. Repeat wash twice and resuspend pellet in ~2ml cold TBS. Divide volume into 3 fast prep tubes. Spin down pellet and aspirate TBS. Freeze at –80.
4. Suspend each pellet in ~600ul of FA-lysis buffer with 140mM NaCl with fresh protease inhibitors added.
5. Break cells with glass beads in fast prep machine for 45 seconds (7 times) with a minute in between each.
6. Puncture tube with needle and spin down in small glass tube in clinical centrifuge at 2K for 3'.
7. Resuspend everything in tube and transfer to Eppendorf.
8. Sonicate each tube at 30% output, 90% duty cycles for 5 pulses. Alternate putting samples from dry ice to ice and sonicate each tube 7-8 times.
9. Spin 5' at 14K in cold room, transfer supernatent to new Eppendorf and spin again for 20'. Transfer supernatent to fresh tube. This is the chromatin solution.
10. Quantitate protein by Bradford Assay.

Immunoprecipitation:

11. IP equal amount from 1mg-2.5mg. Make sure to save enough chromatin for Totals. Use $1/10^{th}$ or $1/20^{th}$ of the amount of protein used in IP for the totals.
12. **If IPing with TBP antibody** add 5ul TBP antiserum, 1.0-2.5mg protein, and bring total volume up to ~500ul with FA-lysis 140 with fresh protease inhibitors. For negative control tube, add 5ul Pre-Immune serum, 1.0-2.5mg protein, and bring total volume up to ~500ul.
13. **If using a protein A tag (TAP)**, IP with about 30ul of washed IgG sepharose beads (Amersham Cat#: 17-0969-01) for 2-4 hours and skip to step 18.
14. **If using a myc tag,** IP with 3ul myc antibody, 1-2.5mg sample, and bring up to ~500ul with FA-lysis 140 with fresh protease inhibitors.
15. IP overnight by rocking at 4C.
16. Spin at 14K for 2' and remove supernatent to tubes with 30ul washed protein A sepharose beads (Amersham Cat# 17-0963-03) **for myc tag and TBP antibody**. To pipet beads, cut end of tip off.
    a. To wash beads: add 1ml FA-lysis 140 and spin at 5K for 30 seconds, suck off buffer. Repeat wash twice.
17. After adding supernatent to washed beads, IP for 2 hours in cold room.
18. Spin down beads at 5K for 30 seconds. Save all of the aspirate.

19. Wash beads once with 1.4ml FA-lysis 140, once with 1.4ml FA-lysis 500, and once with 1.4ml LiCl solution. To wash, add buffer, rotate for 5', spin down for 30 sec. and pipet off supernatent and add next buffer. Do washes at room temp.
20. Elute twice for 15 minutes each with 190ul of 1%SDS and 0.1M NaHCO3 at 65C. Keep both elutions in one tube. Tap occasionally while tube is at 65C.
21. Add 25ul of 5M NaCl to elutions and incubate at 65C for at least 5 hours to reverse the crosslink. DO TO TOTALS TOO!!
22. Also add 380ul of 1%SDS and 0.1M NaHCO3 and 25ul of 5M NaCl to Totals and reverse the crosslink for these at 65C for 5 hours.
23. Precipitate with 1ml of ethanol at –20C overnight.
24. Spin at 14K for 10 min. Aspirate and wash with 75% ethanol. Dry at room temp.
25. Resuspend with 200ul 1XTE with 10ug RNAse A. Incubate at 37 C for an 30-60 minutes.
26. Add SDS to 0.5% (10ul 10%SDS). Add proteinase K to 20ug (1ul). Incubate at 42C for 1 hour.
27. Add equal volume of 2:1 phenol-chloroform, vortex, spin and add 200ul of 1X TE to tube with phenol still in it, vortex, spin and keep supernatent.
28. Add 20ug (1ul) of glycogen (door of freezer) and 100ul of 3M NaAc. Add 1ml ethanol and incubate at –20C overnight.
29. Precipitate at 14K for 10 minutes. Wash with 75% ethanol. Air dry and Resuspend in 100ul of 1X TE.

Quantitative PCR:

1. Dilute total chromatin 1:50 or 1:100.
2. Use a range of dilutions of chromatin (0.1ul, 0.5ul, 1.0ul) and whatever primers of the gene you wish to look at and make total reaction volume 50ul.
3. Continue with regular PCR reagents except for dye (use low conc of BPB) and use 26-28 cycles on the program.
4. Run 20ul on 1.5-2.0% gel made without EtBR.
5. Use buffer from gel and add about 70ul of EtBR and shake in gel tray for 5-10min
6. Put tray and sink and wash with H20 for 10-15min.
7. Take reverse light picture and quantitate with spot density on Alphaimager.

PCR Program:

1. 95 C for 2:00
2. 95 C for 1:00
3. 50 C for 1:00
4. 72 C for 1:00
5. Go to 2 for 26 cycles**
6. 72 C for 7:00
7. 4 C forever

** adjust number of PCR cycles from ~23-32 according to saturation levels

Materials

FA lysis buffer with 140mM NaCl:

	Final Conc.	Stock	Amt/100ml
HEPES	50mM	1M	5ml
NaCl	140mM	5M	2.8ml
Triton X-100	1%	10%	10ml
EDTA	1mM	0.5M	0.2ml
Na Deoxycholate	0.1%	10%	1ml
H2O		55.5M	81ml

			For 5ml
PMSF	0.0087mg/500ul EtOH	100X	50ul
Pepstatin		200X	25ul
Benz.	0.0155mg/500ul H2O	100X	50ul
Chymostatin		500X	10ul

FA lysis buffer with 500mM NaCl:

	Final Conc.	Stock	Amt/100ml
HEPES	50mM	1M	5ml
NaCl	500mM	5M	10ml
Triton X-100	1%	10%	10ml
EDTA	1mM	0.5M	0.2ml
Na Deoxycholate	0.1%	10%	1ml
H2O		55.5M	73.8ml

LiCl Solution:

	Final Conc.	Stock	Amt/100ml
Tris HCl pH 8.0	10mM	1M	1ml
LiCl	250mM	1M	25ml
NP40	0.5%		5ml
Na Deoxycholate	0.5%	10%	5ml
EDTA	1mM	0.5M	0.2ml
H20			63.8ml

1%SDS/0.1M NaHCO3:
10ml
1ml 10% SDS
1ml 1M NaHCO3
8ml H20