Chromatin IP

1. Grow yeast cells in appropriate media till log phase
2. Cross link 50 ml of cells (OD – 0.2 at least) with 1% formaldehyde for 15 min at RT
3. Add Glycine to the final concentration of 125 mM and continue the incubation for 5 more min at RT
4. Harvest the cells and wash at least 3 times with 1X TBS
5. Resuspend cells in 400 ul of lysis buffer (with protease inhibitors)
6. You can refrigerate (4^oC) the sample at this step or continue with the next step.
7. Sonicate the suspension for 14 times,10 sec each incubating on ice in between each sonication.
8. Centrifuge the suspension at 13K for 15 min at 4^0C
9. Take 400 ul of the supernatant for IP and keep 10 ul of the extract for positive control (WCE).
10. Add antibody to the 40 ul extract and incubate at 4^0C / 4hrs on a rocker.
11. Add 60 ul of protein A sepharose CL-4B beads slurry and incubate for 1 hr at 4^0C.
12. Wash the immunoprecipitaes 3times with 1.4 ml of lysis buffer (with protease inhibitors).
13. Resuspend the samples in 200 ul of TE
14. Add 0.25% SDS and 250 ug/ml of proteinase K and incubate the sample at 37^0C / o/n.
15. Treat the samples at 65^0C / 6hrs for reversing the cross linking.
16. Extract the samples with Phenol: Chloroform
17. Back extract the organic phase with one volume (200 ul) of TE
18. Combine the two aqueous phases
19. Extract once with one volume of chloroform : Isoamyl alcohol.
20. Precipitate the DNA with 2.5 volumes of Ethanol and 1/10 vol of Sodium acetate at – 70 for minimum of 3hrs- o/n.
21. Spin at 4^0C at 13K for 30 min. Wash the pellet with 70% ethanol.
22. Vacuum dry the pellet. Resuspend in 30 ul of Tris-HCl.

Lysis Buffer: (Can store this solution add protease inhibitors to a small volume just before you need)

50 mM HEPES-KOH at pH 7.5	(1M stock)
140 mM NaCl	(5M stock)
1 % Triton X-100	(0.5M stock)
0.1 % SDS	(10% stock)

For each experiment make fresh lysis buffer with protease inhibitors (We use protease inhibitors from BM 1tablet/ 10ml of buffer)

TBS
20 mM Tris-HCl at pH 7.6
200 mM NaCl