



Chromatin Immunoprecipitation (ChIPs) Protocol (Fan Lab)

Reminder - Wear Gloves, lab coat and eye protection!!!

Day 1

1. Stimulate or treat at least 1×10^6 cells on a 10cm dish. For LIF, treat at 50 ng/ml to 100 ng/ml for 20 minutes at 37°C.
2. Crosslink histones by adding formaldehyde directly to culture medium for a final concentration of 1 %. For 10cm plate with 10ml media, add 270 μ l of 37% formaldehyde.
3. Incubate at room temperature for 20 minutes.
4. Take off 7 ml of media (leaving 3mL on the plate). Scrape cells in the 3l and transfer to a 15 ml conical tube.
5. Pellet cells for 4 minutes at 2,000 rpm at 4°C.
6. Aspirate off media, add 5 ml of ice cold 1x PBS. Resuspend by vortexing. Pellet for 4 minutes at 2,000 rpm at 4°C.
7. Repeat 2 times.
8. Resuspend pellet in 150 μ l of SDS lysis buffer supplemented with protease and phosphatase inhibitors (PMSF, aprotinin, leupeptin, pepstatin, DTT, NaF, Na_3VO_4). Transfer lysate to eppendorf tube.
9. Incubate on ice for 10 minutes.
10. Sonication
 - Tune sonicator before use.
 - Keep tip almost at bottom but not touching tube.
 - Keep on ice at all times - make a mound around tube to hold it in place.
 - Sonicate 3 times on control setting 3 for 20 seconds each.



11. Centrifuge samples for 10 minutes at 13,000 rpm and add 150 μ l of the sonicated cell supernatant to a new 1.5 ml microcentrifuge tube. Discard pellet.
12. Dilute the sonicated cell supernatant by adding 1,350 μ l ChIP dilution buffer supplemented with aforementioned protease and phosphatase inhibitor (see step 8).
13. Remove 15 μ l of the diluted supernatant to keep as your input fraction. Keep at 4°C until ready to reverse crosslinks.
14. Pre-clear the 1.5 ml of the diluted supernatant with 60 μ l of salmon sperm DNA/protein A-agarose 50 % slurry for 30 minutes at 4°C with rotation (use pipet tip with end that has been cut off).
15. Pellet agarose by spinning for 1 minute at 1,000 rpm and then collect the supernatant fraction.
16. Add the immunoprecipitating antibody. Incubate overnight at 4°C with rotation.

Day 2

17. Add 45 μ l of the slurry and collect the Ab/histone complex by rotating at 4°C for one hour.
18. Pellet agarose for 1 minute at 1,000 rpm at 4°C. Carefully aspirate off non-specific supernatant.
19. Wash by rotating in home-made eppendorf tube rotator for 3-5 minutes with each of the following buffers, spinning down after each with a 1 minute, 1,000 rpm spin at 4°C.
 - a. Low Salt wash buffer (2 times)
 - b. High Salt wash buffer (2 times)
 - c. LiCl wash buffer (2 times)
 - d. 1 x TE (3 times)

Note: Keep buffers at 4°C.

20. Freshly prepare elution buffer (1 % SDS and 0.1M NaHCO₃).
21. Elute by adding 200 μ l of elution buffer to the pelleted complex. Vortex briefly and mix



occasionally for 15 minutes at room temperature.

22. Spin down agarose at room temperature (1 minute, 1,000 rpm) and transfer the supernatant to a new collection tube. Repeat elution to pelleted complex. Combine the 2 eluates.
23. Add 16 μ l of 5 M NaCl to the combined eluate (400 μ l), and 0.6 μ l 5 M NaCl to the saved input fractions.
24. Reverse histone crosslinks by heating at 65°C for 4 hours. (After this step, sample can be stored at -20°C.
25. To the sample add 8 μ l of 0.5 M EDTA, 16 μ l of 1 M Tris-HCl and 1.6 μ l of 10 mg/ml Proteinase K. To the input add 0.3 μ l of 0.5 M EDTA, 0.6 μ l of 1 M Tris-HCl pH 6.5 and 0.6 μ l of a 10 fold dilution of 10mg/mL proteinase K.
26. Incubate for 1 hour at 45°C.
27. Bring up volume of inputs with TE.
28. Extract DNA once with 1 volume of phenol/chloroform. Extract 2 times with $\frac{1}{2}$ volume straight chloroform.
29. Add 10 % 3 M NaOAc. Then add 2 volumes of 100 % EtOH. Add 1 μ l of stock glycogen.
30. Mix vigorously and place at -80°C for at least 1 hour.
31. Spin down at max speed for 20 minutes.
32. Wash pellet with 70% EtOH.
33. Resuspend in 10 μ l of TE.
34. Proceed to PCR.



ChIP Solutions

SDS Lysis Buffer

1ml 10 % SDS
200 μ l 0.5 M EDTA
500 μ l 1 M Tris HCl pH 8.0
8.3 ml ddH₂O
Total Volume: 10 ml

ChIP Dilution Buffer

50 μ l 10 % SDS
0.5 ml Triton-X 100
120 μ l 0.5 M EDTA
835 μ l 1 M Tris HCl pH 8.0
1.67 ml 5 M NaCl
46.8ml ddH₂O
Total Volume: 50ml

Lo Salt

0.5 ml 10% SDS
0.5 ml Triton-X 100
200 μ l 0.5 M EDTA
1 ml 1 M Tris-HCl pH 8.0
1.5 ml 5 M NaCl
46.3 ml ddH₂O
Total Volume 50 ml

Hi Salt

0.5 ml 10% SDS
0.5 ml Triton-X 100
200 μ l 0.5M EDTA
1 ml 1 M Tris-HCl pH 8.0
5 ml 5 M NaCl
42.8 ml ddH₂O
Total Volume: 50 ml

**LiCl**

2.5 ml 5 M LiCl
0.5 ml NP-40
0.5g Deoxychloric Acid
100 μ l 0.5 M EDTA
0.5 ml 1 M Tris-HCl pH 8.0
46.4 ml ddH₂O
Total Volume: 50 ml

1x TE pH 8.0

1 ml Tris-HCl
0.2 ml 0.5 M EDTA
98.8 ml ddH₂O
Total Volume: 100ml

Elution buffer (Freshly prepared)

1 ml 10 % SDS
2 ml 0.5 M NaHCO₃
8 ml ddH₂O
Total Volume: 10ml