Chromatin Immunoprecipitation (ChIPs) Protocol (Fan Lab)

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

Day 1

1. Stimulate or treat at least 1 x 10^6 cells on a 10cm dish. For LIF, treat at 50 ng/ml to 100 ng/ml for 20 minutes as 37ºC.

2. Crosslink histones by adding formaldehyde directly to culture medium for a final concentration of 1 %. For 10cm plate with 10ml media, add 270 µl 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 4ºC.

6. Aspirate off media, add 5 ml of ice cold 1x PBS. Resupend by vortexing. Pellet for 4 minutes at 2,000 rpm at 4ºC.

7. Repeat 2 times.

8. Resuspend pellet in 150 µl 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 µl 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 µl ChIP dilution buffer supplemented with aforementioned protease and phosphatase inhibitor (see step 8).

13. Remove 15 µl of the diluted supernatant to keep as your input fraction. Keep at 4ºC until ready to reverse crosslinks.

14. Pre-clear the 1.5 ml of the diluted supernatant with 60 µl of salmon sperm DNA/protein A -agarose 50 % slurry for 30 minutes at 4ºC 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 4ºC with rotation.

Day 2

17. Add 45 µl of the slurry and collect the Ab/histone complex by rotating at 4ºC for one hour.

18. Pellet agarose for 1 minute at 1,000 rpm at 4ºC. 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 4ºC.

    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 4ºC.

20. Freshly prepare elution buffer (1 % SDS and 0.1M $NaHCO_3$).

21. Elute by adding 200 µl 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 µl of 5 M NaCl to the combined eluate (400 µl), and 0.6 µl 5 M NaCl to the saved input fractions.

24. Reverse histone crosslinks by heating at 65ºC for 4 hours. (After this step, sample can be stored at -20ºC.

25. To the sample add 8 µl of 0.5 M EDTA, 16 µl of 1 M Tris-HCl and 1.6 µl of 10 mg/ml Proteinase K. To the input add 0.3 µl of 0.5 M EDTA, 0.6 µl of 1 M Tris-HCl pH 6.5 and 0.6 µl of a 10 fold dilution of 10mg/mL proteinase K.

26. Incubate for 1 hour at 45ºC.

27. Bring up volume of inputs with TE.

28. Extract DNA once with 1 volume of phenol/chloroform. Extract 2 times with ½ volume straight chloroform.

29. Add 10 % 3 M NaOAc. Then add 2 volumes of 100 % EtOH. Add 1 µl of stock glycogen.

30. Mix vigorously and place at -80ºC for at least 1 hour.

31. Spin down at max speed for 20 minutes.

32. Wash pellet with 70% EtOH.

33. Resuspend in 10uL of TE.

34. Proceed to PCR.

ChIP Solutions

SDS Lysis Buffer

1ml 10 % SDS
200 µl 0.5 M EDTA
500 µl 1 M Tris HCl pH 8.0
8.3 ml ddH_20
Total Volume: 10 ml

ChIP Dilution Buffer

50 µl 10 % SDS
0.5 5 ml Triton-X 100
120 µl 0.5 M EDTA
835 µl 1 M Tris HCl pH 8.0
1.67 ml 5 M NaCl
46.8mL ddH_20
Total Volume: 50ml

Lo Salt

0.5 ml 10% SDS
0.5 ml Triton-X 100
200 µl 0.5 M EDTA
1 ml 1 M Tris-HCl pH 8.0
1.5 ml 5 M NaCl
46.3 ml ddH_20
Total Volume 50 ml

Hi Salt

0.5 ml 10% SDS
0.5 ml Triton-X 100
200 µl 0.5M EDTA
1 ml 1 M Tris-HCl pH 8.0
5 ml 5 M NaCl
42.8 ml ddH_20
Total Volume: 50 ml

LiCl

2.5 ml 5 M LiCl

0.5 ml NP-40

0.5g Deoxychloric Acid

100 µl 0.5 M EDTA

0.5 ml 1 M Tris-HCl pH 8.0

46.4 ml ddH_20

Total Volume: 50 ml

1x TE pH 8.0

1 ml Tris-HCl

0.2 ml 0.5 M EDTA

98.8 ml ddH_20

Total Volume: 100ml

Elution buffer (Freshly prepared)

1 ml 10 % SDS

2 ml 0.5 M $NaHCO_3$

8 ml ddH_20

Total Volume: 10ml