Ren Lab ENCODE2 Chromatin Immunoprecipitation Protocol

Day 1: Preparation of beads, binding primary Ab, followed by binding of chromatin

1. For each sample, add 50 µL IgG Dynabeads (Life Technologies, Anti-Mouse Cat#11201D; Anti-Rabbit Cat# 11204D) to a 1.5 ml tube.
2. Collect beads by placing tubes on magnetic rack (perform all steps with magnetic rack on ice).
3. Once the beads have collected towards the magnet, slowly remove supernatant with a pipette. Avoid disturbing the beads.
4. Wash the beads 3 times with 1 ml cold BSA/PBS (0.5 mg / mL bovine serum albumin in 1x phosphate buffered saline). Perform all washes as follows:
    a. Add solution (BSA/PBS in this case).
    b. Remove tubes from magnet and invert several times to resuspend beads.
    c. Place tubes on magnet and collect beads for 1 min.
    d. Remove supernatant.
5. After the final wash, add cold BSA/PBS (1 ml minus volume of antibody to be added) to the beads.
6. With tubes against the magnet, add 5 µg antibody.
7. Remove tubes from magnet and Incubate at least 4 hours on a rotating platform at 4°C.
8. After the incubation, place tubes on magnetic rack to collect beads.
9. Remove the supernatant with a pipette once the beads have collected.
10. Wash 3 times with 1 ml cold PBS/BSA as above.
11. After the final wash, add 100 µL BSA/PBS to resuspend the beads. Add 300 µL binding buffer (see recipe below) plus 1 ml chromatin (500 µg chromatin – see "Tissue fixation and sonication protocol" -- in a 100 µL volume of 1x TE) to the tube with the beads. Incubate at 4°C overnight on a rotating platform. Save 20 µL for use as input-control (see step #21).

Reagent	Stock Concentration	Final Concentration	Volume per 100 µL
Triton-X	10%	1%	20 µL
Sodium Deoxycholate	10%	0.10%	2 µL
complete EDTA-free protease inhibitor (Roche, Cat#05056489001)	50x	1x	4 µL
TE	1x	--	74 µL

Day 2: Washing beads, elution, and reversal of crosslinks

12. Make RIPA buffer immediately before use. Add the stock solutions in the order listed below and chill on ice.

Reagent	Stock Concentration	Final Concentration	Volume per 1000 µL
Hepes, pH 8.0	1 M	50 mM	50 µL
NP-40	10%	1%	100 µL
Sodium Deoxycholate	10%	0.70%	70 µL
LiCl	8 M	0.5 M	62.5 µL
50x complete EDTA-free protease inhibitor (Roche, Cat#05056489001)	50x	1x	20 µL
EDTA	0.5 M	1 mM	2 µL
dH2O	--	--	695.5 µL

13. Place the tubes containing the chromatin and beads on a magnetic rack on ice. Once the beads have collected towards the magnet, slowly aspirate the supernatant with a pipette without disturbing the beads.
14. Wash the beads with 1 mL cold RIPA buffer 7 times.
15. Wash once with 1 mL cold 1x TE.
16. After removing the TE by aspiration, add 150 µL ChIP elution buffer (recipe listed below).

Reagent	Stock Concentration	Final Concentration	Volume per 500 mL
Tris, pH 8.0	1 M	10 mM	0.5 mL
EDTA	0.5 M	1 mM	0.1 mL
SDS	10%	1%	5 mL
dH2O	--	--	44.4 mL

17. Transfer the beads mixture to a 1.7 ml tube.
18. Incubate at 65°C for 20 minutes at 1300 rpm (or fast enough to keep beads in suspension) on a Thermomixer.
19. After the incubation, spin the tubes briefly to collect condensation from the top.
20. Place on magnetic rack, wait for the beads to collect and transfer supernatant (containing the immunoprecipitated (IP) chromatin) to a new 1.7 mL Eppendorf tube.
21. Incubate samples at 65°C overnight to reverse crosslinks.
    a. For input-control samples, add 20 µL of chromatin to 130 µL ChIP elution buffer and incubate at 65°C overnight with the other samples. Process in parallel with other samples from here on.

Day 3: DNA Precipitation

Proteinase K mix:

1X Sample	x	13				
140ul TE		1820				
3ul Glycogen 10mg/ml		39				
7ul Proteinase K		91				

22. Add 150uL Proteinase K Mix to each tube.
23. Incubate for 2 hours at 37oC.
24. Extract 2X with 300uL phenol. Vortex 20s, spin 5min, remove top layer and place in tube. Repeat. (This can all be done in a single Phase-Lock tube, shake instead of vortex, spin 5 mins)
25. Extract once with 300uL chloroform/isoamyl alcohol.
26. Add NaCl to 200mM final (13uL 5M).
27. Add 700uL EtOH, vortex briefly.
28. Incubate at -80oC for 15-30min, or on dry ice.
29. Spin at 14K rpm for 15 minutes at 4oC.
30. Wash pellet with 1mL cold 70% EtOH, vortex, spin 5min. at 4oC at 14K rpm.
31. Air dry pellet and resuspend in 30uL TE containing 10ug RNase A (33uL of 10mg/mL, RNaseA in 1mL TE or 16.5uL in 500uL) by gentle vortexing.
32. Incubate 1-2 hour at 37oC.
33. Qiagen PCR purify DNA (elute in 50uL), and store at -80oC. OD input DNA.