# Ren Lab ENCODE Chromatin Immunoprecipitation Protocol

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

1. For each sample, add 11 µL IgG Dynabeads (Life Technologies, Anti-Mouse Cat#11201D; Anti-Rabbit Cat# 11204D) to a 200 µl PCR 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 150 µL cold BSA/PBS (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 (150 µL minus volume of antibody to be added) to the beads.
6. With tubes against the magnet, add 3 µg antibody.
7. Remove tubes from magnet and Incubate at least 2 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 150 µL cold PBS/BSA as above.
11. After the final wash, add 100 µL Binding Buffer (see recipe below) plus 100 µL chromatin (20 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 150 µL cold RIPA buffer 5 times.
15. Wash once with 150 µL 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**

22. Add 250 µL 1x TE to each sample.
23. Add 8 µL of 10 mg/mL RNase A (final conc. = 0.2 mg/mL), and incubate at 37°C for 1 hr.
24. Add 8 µL of 20 mg/mL Proteinase K (final conc. = 0.4 mg/mL), and incubate at 55°C for 1 hr.
25. Prepare one Phase Lock tube (5 Prime, Cat#2302820) per IP by spinning down the gel to the bottom of the tube at 20,000 x g for 1 min.
26. Add 400 µL Phenol: Chloroform: Isoamyl Alcohol (25:24:1) alcohol to each Phase Lock tube.
27. Add sample to Phase Lock tube and invert the tube until the contents turn white.
28. Centrifuge for 4 min at max speed. Note: if aqueous phase is cloudy, extract again.
29. Transfer aqueous layer to a new 1.7 mL Eppendorf tube.
30. Add 16 µL of 5 M NaCl (final conc. = 200 mM) and 2 µL of 20 mg/mL glycogen (40 µg total) to each sample and vortex or pipet up and down to mix.
31. Add 920 µL cold 100% EtOH and vortex briefly.
32. Incubate at -80°C for 30 min or until frozen solid.
33. Spin at 20,000 x g for 15min at 4°C.
34. Wash pellet with 1 mL cold 70% EtOH and spin for 5 min at 4°C at 20,000 x g.
35. Remove the 70% ethanol using a pipet without disturbing the DNA pellet.
36. Dry the pellet for 5 min at room temperature.
37. Thoroughly resuspend the pellet in 50 µL 10 mM Tris.
38. IP material can be stored at -20°C for at least 1 month.