Ren Lab ENCODE Chromatin Immunoprecipitation Protocol for MicroChIP

Preparation of beads, binding primary Ab, followed by binding of chromatin

1. Make RIPA buffer 1 immediately before use and chill on ice.

RIPA BUFFER 1			
Reagent	**Stock Concentration**	**Final Concentration**	**Volume per 10 mL**
Tris, pH 7.5	1 M	10 mM	100 µL
NaCl	5M	140 mM	280 µL
EDTA	0.5 M	1 mM	20 µL
EGTA	0.5 M	0.5 mM	10 µL
Triton-X	10%	1%	1 mL
SDS	10%	0.1%	100 µL
dH2O	--		8.39 mL
Sodium Deoxycholate	10%	0.1%	100 µL

2. For each sample, add 5 µL IgG Dynabeads (Life Technologies, Anti-Mouse Cat#11201D; Anti-Rabbit Cat# 11204D) to a 200 µl PCR tube.
3. Collect beads by placing tubes on magnetic rack (perform all steps with magnetic rack on ice).
4. Once the beads have collected towards the magnet, slowly remove supernatant with a pipette. Avoid disturbing the beads.
5. Wash the beads 2 times with 100 µL cold RIPA buffer 1. Perform these washes as follows:
    a. Add solution (RIPA buffer 1 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.
6. After the final wash, add cold RIPA buffer 1 (100 µL minus volume of antibody to be added) to the beads.
7. With tubes against the magnet, add 2 µg antibody.
8. Remove tubes from magnet and Incubate at least 2 hours on a rotating platform at 4°C.
9. After the incubation, place tubes on magnetic rack to collect beads.
10. Remove the supernatant with a pipette once the beads have collected.
11. Wash once with 100 µL cold RIPA buffer 1. Perform this wash as follows:
    a. Add solution (RIPA buffer 1 in this case).
    b. Remove tubes from magnet and incubate at 4°C for 4min on a rotating platform.
    c. Place tubes on magnet and collect beads for 1 min.
    d. Remove supernatant
12. After the final wash, add 190 µL chromatin (1/7 of the ChIP-ready chromatin – see "Tissue fixation and sonication protocol" – divided equally for 1 input and 6 IPs) to the tube with the beads. Incubate at 4°C at least 3 hours (overnight is possible) on a rotating platform.

Washing beads, elution, and reversal of crosslinks

13. Make RIPA buffer 2 immediately before use and chill on ice.

RIPA BUFFER 2			
Reagent	**Stock Concentration**	**Final Concentration**	**Volume per 1000 µL**
Tris, pH 7.5	1 M	10 mM	100 µL
NaCl	5M	300 mM	600 µL
EDTA	0.5 M	1 mM	20 µL
EGTA	0.5 M	0.5 mM	10 µL
Triton-X	10%	1%	1 mL
SDS	10%	0.2%	200 µL
dH2O	--		7.97 mL
Sodium Deoxycholate	10%	0.1%	100 µL

14. 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.
15. Wash the beads with 100 µL cold RIPA buffer 4 times. Perform these washes as follows:
    a. Add solution (RIPA buffer 2 in this case).

b. Remove tubes from magnet and incubate at 4°C for 4min on a rotating platform.
c. Place tubes on magnet and collect beads for 1 min.
d. Remove supernatant

16. Wash once with 100 µL cold 1x TE by incubating at 4°C for 4min on a rotating platform.
17. Spin down briefly and transfer the beads and TE mixture into a new tube.
18. Wash the old tube with 50 µL 1x TE to get any remaining beads and add into the tubes with the previous fraction.
19. Place tubes on magnet and collect beads for 1 min.
20. After removing the TE by aspiration, add 150 µL elution buffer 1.

ELUTION BUFFER 1			
Reagent	**Stock Concentration**	**Final Concentration**	**Volume for 1 mL**
Tris, pH 7.5	1 M	20 mM	20 µL
NaCl	5M	50 mM	10 µL
EDTA	0.5 M	5 mM	10 µL
dH2O	--		860 µL
SDS	10%	1%	100 µL

21. Add 3 µL of 10 mg/mL RNase A (final conc. = 0.2 mg/mL) to each sample and incubate at 37°C for 1 hr, shaking at 1200rpm.
22. Add 1 µL of 20 mg/mL Proteinase K (final conc. = 0.13 mg/mL), and incubate at 68°C for 4 hr, shaking at 1200rpm.
23. After the incubation, spin the tubes briefly to collect condensation from the top.
24. 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.
25. Resuspend the beads in 150 µL elution buffer 1 and incubate at 68°C for 5 min, shaking at 1200rpm
26. Place on magnetic rack, wait for the beads to collect and transfer supernatant (containing any remaining immunoprecipitated (IP) chromatin) to the 1.7 mL Eppendorf tubes containing the first aliquot.

DNA Precipitation

27. Add 190 µL elution buffer 2 for a final volume of 490 µL.

ELUTION BUFFER 2			
Reagent	**Stock Concentration**	**Final Concentration**	**Volume for 1 mL**
Tris, pH 7.5	1 M	20 mM	20 µL
NaCl	5M	50 mM	10 µL
EDTA	0.5 M	5 mM	10 µL
dH2O	--		960 µL

28. 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.
29. Add 490 µL Phenol: Chloroform: Isoamyl Alcohol (25:24:1) alcohol to each Phase Lock tube.
30. Add sample to Phase Lock tube and invert the tube until the contents turn white.
31. Centrifuge for 4 min at max speed. Note: if aqueous phase is cloudy, extract again.
32. Transfer aqueous layer to a new 1.7 mL Eppendorf tube.
33. Add 44 µL of 3 M NaAc, pH7.0 and 11 µL of 5 mg/mL linear acrylamide (Thermo Fisher, Cat#AM9520) to each sample and vortex or pipet up and down to mix.
34. Add 1000 µL cold 100% EtOH and vortex briefly.
35. Incubate at -80°C for 30 min or until frozen solid.
36. Spin at 20,000 x g for 15min at 4°C.
37. Wash pellet with 1 mL cold 70% EtOH and spin for 5 min at 4°C at 20,000 x g.
38. Remove EtOH using a pipet without disturbing the DNA pellet.
39. Dry the pellet for 5 min at room temperature.
40. Thoroughly resuspend the pellet in 50 µL 10 mM Tris.
41. Store the IP material at -20°C or proceed with sequencing preparation using the ThruPLEX DNA-seq 12s kit (Rubicon Genomics, Cat#R400428) according to manufacturer's instructions.