Ren Lab ENCODE Tissue Fixation and Sonication Protocol for MicroChIP

Pulverization of Tissue Using Liquid Nitrogen Cooled Mini Mortar from BelArt (Cat#H37260-0100)

Note: Ensure the samples are kept frozen on dry ice throughout pulverization.

1. Move tissue into a 1.5 mL tube and keep on dry ice.
2. Pour liquid nitrogen into the steel well beneath the mortar and place mortar back on top.
3. Cool down the pestle with liquid nitrogen.
4. Insert the tube with the tissue into the mortar.
5. Use the pestle to grind the tissue inside the tube, turning it to apply pulverizing pressure.
6. Place the sample back onto the dry ice.
7. Clean the pestle before using it for the next sample.

Cross-linking of Tissue

8. Transfer a portion of tissue (roughly 10-20 mg per tissue type was used) into a 1.5 mL tube using a clean spatula or pipette tip.
9. Add cold 1x PBS to 1 mL.
10. Add 0.1 mL crosslinking buffer and rotate the tube at room temperature for 20 min.

CROSSLINKING BUFFER			
Reagent	**Stock Concentration**	**Final Concentration**	**Volume for 5 mL**
NaCl	5 M	0.1 M	0.1 mL
EDTA	0.5 M	1 mM	10 µL
EGTA	0.5 M	0.5 mM	5 µL
Hepes pH8.0	1 M	50 mM	0.25 mL
Formaldehyde	37%	11%	1.5 mL
dH2O	--	--	3.14 mL

11. Stop the crosslinking reaction by adding 55 µL 2.5 M glycine to a final concentration of 0.125 M.
12. Rotate at room temperature for 5 min.
13. Centrifuge samples at low speed (15min at 1000 x g).
14. Decant the supernatant and wash once with cold 1X PBS.
15. Centrifuge at low speed (15 min at 1000 x g).
16. Decant the supernatant.
17. Store cells at -80°C or proceed to sonication.

Sonication of Tissue

18. Resuspend fixed cells in 300 µL lysis buffer.

LYSIS BUFFER			
Reagent	**Stock Concentration**	**Final Concentration**	**Volume for 1 mL**
Tris, pH 8.0	1 M	50 mM	50 µL
EDTA	0.5 M	10 mM	20 µL
SDS	10%	1%	100 µL
dH2O	--		800 µL
cOmplete EDTA-free protease inhibitor (Roche, Cat#05056489001)	50x	1x	20 µL
PMSF	0.1 M	1 mM	10 µL

19. Using a Branson Sonifier 450, sonicate each sample for 10-20 cycles (30 sec ON, 30 sec OFF at power 3, duty cycle 50). Number of cycles depends on the type of tissue and the size of the pellet.
20. Pellet debris at 12,000 x g for 10 min in 4°C.
21. Transfer supernatant to a new tube.
22. Measure DNA concentration (e.g. using NanoDrop).
23. Dilute the amount of SDS in the chromatin down to 0.21% using the chromatin dilution buffer.
24. Remove a portion of chromatin to check fragmentation (steps below). Store the remaining chromatin in 4°C until shearing quality has been assessed. Proceed immediately to immunoprecitpation (see "Chromatin Immunoprecipitation Protocol").

Note: For this experiment, chromatin was divided into 7 aliquots for 1 input and 6 histone mark ChIPs. 190 µL of ChIP-ready chromatin was used per sample.

CHROMATIN DILUTION BUFFER			
Reagent	**Stock Concentration**	**Final Concentration**	**Volume for 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
dH2O	--		8.19 mL
Sodium Deoxycholate	10%	0.1%	100 µL
cOmplete EDTA-free protease inhibitor (Roche, Cat#05056489001)	50x	1x	200 µL
PMSF	0.1 M	1 mM	100 µL

DNA Isolation – Precipitation and Chromatin Fragmentation Check

25. Add enough elution buffer 1 to the chromatin set aside for the fragmentation check so the total volume is 300 µL.

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

26. Add 6 µL 10 mg/mL RNase A (final conc. = 0.2 mg/mL) to each sample and incubate at 37°C for 1 hr.
27. Add 2 µL 20 mg/mL Proteinase K (final conc. = 0.13 mg/mL), and incubate at 68°C for 4 hr.
28. 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

29. 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.
30. Add 490 µL Phenol: Chloroform: Isoamyl Alcohol (25:24:1) alcohol to each Phase Lock tube.
31. Add sample to Phase Lock tube and invert the tube until the contents turn white.
32. Spin for 4 min at max speed. Note: if aqueous phase is cloudy, extract again.
33. Transfer aqueous layer to a new 1.7 mL Eppendorf tube.
34. 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.
35. Add 1000 µL cold 100% EtOH and vortex briefly.
36. Incubate at -80°C for 30 min or until frozen solid.
37. Spin at 20,000 x g for 15 min at 4°C.
38. Wash pellet with 1 mL cold 70% EtOH and spin for 5min at 4°C at 20,000 x g.
39. Remove EtOH using a pipet without disturbing the DNA pellet.
40. Dry the pellet for 5 min at room temperature.
41. Thoroughly resuspend the pellet in 11 µL 10 mM Tris.

42. Measure DNA concentration (e.g. using Qubit dsDNA HS) and check the size of the fragmentation on an agarose gel. If necessary, keep some aside as it can be prepared for sequencing and used as an input-control. Average size of chromatin fragmentation should be 200bp.
43. Store the remaining material at -20°C.