

Mouse Liver Chromatin
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12/02
Modified by Phil Le
6/04

This protocol as written here can be used for pieces of tissue approximately 0.5 cm cubed. It can be used for larger pieces of tissue by scaling up of the ChIP cell lysis buffer and increased sonication.

1. Make 2% formaldehyde/PBS and 2.5 M glycine fresh.
2. Remove liver and either use immediately or freeze immediately in liquid nitrogen.
3. Add 2 ml PBS and chop liver finely with razor or scissors.
4. Push through a 21G needle into 15 ml tube and bring final volume to 5 ml.
5. Add 5 ml 2% formaldehyde/PBS to reach final concentration of 1%.
6. Rotate 10 min at Room Temp (RT).
7. Stop x-linking by adding 500 μ l 2.5 M glycine to final concentration 0.125 M.
8. Rotate 5 min at RT. Pellet by spinning at 2.5K for 2', pour off.
9. Wash 1 x with 5mL cold PBS (Add PBS, vortex, pellet, pour off).
10. Bring up pellet in 1 ml cold ChIP cell lysis buffer + PI (Protease Inhibitor, Roche tablets) and Dounce homogenize on ice. Transfer to 1.5mL eppendorf.
11. Incubate 5 minutes at 4 °C to release nuclei.
12. Pellet nuclei in microfuge at max RPM for 5 minutes at 4 °C. Dump supernatant.
13. Bring up pellet in 2x pellet volume (but not less than 700 μ l) ChIP nuclear lysis buffer + PI.
14. Sonicate on ice 3 x 20 sec pulses, power output (not setting) 4-6 W, allowing the sample to cool between bursts.
15. Spin in microfuge at max RPM at 4°C for 10 minutes.
16. Take off 10 μ l and add it to 190 μ l TE. Heat this at 65 °C for > 4 hrs to un-crosslink, Qiaquick purify, check 5-10 μ l on gel and quantify DNA.
17. Aliquot remainder to ~125 μ l aliquots and freeze on N₂.

Buffers

1. 2% formaldehyde/PBS
 - a. 540 μ l 37% formaldehyde + 9.5 ml PBS
2. 2.5 M glycine
 - a. 190 mg in 1ml H₂O
3. ChIP cell lysis buffer: 10 mM Tris-Hcl, pH 8.0, 10 mM NaCl, 3 mM MgCl₂, 0.5% NP-40
 - a. To make 50 ml:
 - i. H₂O
 - ii. 0.5 ml 1.0M Tris-Hcl, pH 8.1
 - iii. 100 μ l 5M NaCl
 - iv. 150 μ l 1m MgCl₂
 - v. 2.5 ml 10% NP-40
4. ChIP nuclear lysis buffer: 1% SDS, 5 mM EDTA, 50 mM Tris-Hcl, pH 8.1
 - a. To make 50 ml:
 - i. H₂O

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- ii. 5 ml 10% SDS
 - iii. 500 μ l 0.5 M EDTA
 - iv. 2.5 ml 1.0 M Tris-HCl, pH 8.1
5. Protease inhibitor: Roche Complete PI tablets 1 697 498
Dilute in 2mL of water yields 25X.