ChIP From Frozen Pellets - MNase Protocol (Full)

Version 1.0
Date:
Chromatin Prep

Prepare Dynal Beads
1. Mix 25ul A and 25uL G beads per IP (1e7 cells)
2. Wash beads 3X with PBS + 0.5% BSA
3. R/S in 400ul PBS + 0.5% BSA
4. Add antibody
1.
2.
3.
4.
5.
5. Rotate o/n at 4 degrees
6. Wash 2X with PBS + 0.5% BSA; 2X with IP Buffer

Thaw chromatin on ice (usually 1 aliquot / 2 IPs = 1e7 cells)
1. R/S in 1ml Swelling Buffer + 1mM PMSF + 1X PICs (protease inhibitors)
2. Incubate 10 minutes on ice
3. Dounce 20 strokes
4. Pellet nuclei at 2000 RPM X 7 minutes @ 4 degrees
5. R/S in 5mL sucrose A + PMSF + PICs
6. Layer over 5mL sucrose B
7. Spin 3000 RPM X 10 minutes @ 4 degrees
8. Wash in 10 mL digestion buffer
9. Spin 2000 RPM X 7 minutes @ 4 degrees
10. R/S in 1mL/4e7 cells digestions buffer + 3.3uL 1M CaCl2 + PMSF + PICs
Added:
11. Incubate 5 minutes at 37 degrees to pre-warm
12. Add 0.25ul MNase/ 1e7 cells **Added:**
13. Incubate 15 minutes at 37 degrees
14. Add 1/10 volume 0.5M EDTA
15. Store on ice 5 minutes
16. Add 1 volume 2X IP buffer (LB3)
17. Pass 5X through 20G needle then 5X through 25G needle
18. Spin at 13000g x 15 minutes in microfuge @ 4 degrees
19. Save sup = S1 (store on ice)
20. R/S insoluble pellet in 1mL IP buffer (LB3)
21. Incubate 1-2 hours @ 4 degrees rotating (can extend this if desired)
22. Spin at 13000g X 15 minutes in microfuge @ 4 degrees
23. Save sup = S2
24. Combine S1 and S2 (should have 2mL here per original tube of pelleted cells)
25. Adjust volume 2.2mL per original tube
25. Save 100ul as 10% Input
26. Setup IP - add 1mL lysate to conjugated beads from above
27. Rotate o/n at 4 degrees
28. Washes - 1mL each @ 4 degrees for 5 minutes
1. 6 Agilent RIPA washes
2. 1 TE + 50mM NaCl (change tubes before this wash)
29. Elute in 100ul 1% SDS/ 100mM NaHCO3 for 17 minutes at 65 degrees vortexed every 3 minutes
30. add 5ul 5M Nacl
31. Incubate 65 degrees o/n
32. Add 3ul RNAse A - incubate 30 minutes at 37 degrees
33. Add 5ul Proteinase K - incubate 1 hour at 56 degrees
34. Clean up with zymo chip kit - elute in 50uL
35. Check concentration of 1ul on qubit and run 1 and 4ul on 1.5% gel