

ChIP in MTB

Sample preparation

1. Grow fridge/freezer stock and use it to seed a 250ml culture in a 1L roller bottle
2. Grow cells to an OD₆₀₀ of 0.4-0.5 and dilute to an OD₆₀₀ of 0.2 in a sterile spinner flask
3. Connect gas lines to induce hypoxia and incubate cultures for desired time course – expect an evaporation rate of ~10ml per day
4. At appropriate time point, remove a 40ml aliquot and split in to two 50ml tubes (20ml in each) containing 540ul 37% formaldehyde (for a final concentration of 1%).
5. Incubate at room temp. on a shaker for 30 minutes
6. Quench X-linking with addition of 3.3ml of 3M Glycine (for a final concentration of 0.5M)
7. Centrifuge at 3000 rpm for 10 minutes at 4°C. Aspirate supernatant.
8. Resuspend and wash pellet in 20ml ice-cold 7H9 + 174ul of 20mg/ml phenylmethylsulfonyl fluoride (PMSF, for a final concentration of 1mM) – **ADD PMSF JUST PRIOR TO USE**
9. Centrifuge at 3000 rpm for 10 minutes at 4°C. Aspirate supernatant.
10. Resuspend pellet in 500ul ice cold 1X PBS + **fresh protease inhibitors** (5ul 100x Sigma cocktail + 4.35ul 20mg/ml PMSF) and transfer to Lysing Matrix B tubes
11. Bead beat samples 3 x 30s at speed 6.5 in FastPrep with cooling on ice between bead beats
12. Microfuge at full speed for 1 minute and remove supernatant to a clean 2ml Covaris S2 shearing tube
13. Using the “ChIP 16 min” shearing protocol (amplitude = 20%, power = 5, cycles/burst = 200), shear cross-linked DNA on 16 minute program.
14. After shearing, transfer sample to clean 1.5ml screw top tube (note the volume)
15. Decontaminate samples and remove from BL-3

Immunoprecipitation

16. Based on volume of sample removed from BL-3, prepare twice this volume of IP buffer with fresh 1x protease inhibitors (i.e., if the sample volume from shearing is 350ul, make up 700ul of IP buffer with P.I.)
17. Mix IP buffer with sheared sample (note final volume)
18. Add 10 ul of 1ug/ul anti-FLAG antibody (10ug final)
19. Incubate on shaker at 4°C overnight

Day 2

20. Mix stock Protein G Magnetic Bead suspension so that all beads are uniformly resuspended.
21. Determine the number of tubes necessary for IP of all samples and pipette 50 uL of suspended beads in to 1.5 ml microcentrifuge tubes.

22. Place the tubes on to a magnet rack and allow the beads to adhere to the side of the tube. Remove the storage buffer with a pipette.
23. Wash the beads by adding 500 ul of PBS containing 0.1% Tween 20 surfactant and vortexing vigorously for 10 seconds. Quick spin to collect the solution. Return the tube to the magnet and allow the beads to adhere to the side. Remove the buffer with a pipette.
24. Wash the beads two more times (3 times total) with 500 uL of PBS containing 0.1% Tween 20.
25. Remove the buffer with a pipette.
26. Take antibody-coupled samples from overnight incubation and do a quick spin to collect material.
27. Transfer entire antibody-coupled sample to a tube containing washed protein G-coupled beads.
28. Incubate beads and sample for 1 hour at 4°C and 2 hours at 37°C.
29. Magnetically separate beads and remove the supernatant
30. Wash with 500ul IP buffer
31. Wash with 500ul IP buffer
32. Wash with 500ul IP wash buffer #3
33. Wash with 500ul IP wash buffer #4
34. Wash with 500ul IP wash buffer #5
35. Resuspend beads in 100ul IP elution buffer and incubate at 65°C for 15 minutes
36. Separate beads and remove supernatant to a clean microfuge tube

DNA purification & preparation

37. To 100ul sample from above, add 4ul of 20mg/ml stock of pronase (final concentration of 0.8mg/ml) and incubate at 42°C for 2 hours (NOTE: pronase concentration of 1mg/ml has worked as well)
38. To reverse x-linking, increase incubation temperature to 65°C and incubate for 9 hours
39. Purify DNA using Qiagen PCR purification kit