ChIP protocol for procyclic forms

- 1. grow cells to a density of 2×10^7 cells/ml in SDM-79
- 2. spin down (2000g for 10 min) and resuspend in 40 ml SDM-79
- 3. add 1.1 ml 37% formaldehyde to 40 ml cell suspension (final concentration 1%)
- 4. incubate at RT for 5-30 min, gently invert the tube 2-3 times during cross-linking
- 5. add 2.5 ml of 2M glycine solution to stop cross-linking, incubate 5 min at RT
- 6. spin down cells, wash with 20 ml cold PBS, spin down again, place cell pellet on ice.
- 7. resuspend pellet in 2 ml lysis buffer with protease inhibitors, buffer should be at RT: transfer into a 15 ml conical tube or 2 ml Eppendorf tube
- 8. incubate 15 min on ice
- 9. sonicate 6 cycles of 30-60 sec (depends on your sonicator), keep cell suspension on ice all the time, cool cells and tip between cycles, take 50 μ l samples after 2nd, 4th and 6th cycle to check chromatin size, chromatin fragments should be ~ 500 bp
- 10. spin down debris (15,000g for 1 min) and transfer supernatant to new tube.
- 11. start the IP: use 200 μ l of lysate for each IP (should be ~ 4-8 x 10⁷ cells per IP)
- 12. dilute to 1.4 ml with IP buffer with protease inhibitors, add 30 μ l Protein-G-agarose beads and incubate for 90 min at 4 °C, spin down and transfer supernatant into new tube
- 13. add antibody and incubate over night at 4°C, (**remember** to keep an aliquot of lysate as the 'input' control)
- 14. next day, add 25 µl Protein-G-agarose beads and incubate 90 min at 4°C
- 15. wash the beads with 1 ml of buffer A, B, C, TE (at least 15 min each), add protease inhibitors only to buffer A, with last wash step transfer beads to new tube
- 16. resuspend beads with 250 μ l of 1% SDS, 0.1M NaHCO₃, incubate 10 min at RT, pellet beads and transfer supernatant to fresh tube, wash beads again with 250 μ l SDS-carbonate buffer and combine supernatants
- 17. to the 500 μ l supernatant add 20 μ l 5M NaCl and incubate overnight at 65°C to reverse crosslinking
- 18. add 10 µl of 0.5M EDTA, 20 µl 1M Tris/HCl pH 6.5
- 19. add 20 μ g of RNase A, incubate at 37°C for 30 min
- 20. add 40 μ g of Proteinase K, incubate at 45°C for 1h
- 21. extract the solution with 500 μ l CPI, add 20 μ g of glycogen, add 1 ml EtOH to precipitate the DNA, wash 2x with 500 μ l 80% EtOH
- 22. dissolve pellet in 100 μ l water
- 23. slot blot can be used to analyze precipitated DNA if the sequences of interest are repetitive (follow standard protocol e.g. Current Protocols in Molecular Biology, unit 2.9.15 ff)
- 24. Real-time PCR should be used to detect single copy genes

Protocol for bloodstream forms

- 1. Grow cells to $2x10^{6}$ /ml (200 ml).
- 2. Add formaldehyde to a final concentration of 1%; incubate at RT for 5 min. Stop reaction by addition of Glycine (0.125 M, final conc.). Incubate at RT 5 min.
- 3. Spin (10 min, setting 5); wash with cold PBS; spin again. Place pellet on ice.

- 4. Resuspend pellet in 1 ml lysis buffer containing protease inhibitors; incubate on ice 15 min.
- Sonicate (8 cycles of 30 sec each, setting 30%, cell suspension on ice, cool cells and tip between cycles). Take 50 μl samples after 4th, and 8th cycles to check effectiveness of sonication.
- 6. Spin down debris hard for 5 min and transfer supernatant to new tube.
- 7. Start IP: from here, follow the protocol for PF CHIP starting at step 11.

Note: every antibody has to be tested for compatibility with wash buffers. For example: Flagantibody M2 cannot be used with buffers containing SDS (see below)

Solutions

Lysis buffer

1% SDS; 10mM EDTA pH 8.0; 50 mM TrisHCl pH 8.0 add PMSF 1mM; Leupeptin, Aprotinin, Pepstatin $1\mu g/\mu l$ each

IP dilution buffer

0.01%SDS; 1.1% Triton X-100; 1.2 mM EDTA, 16.7 mM TrisHCl pH 8.0; 150 mM NaCl; add PMSF 1mM; Leupeptin, Aprotinin, Pepstatin $1\mu g/\mu l$ each

Beads

Protein G-Agarose, 30 µl per IP, blocked with 5 µg E. coli sonicated DNA and 30 µg BSA

IP wash buffer A

0.1%SDS; 1% Triton X-100; 2 mM EDTA pH 8.0; 20 mM TrisHCl pH 8.0; 150 mM NaCl with 1 mM PMSF; Leupeptin, Aprotinin, Pepstatin $1\mu g/\mu l$ each

IP wash buffer B

0.1%SDS; 1% Triton X-100; 2 mM EDTA pH 8.0; 20 mM TrisHCl pH 8.0; 500 mM NaCl

IP wash buffer C

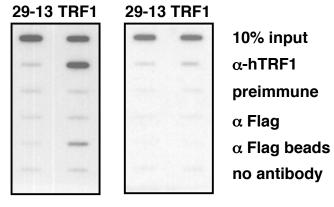
0.25 M LiCl; 1% NP-40; 1% Na-Deoxycholate; 1 mM EDTA pH 8.0; 10 mM TrisHCl pH 8.0

TE wash buffer

10 mM TrisHCl pH 8.0; 1 mM EDTA pH 8.0

CPI: Chloroform/Phenol/Isoamylalcohol (25:24:1 by volume)

Example: C. Janzen, 2001, ChIP with procyclic cells expressing Flag-tagged human TRF1 using polyclonal α -hTRF1 and α -Flag antibodies



TR probe

177bp probe