## ChIP protocol for procyclic forms

1. grow cells to a density of 2 x $10^7$ 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 $\mu$l samples after 2$^{nd}$, 4$^{th}$ and 6$^{th}$ 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 $\mu$l of lysate for each IP ( should be ~ 4-8 x $10^7$ cells per IP)
12. dilute to 1.4 ml with IP buffer with protease inhibitors, add 30 $\mu$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 $\mu$l 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 $\mu$l of 1% SDS, 0.1M $NaHCO_3$, incubate 10 min at RT, pellet beads and transfer supernatant to fresh tube, wash beads again with 250 $\mu$l SDS-carbonate buffer and combine supernatants
17. to the 500 $\mu$l supernatant add 20 $\mu$l 5M NaCl and incubate overnight at 65°C to reverse crosslinking
18. add 10 $\mu$l of 0.5M EDTA, 20 $\mu$l 1M Tris/HCl pH 6.5
19. add 20 $\mu$g of RNase A, incubate at 37°C for 30 min
20. add 40 $\mu$g of Proteinase K, incubate at 45°C for 1h
21. extract the solution with 500 $\mu$l CPI, add 20 $\mu$g of glycogen, add 1 ml EtOH to precipitate the DNA, wash 2x with 500 $\mu$l 80% EtOH
22. dissolve pellet in 100 $\mu$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 2x$10^6$/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.
5. 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: Flag-antibody 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 $\mu$l per IP, blocked with 5 $\mu$g E. coli sonicated DNA and 30 $\mu$g 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