ChIP Staph A Protocol

(For ChIP-PCR only, not for CHIP-seq.
Modified from Farnham lab protocol)

Day 1.

1. **Collect samples** from 10-cm dish of cells in healthy and not dense condition (generally use one 10-cm dish of cells (~1 x 10^7 cells) per antibody per ChIP. If grow cells in 15-cm dishes, cells from one 15-cm can be used for 2-3 ChIPs)
2. Add 270-300 ul of formaldehyde (37% stock) directly to tissue culture media (10-11ml) to a final concentration of 1%, slowly rock (adjust speed to 1.5) on a shaking platform for 10' at RT. If 15-cm dish, add 680 ul formaldehyde into 25 ml medium/dish.
3. Stop the cross-linking reaction by adding 1 ml of 1.25 M glycine to a final concentration of 0.125M continuing to rock/spin for 5' at RT. If in a 15-cm dish, add 2.7 ml glycine.
4. Carefully rinse cells twice with 1X PBS.
5. Aspirate PBS from the dish, add 1ml of PBS+ 1mM PMSF into each dish and scrape cells and transfer into 1.5 ml eppendorf. If 15-cm dish, add 4 ml PBS+PMSF.
6. Spin in microfuge at 3000-4000rpm (1k x g) at 4^{o}C for 3'.
7. Aspirate the supernatant and wash one more time with PBS.
8. Aspirate the supernatant. *Cells can be used immediately for ChIP assay or snap frozen in liquid nitrogen and stored at -80°C*.
9. Resuspend cell pellet in each tube in 0.5-1ml of Cell lysis buffer (with PI), incubate on ice for 20', flick occasionally to resuspend cells well.
10. Spin in microfuge at 5000rpm (2.5k x g) ar 4^{o}C for 5', aspirate the supernatant.
11. Resuspend nuclei in 200 ul Nuclei Lysis buffer (with PI), incubate on ice for <10'.
12. Transfer the cell lysates into Diagenode TPX® Polymethylpentene 1.5 ml Eppendorf tubes (Cat# M-50001. higher sonication efficiency).
13. Weigh glass beads and add 80 mg into each tube, seal the tubes with parafilm.
14. **Sonication**: Sonicate chromatin following the Instruction of Bioruptor to an average length of about 100-500 bp.

    (1) Time: most cells need sonication of 3-4 cycles of 30 min/cycle. Sonication time may vary between cell types. Always test sonication conditions for new cell lines.
    (2) Setting: the setting should be 30" on 30" off for 30 min, total 60 pauses for two tanks each cycle.
    (3) Temperature control: check the cooler's temperature after each cycle. Normally the temperature reaches about 10oC after each cycle. Add some ice into the cooling system and be sure to monitor the temperature for ~5 min until it is stable at 2oC.
    (4) Wait 30 min after every two cycles (1 hr) of sonication to rest the sonicator.
    (5) During the sonication step, you can prepare the preblocked beads for preclear.
    (6) Rinse off used tube holder and accessories with water and then ethanol when finished.
15. While sonicating (or before collecting cells), **prepare Staph A cells** from stocks at -80^{o}C freezer. Each ChIP need 20 ul of Staph A cells (10 ul for blocking and 10 ul for IP).
    (1) Thaw Frozen Staph A cells, for each tube (100 ul) add 10 ul of 10mg/ml BSA and 10 ul of 10mg/ml salmon sperm DNA, rotate at 4oC for >3 hrs.
    (2) Centrifuge at 13,000rpm at 4^{o}C for 2',

(3) Aspirate supernatant, wash twice with 1ml of Dialysis buffer,
(4) Resuspend each tube in 200 ul of Dialysis buffer + 1mM PMSF, use 20 ul for each blocking or ChIP. (These *Staph A cells are can be stored at 4oC for two weeks*)

16. After sonication, microfuge samples at 13,000rpm at 4^0C for 10', transfer supernatant (~170-180 ul) to a new 1.5 ml tube. (Note:1. *This sonicated chromatin can be frozen and used at a later time. 2. Do not leave lysates on ice too long, as the SDS will precipitate.*)
17. **Check DNA size:** DNA sizes are essential for calculation of ChIP. The size should be 100-600bp, centered at 250bp. Discard samples if average sizes are more than 1 KB.
    (1) Take 5 µL sample and add 45 µL IP Elution Buffer
    (2) Reverse cross-link by adding 2 µL of 5 M NaCl (final 0.2M NaCl) and Boil for 15'.
    (3) After returning to RT, add 1 µL of 10 mg/mL RNase A, incubate at 37oC for 15-30'.
    (4) Extract with Phenol/chloroform.
    i. Add an equal volume (50 ul) of buffer-saturated phenol:chloroform (1:1) to the DNA solution. Mix well by vortex.
    ii. Spin at 13,000 rpm for 3 min.
    iii. Carefully remove the aqueous layer to a new tube, being careful to avoid the interface. (Steps 1-3 can be repeated until an interface is no longer visible)
    iv. Ethanol precipitate the DNA if necessary.
    (5) Run 10-15 ul on a 1.5% agarose gel along with 100 bp ladder for 10-15 min.

18. **Dilution**: add 2-4x volume (800 ul) of dilution buffer (with 1mM PMSF) to each 170-200 ul sample, gently mix well.
19. **Preclear**: Add 20 ul of washed Staph A cells (with 1mM PMSF) to each sample, rotate at 4^oC for 10'.
20. Spin at 13,000 rpm at 4^oC for 2', transfer the supernatant to a new tube. *(Avoid taking any beads!)*
21. **Save input**: Save 10 ul of each sample into a new tube as 1% Input. *(Remember to reverse crosslink at later step!)*
22. **IP**: add 1 ug of primary antibody to each sample, rotate at 4^oC for overnight. (Amount of antibody used for ChIP may vary, use 1ug for most transcription factors or enzymes, and 2 ug for histone modifications)

Day 2.

23. If using monoclonal antibody or a polyclonal antibody from a species other than rabbit, add 1-2 ug of appropriate secondary antibody and rotate for 1 h at 4^oC.
24. **Binding**: add 20 ul of washed Staph A cells (with PMSF) to each sample, rotate at RT for no more than 15'.
25. **Wash**: Microfuge at 13,000rpm at 4^oC for 1', aspirate or transfer the supernatant to another tube (*Supernatant of IgG sample can be used as Input if you forgot to save input at earlier step*). Add 1 ml of Dialysis buffer, resuspend the pellet by pipette up and down, invert tubes 20 times at RT, and spin at 13,000 rpm at 4oC for 1'. (Note to use different Dialysis buffers for mouse and rabbit antibodies)
26. Wash with Dialysis buffer one more time.
27. Wash with ChIP Wash buffer 4 times. (Note to use different Wash buffers for mouse and rabbit antibodies)

28. Prepare Elution buffer at RT. *Need 110 ul for each IP sample and 90 ul for each 1% input*.
29. **Elution**: Elute antibody/protein/DNA complexes by adding 55 ul of fresh IP elution buffer at RT, shake on a vortexer for 15' at RT at setting 3.
30. Spin at 13,000 rpm for 3' at RT, transfer the supernatant to a new 1.5 ml tube.
31. Add 55 ul of IP elution buffer and repeat step 25,26, combine supernatant in same new tube (110 ul total).
32. Spin the samples again at 13,000 rpm at RT for 5' to remove traces of Staph A cells by transfer 100 ul of the supernatant to a new 0.5 ml tube. *(Avoid taking any beads!)*
33. For input sample, add 90 ul of Elution buffer to the 10 ul sample (1% input).
34. **Reverse Cross-link**: add 5 ul of 4 M NaCl (0.2 M NaCl final) to each sample and input sample, incubate at 67^{o}C for >8hrs to reverse crosslink.

Day 3.

35. Prewarm samples at 4oC to 37oC for 1' to dissolve SDS.
36. Add 1ul of 10 mg/ml RNase A to each sample, and incubate at 37^{o}C for 30'.
37. Use Qiagen PCR purification kit to purify DNA. Transfer sample to a new 1.5 ml tube, add 500 ul Qiagen buffer PBI and 10 ul of 3 M NaOAc (pH5.2) to adjust pH before binding sample to column. Elute sample with 50 ul of EB twice, and combine the sample (total 100 ul).
38. Use 0.5-1 ul sample for each Real-time PCR reaction (total 10 ul).

Solutions for ChIP-Staph A protocol

Cell Lysis Buffer	Stock	Vol for 500 ml
5 mM PIPES pH 8.0	0.5 M	5 ml
85 mM KCl	3 M	14 ml
1% NP40 (0.5% NP 40 is OK)	10%	50 ml
ddH2O		433 ml
Add PI and PMSF freshly.		

Nuclei Lysis buffer		500 ml
50 mM Tris-Cl pH 8.0	1 M	25 ml
10 mM EDTA	0.5 M	10 ml
1% SDS	10%	50 ml
ddH2O		415 ml
Add PI and PMSF freshly		

Dilution buffer		500 ml
20 mM Tris-Cl pH 8.0	1 M	10 ml
150 mM NaCl	4 M	19 ml
1 mM EDTA	0.5 M	1 ml
1% Trition X 100	10%	50 ml
0.01% SDS	10%	0.5 ml
ddH2O		420 ml
Add PI and PMSF freshly		

ChIP Elution buffer		10 ml
50 mM NaHCO3	1 M	0.5 ml
1% SDS	10%	1 ml
ddH2O	10%	8.5 ml
Make freshly!		

The following buffers are different from ChIP-seq buffers.

ChIP Dialysis buffer-Rabbit		500 ml
50 mM Tris-Cl pH 8.0	1 M	25 ml
0.2% Sarkosyl	20%	5 ml
2 mM EDTA	0.5 M	2 ml
ddH2O		468 ml

ChIP Dialysis buffer-Mouse		500 ml
50 mM Tris-Cl pH 8.0	1 M	25 ml
2 mM EDTA	0.5 M	2 ml
ddH2O		473 ml

ChIP Wash buffer-Rabbit		500 ml
100 mM Tris, **pH 9.0**	1 M	50 ml
500 mM LiCl (MW 42.4)		10.6 g
1% NP40	10%	50 ml
1% Deoxycholic acid (sodium salt. MW 414.5)		5 g
ddH2O		To 500 ml

ChIP Wash buffer-Mouse		500 ml
100 mM Tris, pH 8.0,	1 M	50 ml
500 mM LiCl (MW 42.4)		10.6 g
1% NP40	10%	50 ml
1% Deoxycholic acid (sodium salt. MW 414.5)		5 g
ddH2O		To 500 ml

Stock solutions	Volume	Wright
1.25 M Glycine (MW 75)	1 L	94 g
1M NaHCO3 (MW 84)	50 ml	4.2g
0.5 M PIPES	200 ml	
PIPES		30.2 g
10 M NaOH		17-19 ml