ChIP Protocol
(modified by Ethan Ford from Kouskouti, Epigenome NOE: http://www.epigenome-noe.net/)

X-link Cells

1. Grow two 15cm HeLa plates to confluence (change media aprox. 16 hours before harvesting)
2. Add 690 µl of 36% formaldehyde (final conc. 1%) to each plate and incubate with rocking for 10 min at room temp.
3. Add 1.25 ml 2.5 M glycine to each plate (final conc 0.125 M). Incubate 5 min at room temp with rocking.
4. Wash cells three times with PBS.
5. Harvest cells with 15 ml PBS using rubber policeman.
6. Spin 3k rpm for 7 min at 4° C.

Prepare Nuclei

7. Discard supernatant and resuspend in 15 ml 'Swelling Buffer'.
8. Incubate for 10 min on ice.
9. Dounce homogenize with 20 strokes.
10. Spin at 3k rpm for 7 min at 4° C.
11. Discard supernatant and resuspend in 7 ml Sucrose Buffer A.
12. Dounce homogenize with 20 strokes.
13. Layer cells on top of 7 ml of Sucrose Buffer B in 15 ml tube.
14. Spin 3k RPM for 15 min.
15. Aspirate supernatant from top.
16. Resuspend pellet in 15 ml 'Buffer NUC'.
17. Spin 3k RPM for 7 min.
18. Discard supernatant and resuspend in 1 ml 'Buffer NUC'.

Fragment and solubilize chromatin

19. Add 20 µl 50x protease inhibitor mix (dissolved one protease inhibitor tablet in 1 ml H_2O), 3.3 µl 1M $CaCl_2$ and 5 µl Micrococcal Nuclease (NEB# M0247S 2,000 gel units/µl)
20. Incubate at 37° C for 12 min. The exact time needs to be established empirically. The chromatin preparation should consist of mono-nucleosomes with some di-, tri- and tetra- nucleosomes. >90% of the DNA should be less then 500 bp. The presence of longer DNA fragments causes an increase in background. The amount of Micrococcal Nuclease and the incubation time needs to be optimized empirically. Digestion down to the mononucleosome level seems to work fine but decreases PCR efficiency.
21. Add 1 ml '2x Sonication Buffer X'.
22. Split chromatin into two 1.5 ml tubes and place in ice-water bath.
23. Sonicate for 1.5 min total at 35% in 5 second pulses (19 x 5 seconds) alternating tubes so samples remain ice cold.
24. Spin in microfuge at max speed for 15 min.
25. Pass chromatin through 2 µM syringe filter collecting in a 15 ml tube.

26. Pass an additional 1 ml '1x Sonication Buffer X' through filter into tube with chromatin in order to recover remaining chromatin.

Immunoprecipitation

27. Take a 150 µl aliquot of chromatin and put aside for input control.
28. Divide remaining chromatin into tubes for immunoprecipitation.
29. To each tube add desired amount of antibody.
30. Incubate overnight at 4˚ C.
31. Equilibrate beads. Prepare one 1.5 ml tube for each for each immunoprecipitatin with 1 ml '1x Sonication X Buffer' and desired amount of Protein G-Dyna beads to each tube. Note protein-G beads are a significant source of background so it's best to use as few as possible. The stated binding capacity is 200ng antibody/µl.
32. Mix by inverting and spin at 3k rpm in microfuge briefly to get liquid off top of tube.
33. Place in magnetic rack and remove all liquid.
34. Add the chromatin from each I.P. to the appropriate tube with washed beads.
35. Incubate at 4˚ C for 1.5 hours in tube rotator.
36. Spin briefly in microfuge at 3k RPM. Place in magnetic rack and remove liquid.
37. Use filter tips for all remaining steps.
38. Wash 6 times with 'LiCl Wash Buffer' transferring to new tubes three times (less washes can increase recovery). Wash instructions: Add 1 ml LiCl Wash Buffer, mix by inverting or pipeting. If mixed by inverting, spin briefly to get liquid off the top of the tube. Place in magnetic rack and remove all liquid.
39. Wash one time with 1ml TE pH 8.1.
40. To elute chromatin, add 50 µl 2% SDS, 0.1M $NaHCO_3$ (make fresh) and incubate 45 min at 65˚ C. Vortex every 10 min or so.
41. Spin briefly at 3k rpm and collect eluted DNA by placing in magnetic rack.
42. Collect remaining DNA by resuspending beads in 50 µl 0.1 M $NaHCO_3$, vortex briefly, spin briefly, place in magnetic rack, collect supernatant and combine with previous elution.

Reverse X-linking

43. To ChIP'ed DNA add 6 µl 3M NaCl and 0.5 µl 30 mg/ml RNase A.
    To input DNA add 9 µl 3M NaCl and 0.5 µl 30 mg/ml RNase A.
44. Reverse X-linking by incubating 4 hours to overnight at 65˚ C.
45. To ChIP'ed DNA add 100 µl H_2O, 4 µl 1M Tris-HCl pH 6.5 and 1.5 µl 20 mg/ml Proteinase K.
    To input DNA add 150 µl H_2O, 4 µl 1M Tris-HCl pH 6.5, 12 µl 10% SDS and 1.5 µl 20 mg/ml Proteinase K.
46. Incubate 2 hours at 50˚ C. Vortex a couple times during incubation.
47. Remove 100 µl of input DNA and discard.

Purifiy DNA with Qiagen Minelute columns

48. Add 10 µl 3M NaOAc, pH 5.2 and 1ml Qiagen Buffer PB. Mix by pipeting.

49. Apply 650 µl to Qiagen Minelute column. Spin for 10 sec in microfuge.
50. Remove all liquid from collection tube.
51. Apply remaining DNA to column. Spin 1 min at max speed.
52. Remove all liquid from collection tube.
53. Wash column by adding 750 µl Qiagen buffer PE to column
54. Let sit for 2 min. Spin 1 min at max speed
55. Remove all liquid from collection tube.
56. Spin an additional 2 min to dry column.
57. Place column in new 1.5 ml tube and elute DNA by adding 25 µl Elution Buffer to column, let sit for 5 min and spin 1 min.
58. Transfer eluted DNA to new 1.5 ml tube and add an additional 25 µl of water.

Post ChIP Analysis

59. Use 4 µl of eluted DNA for with QuantIT HS DNA Assay Kit (Invotrogen)
60. Run 5 µl of input DNA on a 2.2% agarose gel.
61. Use 3 µl DNA for qPCR.

Congratulations!!!!

Swelling Buffer

25 mM Hepes, pH 7.8
1.5 mM $MgCl_2$
10 mM KCl
0.1% NP-40
+ 0.5 mM PMSF (must be added fresh)

Sucrose Buffer A
0.32 mM sucrose
15 mM Hepes pH 7.9
60 mM KCL
2 mM EDTA
0.5 mM EGTA
+0.5 mM PMSF

Sucrose Buffer B
30% sucrose
15 mM Hepes pH 7.9
60 mM KCL
2 mM EDTA
0.5 mM EGTA
+0.5 mM PMSF

Buffer NUC
15mM Hepes pH 7.5
60mM KCL
15mM NaCl
0.34mM sucrose
+0.5 mM PMSF

2x Sonication Buffer X
90mM Hepes pH 7.9
220mM NaCl
10mM EDTA
1% NP-40
0.2% Na-deoxycholate
0.2% SDS

1X Sonication Buffer X
Mix 1 volume of 2x Sonication Buffer X with 1 volume Buffer NUC

LiCl Wash Buffer
100 mM Tris-HCl, pH 7.5
0.5 M LiCl
1% NP-40
1% Sodium Deoxycholate

Additional Comments:

1. Sonication of chromatin after MNase treatment may not be required.
2. Several other steps may not be necessary but I haven't tried without them because it works as is.
3. The protocol can easily be scaled up or down. However, researcher must adjust the amount and/or time of MNase treatment.
4. More MNase seems to increase the size the DNA is digested to, i.e. if you have large fragments increase the amount of MNase. Increasing the time and CaCl2 concentration seems have less of an effect on overall size and more of an effect on blurring the definition of the nucleosomes, i.e. when you increase the time/CaCl2 too much you don't get a nice nucleosomal ladder but more of a smear.
5. Good chromatin fragmentation is critical. For ChIP-seq I digest so that at least 90% of the chromatin is mono-nucleosomal.
6. The amount of X-linking can be modified by changing the formaldehyde concentration or incubation time.