18. Spin down to pellet cellular debris (30 sec at 13,000 rpm).
20. Remove 50 μl of each sonicated sample and add to 400 μl lysis solution. This sample is the input. This is used for obtaining DNA concentration for subsequent IP’s (see below) and as control in final PCRs.
22. Store remainder of chromatin on ice at 4°C (stable for a week).

3. Determination of input DNA concentration.
1. Add 5 μl Proteinase K (20 mg/ml) to each sample in lysis solution.
2. Heat with shaking at 65°C for 4-5 hours (or overnight) to reverse crosslinking (can freeze samples here if need to).
3. Phenol:chloroform extract with equal volume, EtOH (2 vols)/NaAcetate (1/10th vol) precipitate with 10 μl glycogen (5 mg/ml) on dry ice.
4. Spin 10 min at 13,000 rpm, wash pellet with 1 ml 70% EtOH.
5. Redissolve in 50 μl TE.
6. Read the OD$_{260}$ (5 μl sample plus 995 μl TE, to give a 200-fold dilution). The concentration of DNA in μg/ml is OD$_{260}$ x 10,000.

4. Chromatin IP
1. For each ChIP experiment, use 25 μg of DNA per IP (this is variable, and you may need to optimize a good amount for your own experiment here).
2. You will need one sample for the specific antibody, one sample for an unrelated antibody, and one sample for the beads-only control (no antibody).
3. Adjust samples to the same volume with ChIP lysis buffer.
4. Dilute each sample 1:10 with dilution buffer (some people use RIPA to dilute to reduce background).
5. IP overnight with rotation at 4°C. Amount of antibody is variable (e.g. 4 μl of ab7312, Rabbit polyclonal to Histone H3 (methylated K9)).
6. Add 20 μl of protein A/G beads (pre-absorbed with sonicated s/s salmon sperm DNA at 1.5 mg/20 μl beads). Incubate for 2 hours with rotation at 4°C.
7. Spin down for 1 min at 8,000 rpm, remove supernatant.
8. Wash beads 3X with 1 ml wash buffer (spin as above).
9. Wash beads 1X with 1 ml final wash buffer (for some IP’s a 1:1 mix of wash buffer and final wash buffer is best e.g. MeCP2/HP1) (spin as above).
10. Elute DNA by adding 450 μl of elution buffer (make fresh each time) and rotate for 15 mins at RT.
11. Spin down and remove supernatant into fresh tube and add 5 μl Proteinase K (20 mg/ml).
12. Heat with shaking at 65°C for 4-5 hours (or overnight) to reverse crosslinking (can freeze samples here if need to).
13. Phenol:chloroform extract with equal volume, EtOH (2 vols)/NaAcetate (1/10th vol) precipitate with 10 μl