18. Spin down to pellet cellular debris (30sec at 13,000 rpm).
19. Transfer S/N to new tube.
20. Remove 50µl of each sonicated sample and add to 400ml 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 5µl Proteinase K (20mg/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 (2vols)/NaAcetate (1/10th vol) precipitate with 10µl glycogen (5mg/ml) on dry ice.
4. Spin 10min at 13,000 rpm, wash pellet with 1ml 70% EtOH.
5. Redisolve in 50µl TE.
6. Read the OD_{260} (5µl sample plus 995µl 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 25µg 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. 4µl of ab7312, Rabbit polyclonal to Histone H3 (methylated K9)).
6. Add 20µl of protein A/G beads (pre-absorbed with sonicated s/s salmon sperm DNA at 1.5mg/20µl beads). Incubate for 2 hours with rotation at 4°C.
7. Spin down for 1min at 8,000 rpm, remove supernatant.
8. Wash beads 3X with 1ml *wash buffer* (spin as above).
9. Wash beads 1X with 1ml *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 450µl of *elution buffer* (make fresh each time) and rotate for 15mins at RT.
11. Spin down and remove supernatant into fresh tube and add 5µl Proteinase K (20mg/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 (2vols)/NaAcetate (1/10th vol) precipitate with 10µl