- Wash cells twice with PBS at room temperature, resuspending to approximately 5 x 10^5 cells/ml (approximately 2 x 10^7 cells total). Add formaldehyde to a final concentration of 1% and incubate at room temperature for 10 minutes.
- Terminate cross-linking reactions by adding glycine to a final concentration of 0.125 M.
- Pellet cells (2,000 RPM, 5 minutes) and wash once with ice cold PBS.
- Resuspend cells in 6 ml **Lysis Buffer** (sc-45000) by mixing gently.
- Collect crude nuclear extract by microcentrifugation at 2,000 RPM, 5 minutes.
- Wash again with PBS. Pellet may be frozen or processing may be continued as follows:
    - Resuspend pellet in ~1.9 ml **Lysis Buffer High Salt** (sc-45001) and transfer to 2 ml microcentrifuge tube for the sonication step.
    - Sonication conditions should be optimized since results may vary using different sonifiers. The following conditions were established by using a Sonics VC130 with a 3 mm tip probe.
    - Sonicate on ice at power output setting = 5–6, continuous mode, 4 times at 30 second intervals.
    - Centrifuge extract for 15 minutes, 10,000 rpm at 4° C and save supernatant (chromatin).
    - Determine protein concentration of supernatant.
    - For the IP step we recommend using 100–500 µg supernatant and 0.1–1 µl TransCruz reagent (0.2–2 µg).

NOTE: Investigators may wish to consider using the primary antibody conjugated to sepharose or magnetic beads as an alternative to using secondary immunoprecipitation reagents (e.g., Protein A-Agarose) as described here. Combining primary antibodies directed to different epitopes of the same protein may be advantageous in some cases.

- Preclear the chromatin solution by adding 50 µl **Protein A/G PLUS-Agarose** (sc-2003) and incubate for 30 minutes at 4° C. Centrifuge at full speed for 5 minutes at 4° C.
- Add primary antibody to the supernatant and incubate overnight at 4° C.
- Add 50 µl **Protein A/G PLUS-Agarose** (sc-2003) and incubate for 2 hrs at 4° C.
- Harvest beads by centrifugations at 12,000 rpm for 20 seconds and place tube in ice.
- Wash beads twice with 1 ml **Lysis Buffer High Salt** (sc-45001).
- Wash pellet four times with **Wash Buffer** (sc-45002). Resuspend beads in 400 µl **Elution Buffer** (sc-45003).
- Reverse cross-links by incubating tube in a 67° C water bath, mixing occasionally over two hours. Remove beads by centrifugation and continue incubating supernatant at 67° C overnight.
- Centrifuge for 3 minutes at 10,000 rpm to remove any residual beads and save supernatant.
- To isolate DNA, extract supernatant once with 500 µl phenol/chloroform/isoamyl alcohol (25:24:1), vortex thoroughly and separate phases by centrifuging tube for 3 minutes at 14,000 rpm.
- Save the aqueous phase, back extract the organic phase once with 100 µl 10 mM Tris, 1 mM EDTA, pH 8.1 (TE) and pool aqueous phases.
- Extract pooled aqueous phase with 600 µl chloroform/isoamyl alcohol.
- DNA may be concentrated by using commercially available kits.