# Co-Immunoprecipitation

1. Wash cells 24-48 hrs post-transfection in cold PBS
2. Collect cells by scraping in cold PBS
3. Spin cells 5 min at 5000 rpm at 4ºC
4. Resuspend cells in NP-40 lysis buffer (~1.0 mL for 60 $mm^2$ dish)
5. Incubate on ice for 15 min
6. Sonicate ~20 times on ice to shear DNA
7. Spin 16,000 xg 15 min at 4ºC to pellet insoluble material
8. Take sample of supernate as WCL
9. Add antibody-beads to supernate (~10 uL FLAG-agarose)
10. Incubate 2 hrs at 4ºC rotating
11. Pellet beads
12. Wash beads 5 times in NP-40 lysis buffer
13. Elute in 30-40 uL 2X sample buffer (or 0.1 mg/mL FLAG peptide for 15 min on ice)

NP-40 Lysis Buffer: 50 mM Tris-HCl, pH 7.7, 150 mM NaCl, 0.5% NP-40, 1 mM dithiothreitol, and 1X protease inhibitor cocktail