ChIP (Whetstine Lab)

Chromatin

- Crosslink cells by adding 1% formaldehyde to the media, 13 min 37 degrees. Stop by adding Glycine to 0.125M.
- Wash cold PBS. Add PBS on dish (2.5 ml for 15 cm dish). Scrape, collect in 15 ml tube, spin 2 min 800 rpm 4 degrees. Resuspend the pellet in cellular lysis buffer-proteases inhibitors (typically 2.5 ml for 1 15 cm dish 293T, 1 ml for 1 10 cm dishes 293T-2 10 cm RPE-1 15 cm RPE), incubate 5 min on ice and spin 2 min 800 rpm 4 degrees. Discard the supernatant and resuspend the pellet in nuclear lysis buffer-proteases inhibitors (typically 300 µl for 1 10 cm dishes 293T-2 10 cm RPE- 1 15 cm RPE). It corresponds to 1-1.2x107 cells per 300 µl.
- **Sonication:** Q800R. 300 µl of chromatin in 0.5 ml thin wall PCR tubes (Brandtech #781312). To get the chromatin <300bp: sonicator at 70% amplitude 15 sec on 45 sec off (RPE in 0.2% SDS 40min. time on; in 1% SDS, 30min. time on). Sonication test. Spin 10 min 14000 rpm 4 degrees for clear the chromatin.

IPs

- Prebind the beads with antibody. In a 1.5 ml tube: 900 µl dilution IP buffer-proteases inhibitors + 25 µl protein A or G magnetic beads (A for rabbit polyclonal Ab, G for mouse monoclonal) + 2 µg Ab. Incubate at least 6h rotator 4 degrees.
- Add 100 µl of wanted amount of chromatin (in nuclear lysis buffer) in the tube, usually 10-30
- µg. Incubate o/n rotator 4 degrees. A preclear step can be added before the IP.
- Washes: two times 1 ml dilution IP buffer, one time TSE buffer, one time LiCl buffer, two times TE. Vortex + incubate couple min each time.
- Elution: add 150 µl elution buffer + 1 µl RNase A 200 mg/ml, incubate 30 min 37 degrees 1000 rpm. Add 1 µl proteinase K 10 mg/ml, 1h 55 degrees 1000 rpm (or vortex sometimes). Treat inputs the same way from this step. The RNase treatment is optional.
- Decrosslinking: remove the samples from the beads and incubate 4h 65 degrees.
- Purification DNA with a PCR clean up system kit.

Buffers

- Cellular lysis: 5 mM PIPES pH8, 85 mM KCl, 0.5% NP40.
- Nuclear lysis: 50 mM Tris pH8, 10 mM EDTA pH8, 0.2% or 1% SDS.
- Dilution IP: 16.7 mM Tris pH 8, 1.2 mM EDTA pH8, 167 mM NaCl, 0.01% SDS, 1.1% Triton X100.
- TSE: 20 mM Tris pH8, 2 mM EDTA pH8, 500 mM NaCl, 1% Triton X100, 0.1% SDS.
- LiCl: 100 mM Tris pH8, 500 mM LiCl, 1% deoxycholic acid, 1% NP40.
- TE: 10 mM Tris pH8, 1 mM EDTA pH8.
- Elution: 50 mM NaHCO3, 140 mM NaCl, 1% SDS.