Jenny DuRose -- Updated 10-26-06

Chromatin IP

Use 3-10cm plates per IP.

Crosslinking:
Crosslink by adding 270μL 37% formaldehyde to media in 10cm plate to achieve 1% FMA. Incubate cells at room temp, 10min while gently shaking.

Wash cells 2X w/ 5mL ice-cold PBS.

Add 0.5mL rinse buffer to each plate and scrape cells off plate and place in eppy. Incubate cells 15min @ 30ºC, and spin down @ 2000*g*, 5min, RT.

- Wash and combine pellets w/ 1mL ice cold PBS, and spin down @ 2000*g*, 5min, 4ºC.
- Lyse cells w/ 1mL buffer I. Incubate on ice 5min, and spin down @ 2000*g*, 5min, 4ºC to collect nuclei.
- Wash nuclei w/ 1mL buffer II, and spin down @ 2000*g*, 5min, 4ºC.

Lyse nuclei w/ 2mL SDS lysis buffer. Can store nuclear extract @ -80ºC or proceed to sonication.

Sonication:
Sonicate using T. Johnson sonicator @ 30% power w/ 10s pulses in a FACS tube. Allow nuclear extract to rest 1min on ice between pulses. Sonicate a total of 3min.

After sonication, spin nuclear extract 10min @ 14,000*g*, 4ºC.

Dilute samples 1:10 in dilution buffer, aliquot 100μL for input. Can store diluted nuclear extracts @ -80ºC or proceed to IP.

Immunoprecipitation:
Use 5mL extract for each IP. Preclear extract 2X 1hr w/ 100μL blocked CL-4B beads while rotating. Remove supernatant from beads and incubate overnight w/ antibody (usually 1-2μg antibody).

Blocking Beads:
Wash slurry at least 3X w/ 1mL dilution buffer to remove EtOH. Add 20μg sonicated salmon sperm DNA per mL of slurry. Rotate at 4ºC for 1h. Store beads at 4ºC.

The following morning add 40μL of block protein G slurry and rotate 2h @ 4ºC.

Washes and Elution:
Spin down IP and remove all supernatant. Use first TSE I wash to transfer beads into eppy.

- Wash 2X 15min w/ 1mL TSE I while rotating at RT.
- Wash 1X 15min w/ 1mL TSE II while rotating at RT.
- Wash 1X 15min w/ 1mL buffer III while rotating at RT.

Remove all traces of wash buffer by rinsing beads 3X w/ 1mL TE.

Elute 3X w/ 100µL of freshly prepared elution buffer. Rotate in elution buffer 5min @ RT, then vortex 30s, and spin down to collect eluate. Combine eluate (300µL total) and then spin down again to remove all beads.

Reverse Crosslinks:
Add 200µL elution buffer to 100µL input. Incubate input and eluate @ 65°C for 4-6h to reverse crosslinks.

After crosslinks are reversed, add 30µL 3M NaOAc pH 5 and 660µL 100% EtOH, and precipitate o/n @ -20°C.

Next day, spin down DNA 30min @ 14,000*g*, 4°C. Resuspend in 38µL TE, add 10µL PK buffer and 2µL (100µg) proteinase K. Digest @ 42°C, 1-2h. Bring up volume to 300µL w/ TE, and add equal volume of phenol choloform. Vortex 15s and spin @ 14,000*g* for 5min @ RT.

Collect 280µL of aqueous layer and precipitate overnight w/ 28µL 3M NaOAc pH 5.0, 626µL 100% EtOH, and 10µg glycogen.

Next day, spin down DNA. Resuspend DNA in 10mM Tris pH 8.5. Use 200µL for input and 20µL for ChIP samples.

Perform PCR w/ 1 and 5µL of DNA.

Rinse Buffer:
100mM Tris pH 8.8
10mM DTT

Buffer I:
0.25% triton
10mM EDTA
0.5mM EGTA
10mM Hepes pH 6.5

TE:
10mM Tris pH 7.4
1mM EDTA

Buffer II:
200mM NaCl
10mM EDTA
10mM Hepes pH 6.5

SDS lysis buffer
1% SDS
10mM EDTA
50mM Tris pH 7.4
protease inhibitors

Elution buffer:
1% SDS
0.1M $NaHCO_3$

Dilution Buffer
1% triton
2mM EDTA
150mM NaCl
20mM Tris pH 7.4
1mM PMSF

TSE I:
0.1% SDS
1% triton
2mM EDTA
20mM Tris pH 7.4
150mM NaCl

TSE II:
0.1% SDS
1% triton
2mM EDTA
20mM Tris pH 7.4
500mM NaCl

Buffer III:
0.25M LiCl
1% NP40
1% Deoxycholate
1mM EDTA
10mM Tris pH 7.4