# Ren Lab ChIP Protocol

Day 1 **DATE:**

**1** ChIP: Preparation of beads

**PURPOSE:**
Isolate modified histones from embryonic stem cells

**MATERIALS & METHODS:**
5mg/mL BSA (0.250g BSA in 50mL 1X PBS) make immediately before use, put on ice

Select beads based on antibody: sheep-anti-rabbit beads (Dynal/Invitrogen), sheep-anti-mouse beads (Dynal/Invitrogen)

Use 50ul of beads per IP

| # | Epitope | Cat# | Lot# | Species | [ab] | Ab amt. | Vol/IP |
|---|---|---|---|---|---|---|---|
| | H3K4me3 | ab8580 | 500368 | rabbit | 1mg/ml | 5ug | 5ul |

1. Place 50ul of resuspended beads in microfuge tube. Use magnetic strip. Remove supernatant with a pipette/vacuum and resuspend in 1mL PBS containing 5mg/ml BSA to each tube (make immediately before use from Sigma BSA powder, cat. A-3350).

2.Wash 3 times. Resuspend beads in 100uL PBS+BSA per tube.

3.Combine antibody and PBS + BSA for a total volume of 900ul then add to 100uL magnetic beads. Incubate overnight with antibody on a rotating platform at 4oC.

Day 2
**2** ChIP: Bead prep and Immunoprecipitation

Make 10% DOC (0.1g DOC-sodium deoxycholate- in 1mL dH2O) immediately before use
Make 5mg/mL BSA (0.125g BSA in 25mL PBS) immediately before use
Make 50X Complete (1 tablet in 1.0mL dH20
Use 500ug of chromatin per IP

1.Remove supernatant with a pipette and resuspend in 1mL PBS+BSA (on ice)

2.Wash 3 times. (let sit on magnet)

3. Resuspend antibody beads in 100uL 1X PBS+BSA

4.Set up IP reactions with crude extract. Adjust cell extract to 1mL (500ug of chromatin) with TE.

5.Add following additional reagents to bring the volume to 1.3 mL with following concentration:

| STOCK SOLUTIONS | Final Concentration | Volume Per Tube | 7X |
|---|---|---|---|
| 10% Triton X-100*** | 1% | 130uL | 910uL |
| 10% DOC | 0.1% | 13uL | 91uL |
| 50X Complete | 1X | 26uL | 182uL |
| 1X TE | 1X | 131uL | 917uL |
| | | 300ul per tube | |

***If chromatin was sonicated in 1%TritonX, then substitute volume with TE.

6.Add 100uL of magnetic beads to each tube and incubate at 4oC overnight in a rotating platform.

# Ren Lab ChIP Protocol

Day 3
**3** ChIP: Washing beads, Elution, and reversal of crosslink

Make 10% DOC (1g DOC in 10mL dH2O) immediately before use.
Make RIPA buffer immediately before use. Add the stock solutions in the order listed.

RIPA buffer:

| Components | STOCK | 50mL |
|---|---|---|
| 50mM Hepes, pH 8.0 | 1M | 2.5mL |
| 1mM EDTA | 0.5M | 100uL |
| 1% NP-40 | 10% | 5mL |
| 0.7% DOC | 10% | 3.5mL |
| dH2O | | 34.8mL |
| 0.5M LiCl | 8M | 3.125mL |
| Complete | 50X | 1mL |

Washing of Beads
1. Use a magnet MPC-E to precipitate the beads (optional: save the 1st supernatant). Keep tubes on ice. Wash 8 times with 1mL RIPA buffer. Remove buffer by aspiration.

2. Wash once with 1mL TE.

3. After removing the TE by aspiration, spin the tubes for 1 minutes at 4000rpm and remove remaining liquid with a pipet.

Elution from beads and reversal of cross links
Elution buffer: (doesn't need to be fresh)

| Components | STOCK | 50mL |
|---|---|---|
| 10mM Tris, pH 8.0 | 1M | 500uL |
| 1mM EDTA | 0.5M | 100uL |
| 1% SDS | 10% | 5mL |
| dH2O | | 44.35mL |

1. Add 170uL of elution buffer, vortex briefly to resuspend the beads and incubate at 65oC for 10 minutes. Vortex briefly every 2 minutes during incubation, or put on thermomixer for 20min (TURN ON AHEAD OF TIME).

2. Spin for 30 seconds at maximum speed and transfer liquid to a new tube.

3. Reverse crosslink at 65oC O/N in incubator.

4. Also reverse crosslink 50uL input chromatin (in 120uL elution buffer).

# Ren Lab ChIP Protocol

Day 4
**4** ChIP: DNA processing/precipitation

Make Proteinase K mix:

| 1X Sample | 7X |
|---|---|
| 140uL TE | 980uL |
| 3uL Glycogen 10mg/mL | 21uL |
| 7uL Proteinase K 20mg/mL | 49uL |

1. Add 150uL Proteinase K Mix to each tube.

2. Incubate for 2 hours at 37oC.

3. Extract 2X with 300uL phenol. Vortex 20s, spin 5min, remove top layer and place in tube. Repeat.

4. Extract once with 300uL chloroform/isoamyl alcohol.
**All extraction can be carried out in one phase-lock tube
5. To aqueous layer add NaCl to 200mM final (13uL 5M).

6. Add 700uL EtOH, vortex briefly.

5. Incubate at -80oC for 15-30min.

6. Spin at 14K rpm for 15 minutes at 4oC.

7. Wash pellet with 1mL cold 70% EtOH, vortex, spin 5min. at 4oC at 14K rpm.

8. Air dry pellet and resuspend in 30uL TE containing 10ug RNase A (33uL of 10mg/mL, RNaseA in 1mL TE or 16.5uL in 500uL) by gentle vortexing.

9. Incubate 2 hour at 37oC.

10. Qiagen PCR purify DNA (elute in 50uL), and store at -80oC. OD input DNA.