Day 1 **DATE:** 01-20-09

**1** ChIP of *** cells: Preparation of beads, binding primary Ab, followed by chromatin

**PURPOSE:** Isolation of modified histones from chromatin

**MATERIALS & METHODS:**
Antibodies (5ug/IP):
Chromatin (use 0.5-0.35mg of chromatin in 1mL):
10% DOC (sodium deoxycholate –Sigma-Aldrich )

5mg/mL BSA (0.125g BSA in 25mL 1X PBS) make immediately before use, put on ice

Sheep-anti-rabbit beads (antibody coupled magnetic beads - Dynal/Invitrogen)/50ul per IP
NOTE: larger volume of beads can be washed at one time for convenience

| # | Epitope | Cat# | Lot# | Species | [Ab] | Ab amt. | Vol/IP | Vol PBS |
|---|---|---|---|---|---|---|---|---|
| **T01** | H3K4me3 | ab8580 | 500368 | rabbit | 1mg/ml | 5ug | 5ul | 995 |
| **T02** | H3K4me3 | ab8580 | 500368 | rabbit | 1mg/ml | 5ug | 5ul | 995 |
| **T03** | H3K4me1 | ab8895 | 133163 | rabbit | .5mg/ml | 5ug | 10ul | 990 |
| **T04** | H3K27me3 | 07-449 | Dam1514011 | Rabbit | 1 mg/ml | 5ug | 5ul | 995 |
| **T05** | H3K36me3 | 9050 | Dam475787 | Rabbit | 1mg/ml | 5ug | 5ul | 995 |
| **T06** | CTCF | 9Mix | 1/23/09 | mouse | | | 25ul | 975 |

1. 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 9XXuL PBS+BSA per tube (see chart).

3.With tube against the magnet, add antibody for a total volume of 1000ul. Incubate for **6-8hrs** on a rotating platform at 4ºC. **NOTE**: if antibody is being replicated, 100ul of beads and 10ug of antibody can be combined in 1000ul for binding, split ab/beads in half before adding chromatin below.

Make 10% DOC (0.1g DOC 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

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

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

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

7.Set up IP reactions with chromatin extract.

| # | Chromatin | Description |
|---|---|---|
| **T01** | XX (0.4mg/ml) | H3K4me3 |
| **T02** | XX (0.4mg/ml) | H3K4me3 |
| **T03** | XX(0.4mg/ml) | H3K4me1 |
| **T04** | XX (0.4mg/ml) | H3K27me3 |
| **T05** | XX(0.4mg/ml) | H3K36me3 |
| **T06** | XX(0.4mg/ml) | CTCF |

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

| Stock Solution | Final Concentration | Volume Per Tube(ul) | 7 X (ul) |
|---|---|---|---|
| 10% Triton-X | 1% | 130 | 910 |
| 10% DOC | 0.10% | 13 | 91 |
| 50X Complete | 1X | 26 | 182 |
| 1X TE | 1X | 131 | 917 |
| | | 300ul per tube | |

8.Add 1.3mL chromatin mix to magnetic beads and incubate at 4ºC overnight in a rotating platform.

Day2 **DATE:** 01-22-09

**2** ChIP of *** cells: Washing beads, Elution, and reversal of crosslink

Make 10% DOC (1g DOC in 10mL dH2O/.35g in 3.5ml/.7g in 7.0ml) immediately before use.
Make RIPA buffer immediately before use. Add the stock solutions in the order listed.

RIPA buffer:

| Components | STOCK | 50ml | 100ml |
|---|---|---|---|
| $dH_20$ | | 34.8ml | 69.6ml |
| 50mM Hepes, pH 8.0 | 1M | 2.5ml | 5ml |
| 1% NP-40 | 10% | 5ml | 10ml |
| 0.7% DOC | 10% | 3.5ml | 7ml |
| 0.5M LiCl | 8M | 3.125ml | 6.25ml |
| Complete | 50X | 1ml | 2ml |

Washing of Beads

1. Use a magnet MPC-E to precipitate the beads (optional: save the 1st supernatant). Keep tubes on ice. Wash **5 times** with 1mL RIPA buffer. Remove buffer by aspiration.

2. Wash once with 1mL TE.

3. After removing the TE by aspiration, quickly spin the tubes or place on magnet and remove remaining liquid with a pipet.

Elution from beads and reversal of cross-link

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 150uL 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 or place on magnet and transfer liquid to a new tube.

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

4. Also reverse crosslink 30uL input chromatin in 120uL elution buffer.
   INPUT: XX

Day 3 DATE: 01-23-09

**3** ChIP: Isolate - DNA precipitation

1. Add 250ul TE to each sample

2. Add 8ul of 10mg/ml RNAse A (final conc. = 0.2mg/ml)

3. Incubate at 37ºC for 1hr.

4. Add 8ul of 20mg/ml Proteinase K (final conc. = 0.4mg/ml)

5. Incubate for 1hr at 55ºC.

6. Prepare Phase Lock tube per IP, spin at 14K rpm for 1min

7. Add 400ul Phenol:Chloroform: Isoamyl alcohol to each tube.

8. Add sample to Phase Lock tube and shake vigorously, do not vortex

9. Spin 4 min at 14K rpm. **Note**: if aqueous phase is cloudy, extract again.

10. Transfer aqueous layer to new tube

11. Add 16ul of 5M NaCl (final conc. = 200mM), plus 30ug of glycogen (1.5ul of 20mg/ml)

12. Add 920uL cold 100% EtOH, vortex briefly.

13. Incubate at -80ºC for 20-30min. Spin at 14K rpm for 15 minutes at 4ºC.

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

15. Resuspend thoroughly in 34ul* of 10mM Tris (EB from qiagen kits). OD input DNA.
    A) Proceed to Library Prep immediately for ChIP-Seq
    B) Store at -80oC.