

Day One

Prior to starting your ChIPs and Shearing:

1. Completely thaw frozen cross linked pellets on ice.
2. Turn on sonifiers and cooling system allow system to reach -1 °C before shearing
3. Cool bench top centrifuge to 4°C
4. Prepare all of your buffers with protease inhibitors in advance
5. **Keep all buffers on ice.**
6. Use DNA low bind tubes throughout protocol (eppendorf cat# 022431021)

Cell and Nuclear Lysis:

Note: For frozen cells, completely thaw on ice. For low cell no.samples use "Alon's SCN v2.0.docx" protocol.

1. Resuspend cross linked cell pellet (1e7) in 1mL of Cell Lysis Buffer (CLB)+ protease inhibitors (pi) (20mM Tris pH 8.0, 85mM KCl, 0.5% NP40).
2. Make sure that sample is well resuspended by pipetting.
3. Incubate 10' on ice, spin for 3.5', 5000xg, 4°C.
4. Remove supt., resuspend in additional 1mL CLB + pi for 5' on ice.
5. Spin 5000xg, 3.5' @ 4°C and remove supt.*
6. Add 1mL Nuclear Lysis Buffer (NLB) + pi. (10mM Tris-HCl pH7.5, 1% NP40, 0.5% Na Deoxycholate, 0.1%SDS).
 - a. **There should be no stickers on your 1.5 ml tube in order to fit in our Branson tube holder**
7. Incubate on ice 10' and proceed to shearing.

*At this point you can freeze material at -80°C; this is called a **nuclear pellet**; Nuclear pellets can be a little more difficult going into solution after they have been frozen and stored.

Day One

Setting Up Your ChIPs:

1. Add the appropriate amount of sheared chromatin to each tube. For instance, If you want 1e6 cells for a ChIP and there are 1e7 cells/mL, you would add 100 λ of sheared chromatin. When diluting your chromatin with CHIP Dilution Buffer, keep in mind that you want your final concentration of SDS to be 0.1%. **Note:** If you shear in the Covaris LE, your sheared material will have a higher concentration of both cells and SDS.
2. Adjust your ChIP vol. with CHIP Dilution Buffer (CDB) plus protease inhibitors then add 1 μ g of your antibody of interest to each tube. (conc. Ab may vary)
3. Rotate your samples end over end O/N at 4°C.

Day Two

In Advance: Prepare thermal cycler for 65°C and have buffers ready so the beads won't dry out during washes. Prepare Blocking Buffer (PBS, 0.1%Tween 20, 0.05% BSA+Protease Inhibitors (pi).

****Note: Dynabeads should constitute \leq 10% of total volume (make vol. adjustments as necessary).**

Dynabeads Preparation and Incubation with ChIP:

1. For each ChIP, aliquot 50 λ of Protein A/G (50/50) magnetic beads into 2ml low bind tube.
2. Place tubes on magnet and remove supernatant.
3. Add 1mL of blocking buffer with protease inhibitors to each 2mL tube containing 50 λ beads.
4. Mix by placing tubes on magnet then turning tubes 360°.
5. Repeat steps 2 and 3.
6. Aspirate blocking buffer and add 100 λ of fresh blocking solution to pre washed beads.
7. Remove ChIPs from rotation, pulse spin and transfer material to 2ml tube with prewashed beads.
8. Incubate for 1hr at 4°C, rotating end over end.

Epigenomics Alternative Mag Bead CHIP Protocol v1.1 exp

Washes:

RIPA Buffer washes x6 (0.1% DOC, 0.1%SDS,1% Triton X-100,140mM NaCl,1mM EDTA, 20mM Tris-HCl pH8.1)

1. Remove ChIPs from rotation and briefly spin tubes to remove any sample from caps., place on magnet and remove supt.
2. Remove tubes from magnet, add 200 λ of RIPA buffer to rinse beads to bottom of tube, transfer beads to 96 well plate on magnetic station. **(Save pipet tips from this step in respective tubes in order to transfer remaining beads to 96 well plate)**
3. Remove supt in wells and add another 200 λ of RIPA buffer to tubes with a clean pipet tip but continue to use "saved" tip to collect remaining beads and add them to their respective wells in the 96 well plate.
4. Move plate on magnet advancing column of wells either left or right. This will help to mix your sample.
5. Repeat steps 3 and 4. If you are satisfied with the bead transfer, you may continue with three more RIPA washes in 96 well plate or continue to wash beads from O/N tubes transferring supt to wells.
6. Wash x2 with 200 λ **RIPA/500mM NaCl Buffer** (0.1% DOC, 0.1%SDS ,1% Triton X-100, 500mM NaCl,1mM EDTA, 20mM Tris-HCl pH8.1)
7. Wash x2 with 200 λ **LiCl Buffer** (0.25M LiCl,1% NP40,1% Na Deoxycholate, 1mM EDTA,10mM Tris-HCl pH 8.1)
8. Wash x2 with **TE**
9. Add 50 λ **ChIP Elution Buffer** and 8 λ reverse x linking mix to each well. (ChIP Elution Buffer: 10mM Tris-Cl pH 8.0, 5mM EDTA, 300mM NaCl, 0.1% SDS), (Reverse Cross Linking Buffer: 250mM Tris-HClpH 6.5, 1.25M NaCl, 62.5mM EDTA, 5mg/ml Proteinase K, 62.5ug/ml RNAse A)
10. Cover plate with film, pulse vortex, spin briefly and reverse x link entire sample including beads at 65°C for 5 hours.
11. Include 10 λ input, 40 λ ddH₂O and 8 λ reverse cross linking mix to additional well on plate (if you don't already have it).

Day Three

SPRI clean up after reverse cross linking -DNA purification:

1. Place overnight reverse cross linking plate on magnet. Transfer supernatants to new well.
2. To each reversed cross linked sample, add ~ 1.8x to 2.2x SPRI beads equilibrated to room temperature (RT)
3. Mix x15, incubate 2' RT followed by 4' on magnet (or until supt is clear). Remove supt.
4. Wash x2 with 200 λ of freshly made 70% EtOH for 30s on magnet
5. Move plate containing samples from left to right on the magnet so beads move through ethanol.
6. After 2nd wash, completely removed ethanol. Spin briefly, return to magnet and remove all traces EtOH .
7. Allow beads to dry, RT ~2'. The pellets should appear shiny and not cracked (over dried).
8. Elute material in 22 λ 10mM Tris-HCl pH 8.0, mix with pipet, incubate RT, 3' and return to magnet.
9. Move cleared material to clean wells. This is your ChIP material that is ready for quantification.

Qubit measurement:

1. Use Qubit dsDNA HS setup
2. Make Qubit master mix
 - a. $(n+2) \times 200 \lambda - (n+2)$ of Qubit dsDNA High Sensitivity Buffer.
 - b. $(n+2) \lambda$ Qubit "Reagent" (pinkish red, in drawer b/c sensitive to light).
3. Aliquot 190λ of master mix to two Qubit Assay tubes and then add 10λ of standard 1 and 2 respectively.
4. Aliquot 198λ of master mix to the remaining tubes.
5. Add 2λ of ChIP material per tube.
6. Mix well via quick vortex, quick spin, let stand 1 – 5 minutes in dark drawer.
7. Measure using Qubit Spectrometer and report values in "ng/ λ "

Epigenomics Alternative Mag Bead ChIP Protocol v1.1 exp

Buffers for Mag Bead ChIP:

Blocking Buffer (4°C): PBS, 0.5% TWEEN 20, 0.5% BSA

Cell Lysis Buffer: 20mM Tris pH 8.0, 85mM KCl, 0.5% NP40

Nuclear Lysis Buffer: 10mM Tris-HCl pH7.5, 1% NP40, 0.5% Na Deoxycholate, 0.1%SDS

Chip Dilution Buffer: 16.7mM Tris-HCl pH 8.1, 167mM NaCl, 0.01% SDS, 1.1% Triton X-100, 1.2mM EDTA

LiCl Wash Buffer: 250mM LiCl, 0.5% NP40, 0.5% Na Deoxycholate, 1mM EDTA, 10mM Tris-HCl pH 8.1

TE Buffer pH 8.0: 10mM Tris-HCl pH8.0, 1mM EDTA pH 8.0

ChIP Elution Buffer : 10mM Tris-Cl pH 8.0, 5mM EDTA, 300mM NaCl, 0.1% SDS

RIPA/140mM NaCl Buffer : 20mM Tris-HCl pH 8.1, 140mM NaCl , 0.1%SDS, 1%Triton X-100, 0.1% DOC, 1mM EDTA

RIPA/500mM NaCl Buffer: 20mM Tris-HCl pH 8.1, 500mM NaCl , 0.1%SDS, 1%Triton X-100, 0.1% DOC, 2mM EDTA

Reverse Cross Linking Buffer: 250mM Tris-HClpH 6.5, 1.25M NaCl, 62.5mM EDTA, 5mg/ml Proteinase K, 62.5ug/ml RNAse A (store in small aliquots @-20 °C)

The dynabeads bind to the Fc region of the antibody and will concentrate your sample on the beads