Day One

Setting Up ChIPs:

From previous optimization, you should know the cell number in your samples.

1. Add the appropriate amount of sheared chromatin, starting at column 1 row 1, to a 96 well 1mL deepwell plate. For instance, If you want 1e6 cells for a ChIP and there are 1e7 cells/mL, you would add 100λ 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%.
2. Adjust your ChIP vol. with ChIP Dilution Buffer (CDB) plus protease inhibitors to desired volume. (16.7mM Tris-HCl pH 8.1, 167mM NaCl, 0.01% SDS, 1.1% Triton X-100, 1.2mM EDTA).
3. Add appropriate amount of your antibody of interest to each well.
4. Seal plate and incubate on plate rotator overnight in the cold room at 4°C
5. Ensure low salt and high salt buffers being used for washes are @ 4°C.

Day Two

Protein A/G Bead Wash:

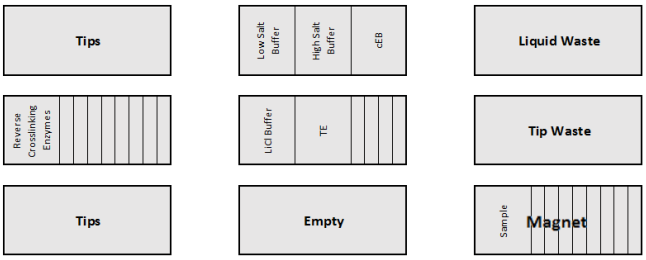
1. Add 55uL of protein A/G beads for every ChIP being run into a microfuge tube (i.e. if you are performing 8 ChIPs, add 440uL of protein A/G beads to the tube).
2. Place beads on magnet and remove supernatant.
3. Add double the original volume of blocking buffer (PBS+0.5% TWEEN, 0.5% BSA + protease inhibitors)
4. Repeat steps 2. and 3. twice.
5. When primary incubation is complete, add 100uL of washed beads to each sample.
6. Cover the plate and place on plate rotator in cold room for at least 1 hour.

ChIP Wash

1. While samples are incubating, stamp volumes of the following reagents onto appropriate plates and keep at 4°C until the protocol is about to be run.

Reagent	For 8 Samples (100uL)
Reverse Crosslinking Buffer	62.5 uL
Proteinase K	25 uL
RNase A	8.33 uL

Reagent	Volume Aspirated (uL)	Volume to Stamp (uL)
LS, HS	170	200
LiCl, TE	170 (x2)	400
cEB	62.5	75
Reverse Crosslinking Enzymes	-	10



2. Cover plate with film and reverse cross link entire sample including beads at 65°C for at least 3 hours.
3. Include 10uL input, 50uL EB and 10uL reverse cross linking mix to additional well on plate (if you don't already have it).

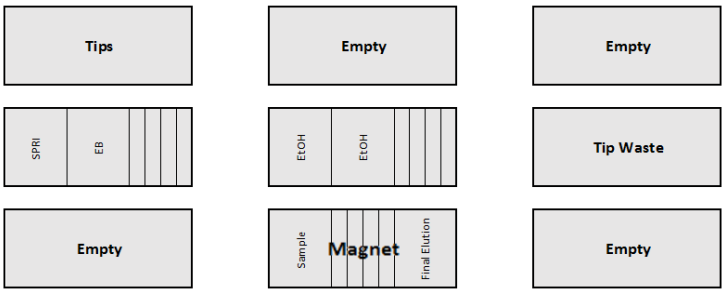
Day Two/Three

SPRI Clean Up After Reverse Cross Linking:

1. After reverse cross-linking, Stamp volumes of the following reagents onto plates according to the table and place them on the Bravo deck according to the diagram below.

   Be sure to move the magnet from position 9 to position 8 before starting and switch profiles

Reagent	Volume Aspirated (uL)	Volume to Stamp (uL)
SPRI	130.5	150
75% EtOH	175(x2)	200
Elution Buffer	40	50



2. When the protocol is finished, the final elution (40uL) will be in columns 9-12 of the original sample plate.
3. Use Qubit to analyze ChIP concentration.

Buffers for ChIP

Blocking Buffer (4°C); PBS+0.5% TWEEN, 0.5% BSA add PI (protease inhibitor cocktail)

ChIP Dilution Buffer (4°C); 16.7mM Tris-HCl pH8.1, 1.1% Triton X-100, and 167mM NaCl, 1.2mM EDTA, 0.01% SDS

Direct ChIP Elution Buffer (RT); 10mM Tris-HCl pH 8.0, 5mM EDTA, 300mM NaCl, **0.1% SDS.** Complete buffer by adding 5mM DTT before adding elution buffer.

RIPA High Buffer (4°C); 0.1% SDS, 1% Triton x-100, 1mM EDTA, 20mM Tris-HCl pH 8.1, 500mM NaCl, 0.1% DOC

RIPA Low Buffer (4°C); 0.1% SDS, 1% Triton x-100, 1mM EDTA, 20mM Tris-HCl pH 8.1, 140mM NaCl, 0.1% DOC

LiCl Wash Buffer (4°C); 250mM LiCl, 0.5% NP40, 0.5% Na Deoxycholate, 1mM EDTA,10mM Tris-HCl pH 8.1

TE Buffer pH 8.0 (4°C); 10mM Tris-HCl pH8.0, 1mM EDTA pH 8.0

Reverse x-linking Buffer (-20°C); 250mM Tris-HCl pH 6.5, 62.5mM EDTA pH 8.0, 1.25M NaCl, 5mg /ml Proteinase K, 62.5ug/ml RNAse A

For 1 Step Lysis:

1% SDS Cell Lysis Buffer 1% SDS, 10mM EDTA, 50 mM Tris-HCL pH 8.1

For 2 Step Lysis:

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

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

Appendix:

Robotic Protocol Overview:

The following sections list the general steps performed by each protocol and the order in which they are performed.

ChIP Wash:

1. Sample is placed on a magnet to magnetize the chromatin/bead complex.
2. Supernatant is removed, leaving behind the chromatin/bead complex.
3. 170uL of low salt buffer are added to the chromatin/bead complex and the complex is resuspended off the magnet.
4. Chromatin/bead complex is magnetized and supernatant is removed.
5. 170uL of high salt buffer are added to the chromatin/bead complex and the complex is resuspended off the magnet.
6. Chromatin/bead complex is magnetized and supernatant is removed.
7. 170uL of lithium chloride buffer are added to the chromatin/bead complex and left on the magnet.
8. Supernatant is removed.
9. Steps 7-8 are repeated.
10. 170 uL of TE are added to the chromatin/bead complex and left on the magnet.
11. Supernatant is removed.
12. Steps 10-11 are repeated.
13. 62.5 uL of ChIP elution buffer are added to the chromatin/bead complex and is resuspended off the magnet.
14. Resuspended chromatin/bead complex is transferred to a plate containing proteinase K and RNase A, mixed and magnetized.
15. The supernatant from the transferred mixture is transferred back to the original plate and mixed to collect any chromatin/bead complex that was left behind before being transferred back to the other plate.
16. Step 15 is repeated two additional times.

1.8x SPRI Cleanup Following Reverse Crosslinking and Deproteination of Chromatin:

1. Sample is placed on a magnet and the supernatant is transferred to a new well in the plate, leaving protein A/protein G beads behind.
2. The plate is moved off the magnet and 130.5uL of SPRI beads are added to the sample, mixed, and incubated for 5 minutes.
3. The plate is moved back on the magnet and the supernatant is removed.
4. The sample is washed twice with 175uL of 75% ethanol and dried for 4 minutes.
5. 40uL of elution buffer are added to the sample, resuspended off the magnet, and allowed to incubate for 2 minutes.
6. Sample is magnetized and supernatant is transferred to a new well.