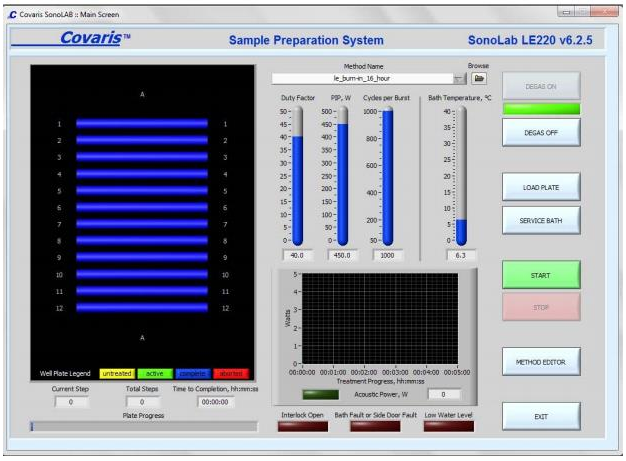
Prior to starting your ChIPs and Shearing:

Cool bench top centrifuge to 4°C and chill all buffers with protease inhibitors in advance.
Use DNA low bind tubes throughout protocol (eppendorf cat# 022431021) unless otherwise stated

Turning Covaris LE on, filling water bath and de-gassing:

1. Sign up for the Covaris LE on the Google Calendar.
2. Follow all rules and instructions posted on the Covaris equipment.
3. If the system is not on, turn on the computer first, then the Covaris LE (the button is on the right side toward the back of the equipment).
4. Once the computer is on, start the Sono Lab software and begin on the main page.
5. If you had to start the software and computer, an initializing screen appears then a homing notice will appear asking you if you want to start homing. Select yes and follow cues.
6. Typically, the Covaris LE is left with the door open and the transducer in the up position. If you do not find it like this, select the “service bath” button on the right side of the screen. This will raise the transducer so you can remove the water bath.
7. Fill water bath below your desired level with ddH_2O at the sink and place bath back into position.
8. Using a clean container (usually there is one under the equipment), continue to fill the bath to the desired level.
9. For the 500191 plate that houses the strip tubes, you want to fill the bath to fill level 6.
10. Turn cooling system on, close instrument door and select “load plate” which lowers the transducer.
11. Next choose “de-gas” for 45’ prior to shearing.
12. Check bath temperature. It should be between 0-4°C.



Software Panel 1: MAIN PANEL (LE220 application shown)

Cell Preparation Using Single Step Lysis Method:

1. Resuspend your pellet of cross linked cells in ~120λ of 1% SDS Lysis Buffer with protease inhibitors to a final volume of 130λ (50mM Tris-HCl pH8.1, **1.0%** SDS, 10mM EDTA).

2. Once you have resuspended your sample, transfer to Covaris micro tubes that have been pre-cooled on ice, incubate for 10' and proceed to shearing.

Covaris Protocol Loading Method:

1. Make sure that the equipment has been de-gassing for 45 min. prior to shearing.

2. From the main screen, select the "method editor" button on the right side of the screen. This button will bring you to the "Method Editor Panel".

3. From this screen, you can either load a pre existing method or create your own method.

4. To load a pre existing method, select the "Load Method" button on the bottom of the screen and find your file where your method is stored. There is an Epigenomics file in the Processes Folder on the "C" drive.

5. Our current program parameters for the microtubes are as follows: DF-30%, PIP-500, CPB-1000, time as previously optimized.

6. Make sure that the program you select is written for the rack you are using. Each rack has its own plate definition file that "homes" the plate and centers your samples.

7. Once you have selected your protocol, select "Load Plate", place rack in equipment, close door, press start.

8. When your samples are finished, select "load plate". Wait for plate to return to its initial position. The green light will indicate that you can open the door and remove your samples. Place your samples on ice.

Post Run Equipment Clean Up:

1. If no one else is using the Covaris after you, then it is your responsibility to clean the equipment.
2. When you are done with your shearing, select “service bath”.
3. Remove bath carefully (you can use tubing to remove some water prior to emptying bath in sink).
4. Replace empty bath in instrument and select “load plate” button.
5. Next, select ”de-gas” until water stops spitting out of the instrument’s tubes. Repeat.
6. Select service bath again, remove bath, clean water tank, wipe system dry, **leave door open**.
7. Don’t forget to shut off the cooling system.
8. Please follow the posted instructions carefully.

Chromatin Clearing and Storage:

1. Pool all chromatin from the same samples in a low DNA binding eppendorf tube.
2. Incubate samples on ice for 10’
3. Spin HS, 15’ at 4°C.
4. Transfer cleared supernatant to clean, labeled tube, leaving residual debris behind (usually SDS that has come out of solution).
5. Remove 3-5λ for reverse cross linking. This will be your input material or Whole Cell Extract ChIP. This is the material you will use for your WCE or control library**.** Clearly label this tube and store appropriately.
6. Add 5M NaCl to your pooled chromatin samples to a final conc. of 167mM.
   This will adjust your ChIPs to the same concentration of NaCl as the ChIP Dilution Buffer (CDB).
7. From this point, you can freeze your chromatin at -80°C or at 4°C if you plan to set up your ChIPs up right away.

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/130λ, you would add 13λ of sheared chromatin.

2. When diluting your chromatin with ChIP Dilution Buffer, keep in mind that you want your final concentration of SDS to be 0.1%. Therefore, in the example above, you would need a minimum total volume of 130λ to dilute the SDS to 1%.

3. Adjust your ChIP vol. with ChIP Dilution Buffer (CDB) plus protease inhibitors then add (1ug of) your antibody of interest to each 1.5 mL tube.

4. 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 ≤ 10% of total volume (make vol. adjustments as necessary).**

Dynabeads Preparation and Incubation with ChIP:

1. For each 500 - 1000λ ChIP, aliquot 50λ 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 50λ beads.
4. Mix by placing tubes on magnet then turning tubes 360°.
5. Aspirate blocking buffer and add 1000λ of fresh blocking solution to pre washed beads.
6. Incubate beads and blocking buffer in cold room with rotation for 1hr or at RT for 10-30'.
7. Remove beads and ChIPs from cold room and pulse spin.
8. Place tubes with beads on magnet and remove supt.
9. Add ChIP material to appropriate tube containing beads.
10. Incubate for 1hr at 4°C, rotating end over end.

Washes:

RIPA/140mM NaCl Buffer washes (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 200λ of RIPA buffer (Low Salt) to rinse beads to bottom of tube. Pipet gently to collect beads and then transfer beads to 96 well plate on magnetic station. **(*IMPORTANT: Save pipet tips from this step in respective tubes in order to transfer remaining beads to 96 well plate)**.

3. Add another 200λ of RIPA buffer to tubes with a clean pipet tip but continue to use "saved" tip to collect remaining beads.

4. Remove supt. in wells, then add beads from above (step 3) to their respective wells in the 96 well plate.

5. Move plate on magnet advancing column of wells either left or right. This will help to mix your sample.

6. If you are satisfied with the bead transfer, you may continue with the following washes, otherwise, continute with bead transfer.

7. Wash x2 with 200λ **RIPA/500mM NaCl Buffer** (0.1% DOC, 0.1%SDS ,1% Triton X-100, 500mM NaCl,1mM EDTA, 20mM Tris-HCl pH8.1)

8. Wash x2 with 200λ **LiCl Buffer** (0.25M LiCl,1% NP40,1% Na Deoxycholate, 1mM EDTA,10mM Tris-HCl pH 8.1).

9. Wash x2 with **TE**. Quickly spin plate and remove residual TE.

10. Prepare a master mix of **ChIP Elution Buffer** containing 8λ reverse x linking mix per sample. (ChIP Elution Buffer: 10mM Tris-Cl pH 8.0, 5mM EDTA, 300mM NaCl, 0.1% SDS, **5mM DTT**), (Reverse Cross Linking Buffer: 250mM Tris-HClpH 6.5, 1.25M NaCl, 62.5mM EDTA, 5mg/ml Proteinase K, 62.5ug/ml RNAse A).

11. Aliquot 60λ of Master Mix per well.

12. Cover plate with film, pulse vortex, spin briefly and reverse x link entire sample including beads at 65°C for 5 hours.

13. Include 10λ input, 40λ ddH2O and 8λ 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:

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 x10, incubate 2' RT followed by 4' on magnet (or until supt is clear). Remove supt.
4. Wash x2 with 200λ 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 2^{cd} 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 22λ 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 and subsequent library prep.

Qubit measurement:

1. Use Qubit dsDNA HS setup
2. Make Qubit master mix
   a. (n+2)*200 λ – (n+2) of Qubit dsDNA High Sensitivity Buffer.
   b. (n+2) λ 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"

Buffers for Covaris Mag Bead ChIP:

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

Chip Dilution Buffer for Covaris: No SDS,16.7mM Tris-HCl pH 8.1, 167mM NaCl, 1.1% Triton X-100, 1.2mM EDTA

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

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

LiCl Wash Buffer: 250mM LiCl, 0.5% NP40, 0.5% Na Deoxycholate, 1mM EDTA, 10mM Tris-HCl pH8.1

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, 5mM DTT

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.