EN-TEx ChIP-seq Protocol – Myers Lab

Tissue Pulverization:

1. Cool hammer, forceps, scalpel, metallic block and Covaris tissueTUBE TT1 extra-thick bags (Covaris; 520001) on dry ice. For each tissue sample, prepare 10 ml of 1X PBS with cOmplete Protease Inhibitor Cocktail (Roche; 11836153001) in a 15 ml conical tube. Warm solution at 37°C. Prepare 40 ml Wash Solution (cold 1X PBS with cOmplete Protease Inhibitor Cocktail) for each sample. Keep Wash Solution at 4°C or on ice.

2. Cut and weigh 150 mg of tissue for each ChIP experiment (always keep tissue frozen). Transfer tissues to Covaris tissueTUBE TT1 bag, attach tube with adaptor and keep on dry ice.

3. To pulverize tissue, dip bag in liquid nitrogen and transfer to metal block. Lay the bag flat on the block while bending the tube upward at a 45-90° angle. Use the hammer gently to break the tissue into fragments, keep tissue near the bottom of the bag (i.e., prevent tissue fragments from getting into the tube). Repeat this snap freeze/hammering process until the tissue is in powder form.

4. Unscrew the tube and add 2 ml of pre-warmed 1X PBS + Protease Inhibitor solution directly into the bag. Resuspend the tissue powder and transfer back into the 15 ml conical with the remaining 1X PBS solution.

5. Crosslink cells at 1% formaldehyde (final concentration) by adding 278 µl of 37% formaldehyde. Incubate on rocker/rotator for 30 minutes at 37°C.

6. Stop crosslinking reaction by adding 541 µl of 2.5M glycine and incubate for 5 minutes at room temperature with gentle rocking. Put samples on ice until all are processed.

7. Spin the samples at 5000 x g for 5 minutes at 4°C. Slowly decant the supernatant and resuspend the tissue in 10 ml cold 1X PBS + Protease Inhibitor. Repeat centrifugation and washes 2 additional times. For the final wash, transfer the tissue to a new 15 ml conical tube. Centrifuge samples, decant supernatant and snap freeze tissue in liquid nitrogen. Store samples at -80°C.

Chromatin Fragmentation by Probe-In Sonication:

1. Resuspend each frozen tissue pellet on ice in 2 ml cold Farnham Lysis Buffer (5mM PIPES at pH 8.0, 85mM KCl, 0.5% NP-40, Protease Inhibitor). Pass the solution several times through an 18 gauge needle to break up larger tissue mass then switch to a 20 gauge needle. Do this approximately 20 times avoiding air bubbles if possible.

2. Centrifuge the lysate at 2,000 rpm for 5 minutes at 4°C. Discard the supernatant and resuspend the crude nuclear prep pellet in 1 ml RIPA Buffer (1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS in PBS at pH 7.4). Transfer the prep into a 1.5 ml Eppendorf tube.

3. Using a probe-in sonicator, sonicate samples at 30% power for 1s on, 1s off for 5 minutes. Keep samples on ice until all samples are processed.

4. Centrifuge samples at 14,000 rpm for 15 minutes at 4°C. Collect supernatant. Snap freeze samples in liquid nitrogen and store at -80°C.

Couple the Primary Antibody to Magnetic Beads:

1. Dispense 1 ml of cold PBS/BSA solution (1X PBS with 5 mg/ml BSA fraction V, Protease Inhibitor; Filtered) into a 1.5 ml Eppendorf tube for each sample.

2. Add 200 μl of resuspended Dynabeads® M-280 (ThermoFisher Scientific; 11201D or 11203D) to each tube on ice. Vortex briefly to mix well.

3. Place tubes on a magnetic rack on ice and remove supernatant. Remove tubes from magnet and resuspend beads in 1 ml cold PBS/BSA solution. Repeat washes 3 additional times.

4. Resuspend magnetic beads in 200 μl PBS/BSA. Add 5 μg of the primary antibody to each magnetic bead solution; do not vortex.

5. Gently mix on a rotator platform at 4°C overnight.

Incubate Bead-Antibody Complex with Fragmented, Cross-linked Chromatin:

1. Thaw chromatin on ice. Wash magnetic beads 3 times with 1 ml cold PBS/BSA solution.

2. Reserve ~10% of chromatin to prepare control library, store at 4°C.

3. Add remaining chromatin to magnetic beads. Incubate on a rotator for 1 hour at room temperature followed by 1 hour at 4°C.

4. Perform a quick spin and put tubes on magnet rack. Remove and discard supernatant.

5. Wash beads 5 times with 1 ml cold LiCl Wash Buffer (100mM Tris pH 7.5, 500 mM LiCl, 1% NP-40, 1% sodium deoxycholate, Protease Inhibitor; Filtered). Mix samples on rotator for 3 mintues at 4°C during each wash step.

6. Resuspend magnetic beads in 1 ml cold TE Buffer (10mM Tris-HCl pH 7.5, 0.1mM Na_2EDTA, Protease Inhibitor; Filtered). Mix samples for 1 minute on a rotator then place on magnet. Discard supernatant. Resuspend beads in 200 μl room temperature IP Elution Buffer (1% SDS, 0.1M $NaHCO_3$; Filtered). Vortex to mix.

7. Incubate magnetic beads in a 65°C bath for 1 hour. Shake or vortex every 15 minutes during the incubation.

8. Spin the samples at 14,000 rpm for 3 minutes at room temperature. Put samples on magnet. Transfer the supernatant to a new 1.5 ml Eppendorf tube.

9. Bring the volume of the saved background control chromatin to 200 ul with 1X PBS. Incubate both the background control chromatin and ChIP'd DNA in a 65°C bath overnight to reverse the crosslinking.

Recover ChIP DNA and Control DNA Samples:

1. Warm EB solution (Qiagen; 28106) at 55°C.

2. To background control DNA, add 20 µl Proteinase K and incubate at room temperature for 2 minutes. Add 4 µl RNase A then 5 volumes of Qiagen PB Buffer (Qiagen; 28106). Incubate at room temperature for 20 minutes.

3. Add 5 volumes of Qiagen PB Buffer to the ChIP'd DNA. Add 10 µl of 3M sodium acetate.

4. Add approximately half of the sample to a QIAquick column (Qiagen; 28106). Centrifuge at 13,000 rpm for 1 minute. Repeat with the other half of the sample.

5. Wash the columns with 750 µl Qiagen PE Buffer. Centrifuge at 13,000 rpm for 1 minute and discard the waste. Perform a second spin to dry the column thoroughly.

6. Elute the DNA from the column with 60 µl of warmed (55°C) Qiagen EB Buffer. Allow the buffer to sit on the column for 3 minutes prior to spinning at 13,000 rpm for 1 minute.

7. Measure the DNA concentration.