Lesch Lab ChIP-seq protocol
updated 11/2/17 for Yale
(reagents and buffers listed at end of protocol)

Day 1

Prepare beads
*Start early in the morning to allow for 8 hours of antibody binding step; work in the cold room

Let the magnetic beads resuspend on a rotating rack for 5 min in the cold room. In the meantime, get everything ready to start the experiment.
1. Add 10 ul Dynabeads to 0.5 ml Eppendorfs (1 tube per IP).
2. Add 100 ul block solution (BS) and resuspend the beads by inversion. Spin down briefly (~3 sec, to get beads off the lid) and remove BS using a magnet made for 0.5 ml tubes.
3. Wash 2x in 150 ul BS (invert to resuspend, spin briefly before removing BS each time)
4. Resuspend in 30 ul BS by pipetting
5. Add antibody (0.5 ug for H3K4me3 ab8580, 1.0 ug for other antibodies)
6. Incubate at 4C, rotating, 8h

Thaw cells and sonicate (start ~1 hour before antibody binding step is done)

1. Thaw (crosslinked) cells on ice.
   *If cells were frozen in solution (not lysis buffer and not pellet alone), wash 1X in 500ul PBS, spin 1400xg for 5 minutes, and remove supernatant.
   *Small quantities of cells (<10^5 cells, if pellet is hard to see) should be frozen in 50 ul of lysis buffer and can be thawed on ice with no wash step.
2. Resuspend cells in 50 ul lysis buffer per IP (if not frozen in lysis buffer). For example, if doing 3 ChIPs from one sample, add 150 ul lysis buffer.
3. Let cells sit 5 min on ice to lyse.
4. Add 100 ul ChIP dilution buffer per IP and transfer to 0.5 mL Eppendorfs (150 ul per tube).
5. Sonicate in Bioruptor (Diagenode) at 4C, 10-15 min* (put on HOLD and set timer), 30 sec on / 30 sec off, setting HIGH.
   *Sonication time and conditions need to be re-optimized for each cell type and sonicator.
6. Move samples into 1.5 ml Eppendorf tubes (combine tubes from same original sample), spin 12000xg, 5 min, 4C. Carefully transfer supernatants to fresh tubes.
7. If doing multiple IPs from the same sample, split into 150 ul aliquots in 1.5 ml Eppendorfs. Bring volume up to 900ul in each tube by adding:
   700 ul dilution buffer
   50 ul lysis buffer
8. Add 100 ul protease inhibitor cocktail (Roche #11836153001) to each tube. Use 1 tablet in 1ml dilution buffer as 10x stock. Make fresh.
9. Set aside 50 ul from each original sample as input DNA and store at -20C.
10. Wash beads (prepared earlier) 3x in 150 ul BS, resuspending and spinning as before.
11. Resuspend beads in 10 ul BS and transfer to tubes with lysate (1 bead aliquot per IP)
12. Incubate overnight, rotating, 4C

Day 2

Make elution buffer fresh:

	for 1mL:
0.1M $NaHCO_3$	100 ul 1M $NaHCO_3$
0.2% SDS	10 ul 20% SDS
5mM DTT	5 ul 1M DTT
bring up volume with TE	885 ul TE

Wash and elute:

All steps performed in cold room. Chill buffers on ice before starting.

13. Collect beads on magnet. Transfer supernatant (unbound fraction) to fresh tubes and store; will use this to confirm fragmentation.
14. Wash:
    *resuspend by inversion; spin 1 min, 1000xg, 4C after each wash
    2x in 700 ul low-salt immune complex wash buffer
    *for H3K27ac, wash 1x in low-salt IC wash buffer, 1x in high-salt IC wash buffer
    2x in 700 ul LiCl wash buffer
    2x in 700 ul TE
15. Spin 3 min, 1000xg, 4C and remove any remaining TE
16. Elute 2x in 125 ul elution buffer
    a. Incubate with elution buffer 10 min, 65C, on shaking heat block. Program to shake for 10 sec every 1 minute.
    b. Spin 1 min, 16000xg, RT
    c. Collect beads on magnet and move supernatant to a fresh tube.
    d. Repeat with another 125 ul elution buffer and combine eluates for each sample (250 ul total)
    *Can combine multiple samples up until this point, but not after crosslink reversal.
17. Crosslink reversal: Incubate eluted chromatin (250 ul), input sample (50 ul), and unbound fraction (~1ml) at 65C, on shaking heat block at slow speed (400 rpm), 8-15 h.
    *Can stop this step after 8h and freeze at -20C
18. Add 5 ul RNAse A (~~100 mg/ml~~ 10 mg/ml stock **note updated concentration, 8/7/19**) to ChIP, 1 ul to input, 10 ul to unbound fraction. Incubate all tubes 2-4h, 37C, shaking slowly (can store at -20C after this step)
19. Add 2.5 ul Proteinase K (20 mg/ml stock) to ChIP, 0.5 ul to input, 10 ul to unbound fraction. Incubate all tubes 2h-overnight, 55C, shaking slowly (can store at -20C o/n after this step)

Purify samples:

20. Purify ChIP and input samples on Zymo ChIP Clean & Concentrator columns:

a. add 1:5 binding buffer to samples (1.25 ml of buffer to 250 ul of ChIP; 250 ul of buffer to 50 ul of input sample)
b. Apply ~800 ul of ChIP sample onto column, spin max speed, 30 sec, RT.
c. Discard the liquid from the collection tubes, place the columns back in the tubes, apply the rest of the ChIP samples onto the columns. Also add entire input sample onto fresh columns. Spin max speed, 1 min, RT.
d. Discard the liquid from the collection tubes, place the columns back in the tubes and wash 1x with 200 ul wash buffer (from kit). (Washing twice will improve the purity of sample, but compromise the yield. When working with small quantities, do one wash for better yield). Spin max speed, 30 sec, RT.
e. Label fresh tubes to elute the samples. For better sample recovery, elute twice (1st elution 7 ul of elution buffer, 2nd elution 6 ul of elution buffer). Spin max speed, 30 sec for first elution, 60 sec for second elution.

21. Spec ChIP and input DNA on Nanodrop or Qubit.
22. Purify unbound fraction by phenol/chloroform:
    a. Add 800 ul phenol/chloroform/IAA and vortex well
    b. Spin 14000xg, RT, 10 min
    c. Move upper aqueous phase to fresh tube (about 800 ul); discard organic phase
    d. Add 5M NaCl to final concentration of 200 mM (4 ul per 100 ul original volume = 32 ul for 800 ul starting volume) and 1.5 ul of 20 ug/ul glycogen (30 ug total).
    e. Add 800 ul 100% EtOH and incubate 30 min, -20C
    f. Spin 10 min, 20,000xg, 4C to pellet DNA
    g. Wash in 200 ul 70% EtOH; vortex to resuspend
    h. Spin 5 min, 20,000xg, 4C
    i. Pipet off EtOH, spin briefly and remove remaining liquid
    j. Air dry ~10 min until pellet is just slightly moist
    k. Resuspend in 10 ul TE or water, volumes can be increased for higher expected yields
23. Spec unbound DNA on Nanodrop or Qubit and run on BioAnalyzer to check fragmentation.

Library Prep

(Previously done by the Genome Core at Whitehead): We use Illumina's TruSeq ChIP library prep kit (#IP-202-1012) or Swift Biosciences Accel NGS 2S-Plus DNA library kit (#21024). We modify the manufacturer's instructions to use 1:50 gDNA adapters instead of the provided 1:40 dilution, and perform size selection after instead of before the PCR step.

Solutions, reagents and kits

Buffers containing detergents should be made fresh every 6 months
Make detergent stocks fresh every 6 months – 1 year

Block solution:

0.5% BSA in PBS *expires in two weeks
make from the stock solution of 1% BSA in PBS

for 10 mL:

50 mg BSA
10 ml PBS

Lysis buffer:	for 10 ml:	
1% SDS	500 ul	20% SDS
10 mM EDTA	200 ul	0.5M EDTA
50 mM Tris-HCl (pH 8.1)	500 ul	1M Tris-HCl
	+ 8.8 ml ddH2O	

Dilution buffer:	for 50 ml:	
0.01% SDS	25 ul	20% SDS
1.1% Triton X-100	5.5 ml	10% Triton
1.2 mM EDTA	120 ul	0.5M EDTA
16.7 mM Tris-HCl (pH 8.1)	835 ul	1M Tris-HCl
167 mM NaCl	1.67 ml	5M NaCl
	+ 41.85 ml ddH2O	

Low Salt Immune Complex Wash buffer	for 50 ml:	
0.1% SDS	250 ul	20% SDS
1% Triton X-100	5 ml	10% Triton
2 mM EDTA	200 ul	0.5M EDTA
20 mM Tris-HCL (pH 8.1)	1 ml	1M Tris-HCl
150 mM NaCl	1.5 ml	5M NaCl
	+ 42.05 ml ddH2O	

High Salt Immune Complex Wash buffer

Same as Low Salt, but with 500mM NaCl

LiCl Wash buffer	for 50 ml:	
0.25M LiCl	2.5 ml	5M LiCl
1% NP40	5 ml	10% NP-40 (Nonidet P-40)
1% deoxycholate	5 ml	10% sodium deoxycholate
1 mM EDTA	100 ul	0.5M EDTA
10 mM Tris-HCl (pH 8.1)	500 ul	1M Tris-HCl
	+ 36.9 ml ddH2O	

TE	for 50 ml:	
10 mM Tris-HCl	500 ul	1M Tris-HCl
1 mM EDTA (pH 8.0)	100 ul	0.5M EDTA
	+ 49.4 ml ddH2O	

Elution buffer (make fresh)	for 1 ml:	
0.2% SDS	10 ul	20% SDS
0.1M NaHCO3	100 ul	1M NaHCO3
+ fresh 5 mM DTT	5 ul	1M DTT
bring up volume with TE	885 ul	TE

**Final concentration of buffer for IP:

0.11% SDS
1% Triton
2 mM EDTA
20 mM Tris-HCl
150 mM NaCl

Protein G Dynabeads (Life Technologies, #10004D)

Protease inhibitor cocktail tablets (Roche #11836153001)

ChIP DNA Clean & Concentrator kit (Zymo Research, cat #D5210)

Glycogen, Roche #10901393001

UltraPureTM Phenol:Chloroform:Isoamyl Alcohol (Life Technologies, #15593-031)

References

Lesch BJ, Dokshin GA, Young RA, McCarrey JR, Page DC. A set of genes critical to development is epigenetically poised in mouse germ cells from fetal stages through completion of meiosis. *Proc Natl Acad Sci U S A*. 2013;110(40):16061-16066.

Lesch BJ, Silber SJ, McCarrey JR, Page DC. Parallel evolution of male germline epigenetic poising and somatic development in animals. *Nat Genet*. 2016;48(8):888-894.