ChIP-seq Protocol for fetal liver tissues **(modified from H. Zhou, UC-Davis) protocol and Diagenode)**	PI	Dr. Chris Tuggle
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Protocol:

I Tissue Harvest and Cross-Linking

1. Grind ~100mg frozen liver tissue into powder using liquid nitrogen in mortar, then transfer the liver powder to a clean 15-mL tube containing 3 mL of ice-cold PBS with 1% formaldehyde.

2. Incubate the tube on the rotator at **room temperature** for **8 min.**

3. Quench the cross-linking by adding **2.5 M glycine** (**150 mM final**) and incubate the tube on the rotator at **room temperature** for an additional **10 min**.

4. Harvest the tissue pellet by centrifugation at **2000g** for **10 min at 4°C**.

5. Remove the supernatant and add **3 mL** of ice-cold **PBS**.

6. Harvest the tissue pellet by centrifugation at **2000g** for **10 min at 4°C** and discard the supernatant. Then flash freeze the tissue pellet in liquid nitrogen and store at -80°C or proceed to Step 7 without freezing.

II Chromatin Preparation

7. Resuspend by flicking the frozen cross-linked tissue pellet in **900 µL of cell lysis buffer**. Incubate the tube on **ice for 20 min**.

8. Transfer the tissue suspension to a prechilled Dounce homogenizer (tight pestle B) and homogenize with 30-40 strokes with the tight pestle. **Keep samples cold.**

9. Transfer the homogenate to a fresh 15-mL tube, rinse the Dounce homogenizer with

1500 µL of cell lysis buffer, and combine.

10. Harvest the nuclei by centrifugation at **2000g for 5 min at 4°C**.

11. Remove the supernatant and resuspend the nuclei in **960 µL of nuclear lysis buffer**

12. Incubate the tube on **ice for 20 min**.

13. Take a **5-µL aliquot** from each sample and dilute it with **15 µL of TE** for an analytical gel.

14. Aliquot **300 uL** into the 1.5 mL TPX tubes（Diagenode）for chromatin shearing. Proceed to sonication steps.

15. Schedule a Bioruptor® Plus in DNA facility, observe and adjust the water level for correct height. Then, adjust settings to match the following: H level; 25 second on, 35 second off

16. Perform 12 - 16 cycles for each sample to allow for choice for optimized amount of correct DNA fragments. Every 5 cycles should add ice into the Bioruptor.

17. Once completed, remove the samples from the Bioruptor and store on ice or store at 4**°C refrigrator**.

- **QC for Chromatin shearing:**

18. Take a **5-µL aliquot** and dilute it with **15 µL of TE** for an analytical gel.

19. Treat all reserved aliquots with **2 µL RNAse A** (10mg/mL, #EN053) **for 30min at 37°C**, then add **20 µg or 2 µL** of **proteinase K** (600mAU/mL, cat:71049-4) for **4 hours or overnight at 65°C.**

20. Add loading dye to each sample, and separate samples on a 1% agarose gel in 1× TBE running buffer.

21. Visualize DNA samples on the agarose gel. ***(Sheared chromatin should range in size from 200 to 600 bp**.)*

22. Additionally, purify the DNA using Quick-DNA Miniprep Plus kit, measure the DNA concentration by Nonadrop, run on High Sensitivity DNA Bioanalyzer chip in DNA facility.

III Chromatin Immunoprecipitation:

Chromatin immunoprecipitation was performed according to iDeal ChIP-seq kit for Histones (Diagenode, Cat. No. C01010051)

Recommended antibody and the volumes used in the ChIP assay are as following:

H3K4me1(Diagenode catalog: C15410194), 0.66 µL

H3K4me3 (Diagenode, catalog: C15410003), 0.77 µL

H3K27me3 (Diagenode, catalog: C15410195), 0.69 µL

H3K27ac (Diagenode, catalog: C15410196), 0.36 µL

IgG (Diagenode catalog: C15410206), 1 µL

Recommended chromatin input into IP: 1 µL

- **QC for Chromatin Immunoprecipitation:**

Perform qPCR with a positive and negative target for each mark on each sample. The enrichments were evaluated using the percent input method with the following formula: % recovery=2^($Ct_{Input-6.64}$)-Ct_{Sample}) x 100%

Current available primers:

Gene	Sequence (5'-3')	H3K4me1	H3K4me3	H3K27me3	H3K27ac
RPL30	F: GGATCCAGTTTTGAGCGGTA R: GGAGCCGAGAGTTGATTGAC		+	-	+
WNT10A	F: TGCATCTCTTTGCAGGTGAG R: CCAGAAGCTGGGACTTATGC		-	+	-
FETUB	F: GCTGGCCCTCTGTATGCTAA R: GCTTCAACCCCACATCATCT	+			
Desert region	F: CGCGTACACCTTTCCATTCT R: GCGACAGAGACAATGCTGAA	-	-	-	-

VI Library prep kit:

The ChIP-seq libraries of the IPed DNA and the matched Input DNA were prepared using the NEBNext® Ultra™ II DNA Library Preparation Kit for Illumina and sequenced on the Illumina HiSeq 3000 platform at 2x100bp.

Reagents list

- **Cell Lysis Buffer:**

50 mM Tris (pH 8.0)
140 mM NaCl
1 mM EDTA
10% glycerol
0.5% NP-40
0.25% Triton X-100
Filter-sterilize using 0.2 um filter system. Store for up to 1 yr at 4°C.
***In addition, if immunoprecipitating with an antibody against the histone modification H3K27ac, include the histone deacetylase inhibitor sodium butyrate at a final concentration of 5 mM. Other inhibitors are generally not necessary when immunoprecipitating transcription factors but should be considered when using antibodies against posttranslational covalent modifications.

- **Nuclear Lysis Buffer:**

10 mM Tris (pH 8.0)
1 mM EDTA
0.5 mM EGTA
0.2% SDS concentration can be increased to (0.5% SDS as required)
Filter-sterilize. Store for up to 1 yr at 4°C

- **TE Buffer (**Tris-HCl 10 mM, EDTA 1 mM, pH 8.0**)**
- **PBS (**8g of NaCl；0.2g of KCl；1.44g of Na_2HPO_4；0.24g of KH_2PO_4;dissolve to 1L ddH2O;pH7.4**)**

*** **Add one protease inhibitor tablet according to product description at day of use to all buffers.**

- **proteinase K: 600mAU/mL, cat:71049-4**
- **RNAse A: 10mg/mL, DNase-free #EN053**
- **Dilution buffer:** *0.01% SDS 1.1% Triton X-100 1.2 mM EDTA 16.7 mM Tris(pH 8.1) 167 mM NaCl*
- **16% Formaldehyde**
- **2.5M Glycine**

- **Diagenode ----iDeal ChIP-seq kit for Histones Cat. No. c01010051**

- H3K4me1(Diagenode catalog: C15410194)
- H3K4me3 (Diagenode, catalog: C15410003)
- H3K27me3 (Diagenode, catalog: C15410195)
- H3K27ac (Diagenode, catalog: C15410196)
- IgG (Diagenode catalog: C15410206)

Equipment and materials list

- **Step1- Tissue Harvest and Cross-Linking:** mortar, rotator, 15mL tube, centrifuge
- **step2- Chromatin Extraction and Shearing:** Dounce homogenizer with a tight pestle (pestle B), nonstick 1.5-mL, Incubator 65℃, TPX tube (Cat. No. C30010009), Gel electrophoresis apparatus, centrifuge
- **step3- Preparation of Antibody Beads:** magnetic stand or rack
- **step4- IP of Chromatin:** rotator in cold room, magnetic stand or rack
- **step5- Purification of Chromatin:** NanoDrop 3300
- **step6- Analysis by qPCR:** qPCR apparatus, Gel electrophoresis apparatus