**Supplemental Material**

**Chromatin immunoprecipitation (ChIP) protocol for low abundance embryonic samples**

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**DNA extraction protocol and verification of sonication efficiency**

1. **Phenol-Chloroform extraction of DNA** (at room temperature):
	1. Add 1 volume of 25:24:1 Phenol-Chloroform-Isoamyl alcohol: Add 240 µL Phenol-Chloroform-Isoamyl alcohol to 240 µL of input control sample and 400 µL of Phenol-Chloroform-Isoamyl alcohol to 400 µL of ChIP sample.
	2. Spin 5 min at 21000 x g at room temperature to separate layers.
	3. Remove upper aqueous layer and transfer to a fresh tube.
	4. Repeat the above process with chloroform.

**CAUTION:** Phenol-Chloroform-Isoamyl alcohol and Chloroform are acute toxic (Skin and eye damage, hazard to the aquatic environment and respiratory sensitization).

1. **Ethanol precipitation of DNA** (on ice)
	1. Add the following in order and vortex well after adding each component:
		1. 20 mg/mL glycogen: Add 1.2 µL of 20 mg/mL glycogen to 240 µL of control sample and 2 µL of 20 mg/mL glycogen to 400 µL of ChIP sample.
		2. 1/10th volume 3M NaOAc : Add 24 µL of 3M NaOAc to 240 µL of control sample and 40 µL of 3 M NaOAc to 400 µL of ChIP sample.
		3. Add 2-3 volumes (fill up tube) ice cold 100% ethanol.

**CAUTION:** Ethanol is flammable.

* 1. Incubate for 30 min at -80 °C to precipitate the DNA. (check after 15 min – samples should NOT freeze).
	2. Spin 30 min at 21,000 x g at 4 °C to pellet DNA (small white pellet).
	3. Pour off ethanol and remove the remainder with a gel loading tip.
	4. Add 0.5 mL ice cold 70% ethanol.

**CAUTION:** Ethanol is flammable.

* 1. Pour off the EtOH and remove the remainder with a gel loading tip.
	2. Airs dry the pellet at room temperature on the bench for ~10 min (until pellet turns clear) by keeping the lid of tube open.
	3. Resuspend the pellet of input and ChIP reactions in the desired volume (e.g. 25 µL) of dH20 at room temperature by vortexing.

Note: Input control samples can also be used to verify the efficiency of sonication (see the step 11). Instead of phenol:chloroform extraction and ethanol precipitation, DNA can be purified using commercially available columns, which can be used directly after step 8.5.

1. **Verification of sonication efficiency**
	1. Load 15 µL of the input control DNA to a 1% agarose gel to test the efficiency of sonication (Figure 3).

Note: Optimization of sonication conditions is fundamental and must be determined separately for each cell or tissue type, and sonicator model. The optimal DNA fragment size after shearing should be examined by gel electrophoresis and the ideal DNA fragment size after sonication is 200 – 500 bp. For reproducible results these parameters should be kept constant for the same tissue or cell type.

Note: please refer to Troubleshooting Table 1.

* 1. Store the DNA at -20 °C until needed for subsequent steps.

**Buffer Recipes**

Locke Solution (1000mL)

Stock Solutions:

NaCl: 94, 27 g in 1000 mL dH2O

KCl: 12, 0253 g in 1000 mL dH2O

CaCl2.2H2O: 15, 5092 g in 1000 mL dH2O

Mix 100 mL NaCL stock solution, 37 mL KCl stock solution and 21 mL CaCl2.2H2O stock solution.

Fill up to 1000 mL with dH2O.

After sterilizing in the autoclave add penicillin-G.

Wash Buffer (500 µL) [prepare freshly]

465 µL PBS (cold) 1 x PBS

20 µL Protease Inhibitor Concentrate (25x) 1 x Proteinase Inhibitor

10 µL 1 M Na-butyrate 20 mM Na-butyrate

5 µL 100% PMSF 1% PMSF

Block Solution (50 mL) [store at 4°C]

250 mg BSA powder 0.5% BSA (w/v)

50 mL PBS (1x) 1 x PBS

Lysis Buffer – LB (50 mL) [store at room temperature]

0.5 mL 1 M Tris-HCl pH 8.0 10 mM Tris

1.0 mL 5 M NaCl 100 mM NaCl

100 µL 0.5 M EDTA 1 mM EDTA

50 µL 0.5 M EGTA 0.5 mM EGTA

0.5 mL 10% Na-Deoxycholate 0.1% Na-Deoxycholate

1.25 mL 20% N-lauroylsarcosine 0.5% N-lauroylsarcosine

46.60 mL dH20

RIPA Wash Buffer (250 mL) [store at room temperature]

12.5 mL 1 M Hepes-KOH pH 7.5 50 mM Hepes

25.0 mL 5 M LiCl 500 mM LiCl

0.5 mL 0.5 M EDTA 1 mM EDTA

25.0 mL 10% NP-40 1% NP-40

17.5 mL 10% Na-Deoxycholate 0.7% Na-Deoxycholate

169.5 mL dH2O

Elution Buffer (50 mL) [store at room temperature]

2.5 mL 1 M Tris-HCl pH 8.0 50 mM Tris

1.0 mL 0.5 M EDTA 10 mM EDTA

5.0 mL 10% SDS 1% SDS

41.5 mL dH2O

50X Tris-Acetate-EDTA Electrophoresis Buffer (TAE Buffer)

242g Tris

57,1 mL Acetic acid

100 mL 0.5 M EDTA pH8

1000 mL dH2O (Final pH8.3)