

DNA extraction from mouse tissue
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1. Lysis
 - a. Place mouse tissue (e.g. tail tips, skin) in 500 μ l tail lysis buffer + 10 μ l Proteinase K (10 mg/ml stock). Incubate at 55 °C overnight.
2. Removal of proteins and lipids
 - a. Centrifuge the solution and collect 400 μ l of the supernatant, add 500 μ l of Phenol: Chloroform: Isoamyl alcohol (IAA) (25:24:1) to supernatant, mix by inverting the tube (do not vortex) and centrifuge at max speed for 5-8 minutes
 - b. (Optional) Take 400 μ l of the supernatant and add 500 μ l of Chloroform:IAA (24:1), mix by inverting the tube (do not vortex) and centrifuge at max speed for 5-8 minutes
3. DNA precipitation
 - a. Add 400 μ l of the supernatant to 500 μ l of 100 % Isopropanol or 100 % cold Ethanol, mix by inverting the tube (do not vortex) and centrifuge at max speed for 5-8 minutes
 - b. Decant the Isopropanol or Ethanol, you should see a small, white, DNA pellet on the bottom of the tube. Add 900 μ l of 70 % Ethanol, mix by inverting the tube (do not vortex) and centrifuge at max speed for 5-8 minutes. This step clears the salt content in the DNA pellet.
 - c. Decant the 70 % Ethanol, air dry the pellet, and then add 30-50 μ l ddH₂O (depending on the size of the pellet) to the pellet.

Reagents:

- 1) Tail lysis buffer: (10 mM Tris pH 8.0, 100 mM NaCl, 10 mM EDTA pH 8.0, 0.5% SDS)
To make 500 ml:

Stock soln	mL needed	Final conc
1 M Tris pH 8.0	5	10 mM
5 M NaCl	10	100 mM
0.5 M EDTA pH 8.0	10	10 mM
10 % SDS	25	0.5 %
dH ₂ O	450 mL	

- 2) Proteinase K: stored at 10 mg/ml stocks at -20 °C
- 3) Phenol: saturated in 10 mM Tris pH 8.0...stored at 4 °C
- 4) Chloroform and IAA: stored in flammable cabinet