DNA extraction from mouse tissue
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1. Lysis
   a. Place mouse tissue (e.g. tail tips, skin) in 500 μl tail lysis buffer + 10 μl Proteinase K (10 mg/ml stock). Incubate at 55 °C overnight.

2. Removal of proteins and lipids
   a. Centrifuge the solution and collect 400 μl of the supernatant, add 500 μl 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 μl of the supernatant and add 500 μl 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 μl of the supernatant to 500 μl 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 μl 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 μl 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:

<table>
<thead>
<tr>
<th>Stock soln</th>
<th>mL needed</th>
<th>Final conc</th>
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</thead>
<tbody>
<tr>
<td>1 M Tris pH 8.0</td>
<td>5</td>
<td>10 mM</td>
</tr>
<tr>
<td>5 M NaCl</td>
<td>10</td>
<td>100 mM</td>
</tr>
<tr>
<td>0.5 M EDTA pH 8.0</td>
<td>10</td>
<td>10 mM</td>
</tr>
<tr>
<td>10 % SDS</td>
<td>25</td>
<td>0.5 %</td>
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<tr>
<td>dH2O</td>
<td>450 mL</td>
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</tbody>
</table>

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