DNA extraction from mouse tail to genotyping
(NO ORGANIC SOLVENTS EXTRACTION)

1. Fast extraction

1. Obtain the last 2 mm of the tail and place directly into 75 μl alkaline lyse reagent in a PCR tube. (Tails can be stored at frozen in PBS or PBND until use).
2. Samples heated at 95°C for 10 min to 1 h. After heating, samples are cooled at 4°C and 75 μl Neutralization reagent are added to each sample.
3. 1 to 5 μl of the final preparation is used per each PCR reaction.

**Alkaline lysis reagent:**

NaOH [25 mM] 0.5 g

Na₂-EDTA 2H₂O [0.2 mM] 0.03722 g

pH: around 12 (not adjust)

FINAL VOLUME = 500 ml

**Neutralization reagent:**

Tris-HCl [40 mM] (Not Tris-base) 3.152 g

pH: around 5 (not adjust)

FINAL VOLUME = 500 ml

Original paper: Biotechniques 2000 Vol 29, No.1 p. 52-54
B) Pure extraction

1. Obtain the last 1 to 3 mm of the mouse tail and place directly into an eppendorf.
2. Add 200 µL of Digestion buffer.
3. Add 2 µL of Proteinase K (10 mg/mL)
4. Stir at 800 rpm for +/- 6 hours or 600 rpm overnight, both at 55 ºC.
5. Centrifuge at 14000 rpm for 15 min.
6. Remove supernatant (= 180 µL) and put it in a new eppendorf. Note: while you remove it, avoid bring together mouse piles.
7. Add 180 µL of isopropanol (volume 0,7). Note: isopropanol is easily evaporated, so do not let flask open for big time.
8. Mix well by vortex, first, and inversion, after, until you see DNA precipitate. Note: if you do not see it, try to centrifuge at 14000 rpm for 25 min; it can also be adhered to the eppendorf cover or wall.
9. Remove DNA with a tip (Note: avoid mouse piles or possible unknown floccules), block superior extremity of the tip to block air passage and allow liquid removal or pass DNA to another new tip. Let DNA dry for 30 minutes (Note: no more than 2 hours).
10. Resuspend with 200 µL of Milli Q water or Tris EDTA.
11. Let dissolve at 37 ºC for +/- 2 hours. If this does not work, try vortex at low speed for small time or do a freeze-defrost cycle. Note: do step 11 only if step 10 doesn’t work.
12. Save your DNA extract at 4 ºC until use.

Digestion Buffer (for 500 mL):

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milli Q Water</td>
<td>415 mL</td>
</tr>
<tr>
<td>TrisHCl [1M] pH 8.0</td>
<td>50 mL</td>
</tr>
<tr>
<td>EDTA [0.5M]</td>
<td>5 mL</td>
</tr>
<tr>
<td>SDS 10%</td>
<td>10 mL</td>
</tr>
<tr>
<td>NaCl [5M]</td>
<td>20 mL</td>
</tr>
</tbody>
</table>