Chloroform-ethanol Isolation of Genomic DNA from Mouse Tail

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[Abstract] This protocol describes a simple and fast method to purify genomic DNA from mouse tails using chloroform.

Materials and Reagents

1. Proteinase K (20 mg/ml) (Life Technologies, Invitrogen™)
2. KAcO (Sigma-Aldrich)
3. Chloroform (Mallinckrodt)
4. Ethanol (Pharmco-Aaper)
5. Tris
6. NaCl
7. EDTA
8. SDS
9. Lysis buffer (see Recipes)

Equipment

1. Centrifuges
2. Incubator

Procedure

1. Dilute protease K to 1 mg/ml with lysis buffer (1:20 dilution).
2. Add 400 μl to each tail, and incubate at 55 °C overnight. After this step the lysate can be still stored in -20 °C, and thaw it completely in water bath and spin down before next step.
3. Add 75 μl 8 M KAcO, invert.
4. Add 500 μl cold chloroform (store at -20 °C). Invert 5 times.
5. Put the tubes in -20 °C for 10 min.
6. Spin at 13,000 x g for 10 min at 4 °C, transfer 300 μl supernatant to fresh tubes.
7. Add 617 μl cold ethanol (100%, store at -20 °C) and invert 5 times.
8. Spin at 13,000 x g for 10 min at 4 °C. Genomic DNA pellete should be visible.
9. Suck the supernatant and wash the pellete with 700 μl 70% ethanol.
10. Spin at 13,000 x g for 5 min at 4 °C.
11. Suck the supernatant, air dry for 10 min. Should be no visible liquid.
12. Add 100 μl 10 mM Tris (pH 7.4), if DNA not visible, then quantify.
13. Store genomic DNA in -20 °C.

Recipes

1. Lysis buffer: (50 ml)

<table>
<thead>
<tr>
<th>Stock</th>
<th>Volume</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M Tris (pH 8.0)</td>
<td>2.5 ml</td>
<td>0.05 M</td>
</tr>
<tr>
<td>4 M NaCl</td>
<td>1.25 ml</td>
<td>0.1 M</td>
</tr>
<tr>
<td>0.2 M EDTA</td>
<td>0.75 ml</td>
<td>3 mM</td>
</tr>
<tr>
<td>10% SDS</td>
<td>2.5 ml</td>
<td>0.5%</td>
</tr>
<tr>
<td>H₂O</td>
<td>Make up to 50 ml</td>
<td></td>
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</tbody>
</table>

Acknowledgments

This protocol was adapted from the original DNA isolation protocol as implemented in the Eric Sibley lab, Department of Pediatrics, Stanford University, CA, USA. Funding from the NIH supported this work.

References