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)

Stock	**Volume**	**Final**
1 M Tris (pH 8.0)	2.5 ml	0.05 M
4 M NaCl	1.25 ml	0.1 M
0.2 M EDTA	0.75 ml	3 mM
10% SDS	2.5 ml	0.5%
H_2O	Make up to 50 ml	

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References

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