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# DNA extraction - mouse tails - phenol-chloroform

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ABSTRACT

This protocol is taken from the URL indicated below from the Jackson Laboratories

This protocol yields a highly purified DNA preparation from mouse tail biopsies suitable for next-generation sequencing.

EXTERNAL LINK

https://www.jax.org/jax-mice-and-services/customer-support/technical-support/genotyping-resources/dna-isolation-protocols

PROTOCOL CITATION

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KEYWORDS

DNA extraction



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MATERIALS TEXT

DNA digestion buffer:

- 50 mM Tris-HCl pH 8.0
- 100 mM EDTA pH 8.0
- 100 mM NaCl
- 1% SDS
- proteinase K added to 0.5 mg/ml final

phenol/chloroform/iso-amyl alcohol 25:24:1

100% ethanol

70% ethanol (put at -20C prior to use)

EQUIPMENT

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Mini-centrifuge | S67601B | |
| Centrifuge | 5405000441 | |

SAFETY WARNINGS

Phenol and chloroform are toxic substances. Full PPE must be used and all steps are to be performed in a fumehood.

DISCLAIMER:



1 Remove 0.5 mm of tail into polypropylene microfuge tube (do not mince). (The tubes must have tight-fitting caps, so that there are no leaks in steps 3 and 7 below.)

2 Add 0.5 ml DNA digestion buffer with proteinase K added to 0.5 mg/ml final concentration. (0.5 mg/ml is a high concentration and can probably be reduced.) DNA digestion buffer:
• 50 mM Tris-HCl pH 8.0
• 100 mM EDTA pH 8.0
• 100 mM NaCl
• 1% SDS

3 Incubate overnight at 50-55 °C with gentle shaking. (At this step, mechanical agitation greatly aids complete disruption of the tail.) **Overnight**

4 Quick-spin tubes to get solution off inside of cap.

Mini-centrifuge
Centrifuge

**Fisher** **S67601B**

Any standard mini centrifuge with adapters for different tube sizes will suffice

5 Fill inside well of microfuge tube cap with vacuum grease. (We use Dow Corning high-vacuum grease and a 10cc syringe to dispense.)

6 Add 0.7 ml neutralized phenol/chloroform/iso-amyl alcohol (25:24:1). **700 µl**

7 Mix fairly vigorously at 4C. (Do NOT vortex; we use a clinical rotator for 1 hour.) **01:00:00**

8 Spin in microfuge **00:05:00** at top speed 5 minutes and transfer 0.5 ml of the upper phase to new microfuge tube. (Use P1000 for transfer, and draw the aqueous phase gently through tip several times after transfer if the DNA is still in large, gelatinous mass.)

Centrifuge
Benchtop Centrifuge
**Eppendorf** **5405000441**
Any benchtop centrifuge will suffice

9 Add 1 ml 100% ethanol at room temperature and invert (using clinical rotator if you wish) until DNA precipitate forms. (approximately 1 minute). **1 mL**

10 Spin in microfuge 5 minutes and carefully rem

Centrifuge
Benchtop Centrifuge
**Eppendorf** **5405000441**
Any benchtop centrifuge will suffice

ove and discard supernatant. **00:05:00**

11 Add 0.5-1 ml 70% ethanol (-20 °C) and invert several times.

12 Spin in microfuge 5 minutes

Centrifuge
Benchtop Centrifuge
Eppendorf 5405000441
Any benchtop centrifuge will suffice

and care **00:05:00** fully remove and discard supernatant.

13 Quick-spin tubes and remove last drop of ethanol solution with 25 µl capillary tube.

Mini-centrifuge
Centrifuge
Fisher S67601B
Any standard mini centrifuge with adapters for different tube sizes will suffice

14 Air dry at room temperature or in dessicator (overnight if you wish).

15 Add 100-200 µl TE buffer and incubate at 65 °C for 15 minutes to resuspend DNA. Draw DNA through P1000 tip after 65 °C incubation to aid in suspension if you wish.

16 Use 10-20 µl for restriction enzyme digest.

17 Total yield is approximately 20-50 µg DNA, 0.1-0.25 µg/µl.