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Protocol

Preparation of Polymerase Chain Reaction Template DNA from Mouse Tail Tissue

Richard Behringer, Marina Gertsenstein, Kristina Vintersten Nagy, and Andras Nagy

A simple cell or tissue lysate can provide a sufficient quality and amount of template DNA for polymerase chain reaction (PCR). In this protocol, a small piece from the tip of the tail is removed and processed using hot sodium hydroxide and Tris (HotSHOT).

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MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

Mouse tail tissue (1–2 mm)
Sodium hydroxide (NaOH) (50 mM)
Tris (1 M, pH 8)

Equipment

Heating block (95°C)
Microfuge
Microfuge tubes (1.5 mL)
Vortex

METHOD

1. Cut a 1- to 2-mm piece of tail tissue and place it into a microcentrifuge tube.
2. Add 200–300 µL of 50 mM NaOH.
3. Heat for 10 min to 95°C (if the piece is more than 2 mm, incubate longer).
4. Vortex to mix.

From the Manipulating the Mouse Embryo collection, edited by Richard Behringer, Marina Gertsenstein, Kristina Vintersten Nagy, and Andras Nagy.

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5. Add 50 µL of 1 M Tris (pH 8) to neutralize the NaOH. The pH can be checked at this step; it should be 7.
6. Centrifuge the tube at high speed (e.g., 12,000 rpm) for 6 min.
7. Transfer the supernatant to a new tube.
8. Use 1 µL of the supernatant for PCR, or dilute as needed.

RELATED INFORMATION

This very popular protocol was developed by Truett et al. (2000). For an alternative procedure that includes advice on tail-tip excision, see Protocol: **Isolation of High-Molecular-Weight DNA from Mouse Tail Tips** (Behringer et al. 2019).

REFERENCES

Behringer R, Gertsenstein M, Vintersten Nagy K, Nagy A. 2019. Isolation of high-molecular-weight DNA from mouse tail tips. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot092692.

Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman ML. 2000. Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques* **29:** 52–54.

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