



JUN 14, 2023

Genotyping mice from ear clips

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ABSTRACT

This is a protocol that describes the genotyping procedure from ear clip samples. This includes a general PCR protocol for primers with annealing temperatures in the range 55-70 degrees C. For other primers, the thermal cycling should be adjusted.

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.5qpvo3xobv4o/v1

Protocol Citation: Elizabeth P. 2023. Genotyping mice from ear clips. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.5qpvo3xobv4o/v1>

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Protocol status: Working
We use this protocol and it's working

Created: Jun 09, 2023

Last Modified: Jun 14, 2023

PROTOCOL integer ID:
83164

Keywords: ASAPCRN

Tissue Collection

- 1 Scruffing mouse securely, snip a tissue sample approximately 4mm² in size from the edge of its ear using a punch or scissors.
- 2 Store and transport samples separately in clearly-labelled 1.5mL tubes.

DNA Extraction

- 3 Add 200uL of 20mM NaOH to each tissue sample.
- 4 Heat samples to 100 degrees C for 15 minutes, or until tissues are thoroughly dissolved.
- 5 Add 50uL of 30% TRIS to each sample and centrifuge on high speed for 6 minutes.
- 6 Withdraw 200uL of supernatant from each sample, store in 1mL tube. Use 1uL of this DNA solution for each reaction.

PCR amplification

- 7 Create master mix. Scale depending on how many reactions you are conducting.
 - 7.1 To create the mix, for each reaction add in order:
 - 17 uL of autoclaved water

- 5 uL of GoTAQ buffer
- 0.5uL of dNTP
- 0.5uL of each primer
- 0.5uL of TAQ

- 8 Shake gently and add 24uL of this solution to DNA in a PCR plate.
- 9 Thermal cycle; the PCR protocol will vary according to primers, but cycling between 60 and 94 degrees Celsius has been effective for our lab.

Gel electrophoresis

- 10 Prepare gel:
 - 10.1
 - Heat 3g of agarose in 200mL TAE buffer solution.
 - Once cooled to 55 degrees C, add 12uL SYBR Safe DNA Gel Stain
 - Pour the gel onto the mold, add combs, and allow it to solidify for 15 minutes.
- 11 Place solid gel into the electrophoresis chamber. Load PCR product from the plate into the wells, one per well, leaving a gap between each well. Add a ladder before the first and after the last well, or as desired.
- 12 Switch on the electrical current (around 90 volts is recommended). Run the gel for 15-30 minutes depending on desired band size.
- 13 Remove the gel and image. Using an Anxygen gel reader is recommended.

