

SOP: Rodent Genotyping (Ear Punch and Tail Biopsy)

Objective:	Establish procedures for genotyping rodents
Author:	Attending Veterinarian, Laboratory Animal Resources
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Purpose

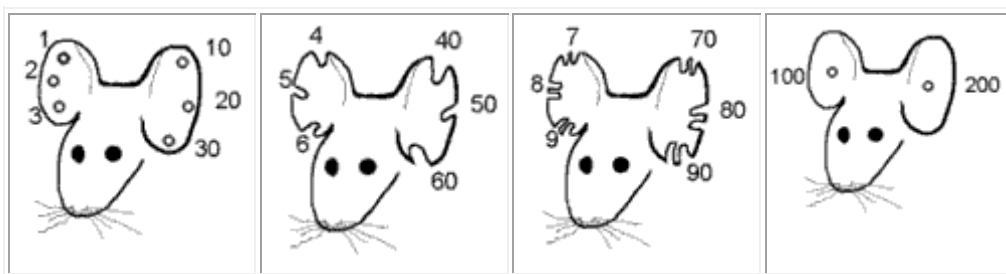
Genotyping of animals in a litter is critical to the efficient pursuit of research and in reducing the number of animals involved in a research project. Most often the genotype is determined by analysis of DNA extracted from tissues of young mice. The amount of tissue removed depends on the amount of DNA required to complete the experiment. For genotyping alone, the UNC Charlotte IACUC recommends using the ear punch procedure or blood samples. The UNC Charlotte IACUC recommends using tail biopsies as the last option.

Procedures

Ear Punch Procedure ¹

1. Restrain the mouse by the scruff and using the ear punch, make holes and/or notches in the ears, following an identification chart (see sample below).
2. Whenever possible, use a simple code to limit the number of notches/punches.
3. Have the identification chart readily available in the animal room to allow prompt identification of individuals.
4. If possible, use the excised tissue as a sample for genotyping, replacing the need for a tail biopsy.

Ear notch punch code:



DNA sample prep from mouse ear punches for PCR screening ²

1. Clip ear and save in labeled tube
2. Add 5 µl of 3 µg/µl Proteinase K
3. Let sample sit at room temperature for 30 min
4. Heat 95°C for 3 min
5. Spin debris to bottom
6. Samples are ready for PCR analysis or store in 4°C

This procedure and others (BioTechniques 29:52-54 (July 2000)) are very time efficient especially when large numbers of mice need to be genotyped.

Other Procedures

Depending on the requirements of the study, investigators are required to consider all alternatives to a tail biopsy. Tail biopsies should be used only when the investigator has demonstrated in the past that he/she cannot obtain sufficient amounts of DNA for the specific test being conducted.

Obtaining tissue from a mouse for DNA analysis via tail biopsy is a safe, effective and humane procedure that causes minimal or transient pain and distress when performed properly. DNA prepared from tail biopsies is suitable for analysis by either Southern Blot or Polymerase Chain Reaction (PCR). PCR analysis requires the least amount of DNA and tissue for this type analysis can also be obtained from ear punches, hair samples or oral swabs. Larger amounts of DNA are required for Southern Blot analysis.

Rodent Tail Clipping (Biopsy) Guidelines

- Animals should be 10-17 days of age when performing tail clipping.
- Scientific justification must be provided in the protocol if alternative methods cannot be used.
- A one time sample of 2 mm may be taken without anesthesia. No repeat cutting will be allowed without anesthesia.
- Alternatives to tail clipping must be considered for animals older than 17 days of age*.
- After 17 days of age, anesthesia must be used.
 - Repeated tail biopsies require general anesthesia and must be justified in the IACUC protocol.
 - Anesthesia must be used for sample collection greater than 2 mm. Removal of a sample greater than 2mm is considered a surgical procedure.
- The maximum total tail length per animal that can be taken is 5mm. For samples greater than 5 mm, an exception must be requested and justified in the IACUC protocol.
- If proper procedures are followed, the DNA yield from 5 mm of tail should exceed 50 micrograms, enough for multiple analyses. The DNA yield does not increase proportionally with tail fragment size. If only small amounts of DNA are required, investigators should consider taking only 2 mm of tail.

***NOTE:** Performing tail clips on animals over 17 days of age is considered a painful procedure due to the likelihood of bone involvement. Hemostasis is also a problem. If tail clips are necessary, along with the scientific justification, animals are to be placed in USDA Pain Category D, a description of anesthesia, post-procedural analgesics, and monitoring must be provided.

Tail Clipping Procedure

Prior to beginning:

Determine numbering system to be used in identification of animals and any specific records to be kept.

Materials Needed:

Straight Iris Scissors or disposable razor blades
Cautery Pen or Silver Nitrate Sticks
Small Forceps
Eppendorf or other tubes to hold samples
Fine tip permanent marker to label tubes
Ear punch or ear tags and applicator
Ice Bucket, Ice
Paper Towels
Non-sterile Gloves

Disinfectant solution (Clidox, Nolvasan, Alcohol, etc)

Clean cage for transfer of animals

Recommended Method for Tail Clip:

1. Lay out materials and place paper towels on a work surface.
 - If animals are older than 17 days, include anesthesia and post-procedural analgesics
2. Bring one cage at a time to the work area to avoid confusion.
3. Count number of animals in the cage and write their designated ID numbers on cage card. As you work it may also be helpful to indicate coat color of each mouse along with its number for future reference.
4. Label vials with corresponding cage animal numbers
5. Restrain the first mouse by scruff of neck and ear punch or tag with appropriate number
6. Verify that animal ID, vial and cage card number are the same. This is extremely important.
7. Place the mouse on cage top so that it grips it as you hold the animal by its tail, approximately 2 cm from the end.
8. Excise a total maximum per animal of 5 mm piece from the end of tail with iris scissors or razor blades, allowing it to fall onto paper toweling.
9. Cauterize the end of tail to stop bleeding; once stopped place the mouse into a transfer cage
10. Pick up the tail sample with forceps and place into the corresponding-numbered vial
11. Place the vial on ice for holding.
12. Continue with each mouse in the cage.
13. Return mice to the original cage, check again for any bleeding and return to same position on the rack.
14. To avoid wound and DNA cross-contamination, disinfect scissors or razorblades between animals with a disinfectant that is compatible with the assay you need to run.
15. Continue until all animals requiring testing have been sampled.

Thoroughly wash instruments and either disinfect chemically with Clidox solution, rinsing again afterwards to remove chemical contaminants OR sterilize by steam or ethylene oxide gas.

Rodent Tail Biopsy

Ideally, mice should be 10-17 days of age. For mice this age, local anesthesia to the tail is used prior to clipping. Local anesthesia can be achieved through tail immersion in ice cold ethanol for 10 seconds, or by disinfecting the tail with 70% ethanol, allowing it to dry, followed by application of ethyl chloride spray. For mice greater than 17 days old, a local or general anesthetic is required. In general, no more than 5mm total of tail should be excised per animal. Repeated tail biopsies require general anesthesia and must be justified in the IACUC protocol.

Sources

1. Cornell University: www.research.cornell.edu/Care/documents/SOPs/CARE552.pdf
2. This protocol was adapted from the Lab of Dr. Yvette Huet, Dept. of Biology, UNC Charlotte

Other sources

University of California San Diego: <http://iacuc.ucsd.edu/policies/Policy6.03.pdf>

Emory University: <http://www.emory.edu/IACUC/pdfs/BiopsyPolicy.pdf>

University of Pennsylvania: <http://upenn.edu/regulatoryaffairs/animal/guides/28RodentIdentification.pdf>

University of Iowa: <https://animal.research.uiowa.edu/iacuc-policy-rodent-tail-snipping-genotyping>

University of California Davis: <https://research.ucdavis.edu/wp-content/uploads/IACUC-32.pdf>

University of California San Francisco: <http://www.iacuc.ucsf.edu/Policies/awSPRodId.asp>

Revision History

Approved April 23, 2012

Re-approved August 31, 2015

Re-approved June 18, 2018

Re-approved April 26, 2021

Administrative changes September 17, 2022

Administrative changes October 17, 2023