UNIVERSITY OF NORTH CAROLINA
CHARLOTTE
Animal Care and Use Program

SOP: Rodent Genotyping (Ear Punch and Tail Clip Biopsy)

Objective:	Establish procedures for genotyping rodents
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I. 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.

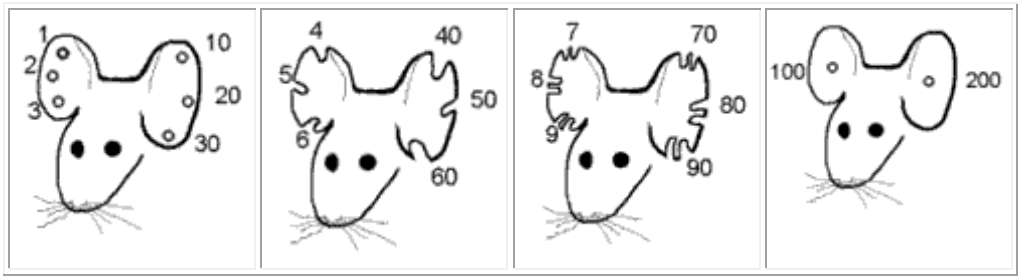
II. Procedures

- **Prior to beginning:** Determine numbering system to be used in identification of animals and any specific records to be kept.

A. 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 clip biopsy.

Ear notch punch code (for example):



B. DNA Sample Prep from mouse ear punches for PCR screening [2]

1. Clip the ear and save the tissue in a labeled tube.
2. Add 5 µl of 3 µg/µl Proteinase K.
3. Let the sample sit at room temperature for 30 min.
4. Heat at 95^{0}C for 3 min.
5. Centrifuge debris to the bottom.

6. Samples are ready for PCR analysis or storage in 4^0C.

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

C. Rodent Tail Clipping (Biopsy)

a. Consideration of Alternatives

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 via alternative methods (e.g., ear punch).

DNA samples are suitable for analysis by either Southern Blot or Polymerase Chain Reaction (PCR). PCR analysis requires the least amount of DNA, and tissue for PCR can be obtained from ear punches, hair samples or oral swabs. Larger amounts of DNA are required for Southern Blot analysis.

Obtaining tissue from a mouse for DNA analysis via tail clip/biopsy is an option when larger quantities of DNA are needed. This is a safe, effective, and humane procedure that causes only minimal or transient pain and distress when performed properly.

b. Guidelines

- Animals should be 10-17 days of age when 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.
- Anesthesia must be used:
  - In animals older than 17 days of age.
  - For repeated tail biopsies ***and*** must be justified in the IACUC protocol.
  - For sample collection 2 mm – 5mm or greater, ***and*** in these cases, removal is considered a surgical procedure (i.e., amputation).

  ****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 more difficult, too. If tail clips are necessary, along with the scientific justification, animals are to be placed in* ***USDA Pain Category D****, and a description of anesthesia, post-procedural analgesics, and monitoring must be provided.*
- 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.
- The DNA yield does not increase proportionally with tail fragment size. The DNA yield from 5 mm of tail should exceed 50 micrograms, enough for multiple analyses. If only small amounts of DNA are required, investigators should consider taking only 2 mm of tail.

c. Procedure (recommended method):

- **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

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 the number of animals in the cage and write their designated ID numbers on the cage card. As you work it may also be helpful to indicate the coat color of each mouse along with its number for future reference.
4. Label vials with corresponding cage animal numbers.
5. Anesthesia:
   - For mice 10-17 days of age:
     - This is the ideal age for tail clipping.
     - 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 (per above) or general anesthetic (see guideline requirements above) is required.
     - 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.
6. Restrain the first mouse by the scruff of the neck and ear punch or tag with appropriate number.
7. Verify that the animal ID, vial, and cage card number are the same. This is extremely important!
8. Place the mouse on a cage top so that it grips the cage as you hold the animal by its tail, approximately 2 cm from the end and place a paper towel under the tail.
9. Excise a <u>maximum per animal of 5 mm</u> from the end of the tail with iris scissors or razor blades, allowing it to fall onto paper toweling. Use less if possible.
10. Cauterize the end of the tail with a cautery pen or silver nitrate stick to stop bleeding. Once stopped, place the mouse into a transfer cage.
11. Pick up the tail sample with forceps and place it into the corresponding-numbered vial.
12. Place the vial on ice for holding.
13. Continue steps 5-12 with each mouse in the cage.
14. Re-check all mice in the transfer cage for bleeding.
15. Return mice to the original cage, check again for any bleeding, and return the cage to the original position on the rack.
16. To avoid wound and DNA cross-contamination, disinfect scissors or razorblades <u>between animals</u> with a disinfectant that is compatible with the assay you need to run.
17. Continue until all animals requiring testing have been sampled.

18. 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.

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

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