Institutional Animal Care Program (IACP)	
Title: **Tissue Collection for Rodent Identification and Genotyping**	
Policy number IACP 002	**Date in Effect**: 03/17/09
Revision 4 with title change	**Revision Date**: 11/14/25
In Effect ☒ **Rescinded** ☐	**Date Rescinded**:

A) RESPONSIBILITIES

It is the responsibility of all personnel using animals in approved research and teaching Institutional Animal Care and Use (IACUC) protocols to ensure that tail biopsies, toe clipping and ear notching of mice or rats meet the standards outlined below. It is the responsibility of the IACUC to review for approval properly justified requests for an exception to this policy.

B) APPLICATION

1) This policy applies to all mice and rats used in research and teaching at UT San Antonio and outlines the procedures of tissue collection for identifying and genotyping laboratory mice and rats via tail biopsy, toe clipping and ear notching. These procedures are consistent with the principles and recommendations established in the *Guide for the Care and Use of Laboratory Animals, 8th Edition* (National Research Council, 2011) and the *NIH Office of Animal Care and Use's Guidelines for Tissue Collection for Genotyping of Mice and Rats* (NIH, 2024).

2) Be aware that handling pre-weanling rodent pups may lead to rejection by the dam in some cases; therefore, investigators may want to consider performing tail biopsies and ear punching closer to or after the weaning period.

DEFINITIONS

1. **Tail Biopsy** – a procedure to remove the distal end of the tail to obtain small amounts of tissue or blood from a mouse or rat. It involves the removal of a 5 mm or smaller piece at the tip of the tail, which leads to bleeding at the site.
2. **Toe Clipping** – used on neonatal rodents up to 7 days old as a means of uniquely identifying animals for long term studies. This method may be necessary when other forms of identification are not feasible due to the size of young mice), low tissue volume, lack of permanency and side effects of other makings.
3. **Ear Punching** – minimally invasive technique used in weaned and older mice (≥ PND14) for identification and tissue collection when toe clipping is no longer appropriate. This method serves as both a form of individual identification and a source of DNA.
4. **PND** – post natal day.

C) SAMPLING PROCEDURES

1) <u>Tail Biopsy</u>

a) Only the minimum amount of tissue necessary for analysis should be taken.

b) Tail clipping is not considered a surgical procedure. The following steps should be followed when doing a tail biopsy:

1) Wipe down the biopsy site with 70% ethanol/isopropanol.
2) Instruments must be sharp, sterile or disinfected and clean of visible debris. At a minimum instrument must be wiped

down with 70% ethanol/isopropanol. Ideal methods of sterilizing/disinfecting instruments include exposure to autoclave, glass bead sterilizer, or chemical disinfectants.

c) Bleeding from the sampling site may stop spontaneously, however if required, adequate hemostasis can be achieved via a styptic pen, silver nitrate, tissue adhesive, gauze, cotton ball, etc.

d) Data[1] indicates that significant ossification of the distal end of the tail occurs by PND 17. Based on this data, **appropriate analgesia/anesthesia is always required when:**

1) Animal is ≤ 16 days old and more than 2 mm of the tail will be biopsied.

2) Animal is ≥ 17 days old regardless of the amount of tail that will be biopsied.

e) Analgesia/anesthesia is NOT required when:

1) Animal is ≤16 days old and 2 mm or less of tail is biopsied.

f) Only one biopsy sample per animal should be performed unless it is scientifically justified and approved by the IACUC.

g) Other less invasive alternatives should be considered in lieu of tail biopsy. Examples of alternatives are small ear punch, small quantity of blood from peripheral veins, and PCR analyses using saliva or hair.

h) **Scientific justification** for doing **tail biopsies is required when:**

1) Analgesia/anesthesia cannot be provided, and the rodent is ≥17

[1] [1] Hankenson F. Claire, Laura M. Garzel, David D. Fischer, Bonnie Nolan and Kurt D. Hankenson. "Evaluation of Tail Biopsy Collection in Laboratory Mice (Mus musculus): Vertebral Ossification, DNA Quantity, and Acute Behavioral Responses." Journal of the American Association for Laboratory Animal Science 47.6 (2008): 10-18.

days old.

2) Anesthesia/analgesia cannot be used, and the sample is to be >2 mm.
3) Sample is >5 mm.
4) More than one biopsy is needed for each animal.
5) Other less invasive procedures cannot be used.

i) **Procedure:**

1) Restrain the animal by grasping the skin along its back with your non-dominant hand.
2) Disinfect tail end with alcohol.
3) Apply topical anesthetic if indicated (see above).
4) With a sterile or disinfected scalpel, razor blade, or scissors, excise the distal tail.
5) Apply any of the methods mentioned above to stop any bleeding.

Toe Clipping

Toe clipping will be employed only for neonatal mice (PND 1-7) that are part of large, in-house breeding colonies. This method is used to simultaneously provide permanent identification and tissue for genotyping, particularly in cases where genotyping is time-sensitive.

NIH Guidelines recognize that distal phalanx biopsy (toe clipping) "may be the preferred method for neonatal mice up to seven days of age, especially if toe clipping and genotyping can be combined" (NIH, 2024, p. 3). Published studies

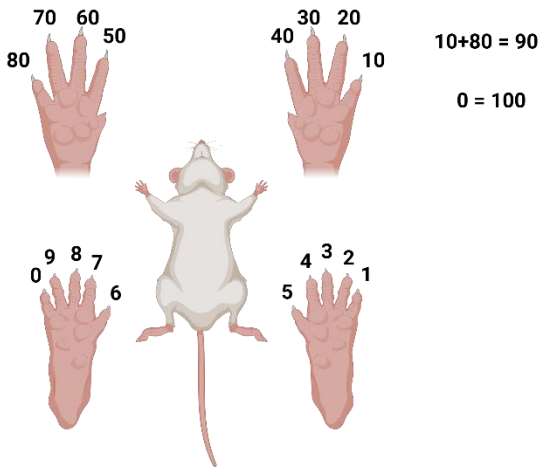
show that when performed correctly in this age window, toe clipping results in no greater pain or distress than other identification methods and no long-term behavioral or physiological effects (Castelhano-Carlos et al., 2010; Paluch et al., 2014; Schaefer et al., 2010; Dahlborn et al., 2013).

Every reasonable effort should be made to minimize pain or distress, including limiting the number of digits clipped to one digit per rodent. It is preferable to remove a digit from a hind paw rather than a forepaw, especially if the animal will be used in studies that include grip strength testing (3, 10). If the forepaw must be used, it is preferable to not cut the gualux ("dew claw" or "little toe" of the forepaw) as this may decrease the rodent's grasping ability. To ensure pain and distress is minimized, small sharp scissors should be used and personnel performing the procedure should be trained and proficient in the technique.

The following approach is consistent with the *Guide*'s emphasis on minimizing pain and distress while allowing for necessary scientific procedures (National Research Council, 2011, p. 120).

Materials:

- Suture scissors
- 70% Ethanol
- Labeled PCR tubes
- Bucket with ice
- Paper towels



Procedure:

a) Separate pups by sex and place them in a temporary holding cage with a

folded, clean paper towel at the bottom to reduce stress and heat loss.

b) Gently retrieve one pup at a time.

c) Using suture scissors disinfect with 70% alcohol, clip distal phalanx according to chart.

d) The clipped toe is placed in a labeled tube for genotyping and put on ice

e) If bleeding occurs, apply digit pressure with clean gause or paper towel until homeostasis is achieved.

f) Promptly return the pup to nest and observe for reintegration.

g) Disinfect scissors between each animal with 70% alcohol.

h) Record mice numbers, date, and breeding information on breeding record/cage card and genotyping form.

3) **Ear Punching**

Ear punches can be used in weaned and older mice (≥ PND14) for identification and tissue collection. This method serves as both a form of individual identification and a source of DNA. According to the *NIH Guidelines*, ear punching is a minimally invasive method that results in "minimal or transient associated pain and distress" and does not require anesthesia or analgesia when performed correctly (NIH, 2024, p. 2). The following method supports refinement and reduction principles by enabling both identification and genotyping through a single intervention.

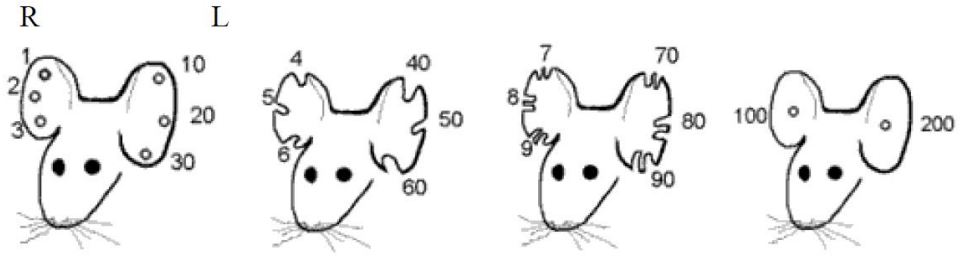
Materials:

- Ear punch tool (2 mm or smaller)
- 70% alcohol

- Labeled PCR tubes
- Bucket with ice
- Paper towels

Procedure:

1. Separate mice by sex (if still housed in breeding cage) and place them in a temporary holding cage with a folded, clean paper towel at the bottom to reduce stress and heat loss.
2. Retrieve one mouse at a time, using manual scruffing.
3. Using ear punch tool cleaned with 70% alcohol, punch the ear according to chart below (contact Laboratory Animal Resource Center, LARC for further guidance).
4. Collect tissue in a labeled tube for genotyping and put on ice.
5. If bleeding occurs, apply pressure with clean paper towel until homeostasis is achieved.
6. Promptly return the mouse to its home cage and observe.
7. Disinfect ear punch between each animal with 70% alcohol.
8. Record mice numbers, date, and breeding information on breeding cage card (if still housed in breeding cage) or cage card (if not in home cage), and on genotyping form.



Citations

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