**Rodent Genotyping and Identification**

Last Reviewed: March 13, 2024

**I.  Purpose**

The purpose of this standard operating procedure (SOP) is to provide guidelines to researchers regarding acceptable methods for identifying individual animals (e.g., ear punching) or collecting tissue for the purpose of rodent genotyping.

**II. Policy**

It is LAR policy to meet or exceed all federal, state, and local regulations and guidelines and to comply with all institutional policies and procedures as they apply to the use of animals in research. Personnel must attend any applicable training in animal care and use, occupational health and safety, equipment operation, and SOPs prior to performing activities outlined in this SOP or work under the direct supervision of trained personnel.

**III. Procedures**

The Institutional Animal Care and Use Committee (IACUC) must approve all invasive methods for rodent identification or tissue collection prior to performing procedures on animals.

**A. Ear Tagging**

Ear tagging involves the placement of a metal or plastic tag with a unique identification number or code to the base of the rodent’s ear.

1. Animals must be 14 days or older.
2. Ear tags should be sterile or disinfected prior to placement.
3. The ear should be disinfected prior to placement with chlorhexidine or 70% alcohol.
4. Ear tags should be placed in the lower outer third of the ear to facilitate normal ear positioning and to avoid areas with the highest concentration of capillaries.
5. Ear tags must be placed such that they do not cause a bend in the pinna, interfere with the animal’s mobility, or be placed in such a manner that they can catch on any part of the caging.

**B. Ear Punching and Notching**

This Identification method involves punching a hole or making a notch in the ear pinna. Commercial ear punches are available and inexpensive. Ear notch or punch tissue remnants can usually provide enough tissue for genotyping.

1. Ear notching or punching may not be performed on animals ≤ 14 days.
2. Ear punching or notching does not require the use of anesthetics or analgesics, however, for identification purposes the animal must be appropriately restrained to
ensure proper technique.
3. The ear punch device must be disinfected between animals (these devices can be autoclaved).

**C. Tail Clipping**

This tissue collection method involves amputating a very small segment of the distal tail. At < 21 days of age, the degree of ossification of the coccygeal vertebrate in the distal 5 mm is much less than that at 1 cm. After 21 days of age, the degree of ossification is similar along the entire length of the tail. The perception of pain is assumed to be more likely in bony vs. cartilaginous tissue. Tail clipping on mice or rats ≤ 21 days of age does not require anesthesia.

1. Animals must be appropriately restrained during the procedure to minimize trauma
2. Sterile sharp scissors (must be disinfected between uses) or a sterile blade per animal can be used for the procedure.
3. Only the distal 5 mm of the tail should be amputated.
4. Hemostasis can be achieved by using a silver nitrate stick, Quick Stop powder, or by applying a gauze sponge over the site with gentle pressure until bleeding stops.
5. Animals > 21 days of age or animals requiring a second tail sample must be appropriately anesthetized using ketamine/xylazine, isoflurane, or a local anesthetic.
6. Animals > 35 days of age that require tail clipping must be anesthetized (ketamine/xylazine, isoflurane, or local anesthetic) and administered a systemic analgesic given at least once following the procedure.
7. If multiple tail clippings are required a maximum of 1 cm total tail length may be amputated.

**D. Toe Clipping**

Toe clipping, as a method of identification of small rodents, should be used **only** when no other individual identification method is feasible and should be performed only on altricial neonates and when combined with genotyping. The specific method(s) must be described in the Animal Use Protocol (AUP) and appropriate scientific justification provided. Under all circumstances, aseptic technique must be followed.

This method involves removal of the distal phalangeal bone of one or more limbs. Toe clipping has the potential to induce pain and distress, and alter the animal’s gait and ability to feed.

1. Only one toe per foot may be amputated.
2. Sterile sharp scissors can be used for this procedure and must be disinfected between
 uses.
3. Hemostasis can be achieved using a silver nitrate stick, Quick Stop powder, or by placing a gauze sponge over the amputation site and applying gentle pressure until bleeding has stopped.
4. Toe clipping may only be performed in mice ≤ 12 days and rats ≤ 7 days.

**E. Other Methods of Identification**

1. Micro Chipping: A small microchip transponder is injected subcutaneously between the scapulae of the rodent.
	1. The microchip is detected by use of an electronic reader.
2. Micro-tattooing: A permanent mark using a needle and ink which is applied to the tail, toes, or foot pads.
	1. The tattoo equipment must be disinfected between animals.
3. Non-toxic markers: Sharpies can be used to mark the tail or fur of rodents; however, the marks must be reapplied every 24 hours (or as needed) to ensure the mark remains visible.
4. Animal Marker™ is another product available which can be used on rodent fur.
	1. The Animal Marker™ can remain visible for 6 -12 weeks.

**IV. References**

1.  Guide for the Care and Use of Laboratory Animals, 8thEdition  pg. 75.

2.  Hankenson FC Laire, Garzel LM, Fischer DD, Nolan B, and Hankenson KD.
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     ossification, DNA quantity, and acute behavioral responses. J Am Assoc Lab Anim
     Sci 47:10-18, 2008.

3. Vachon P. Anatomical and histological observations of fore- and hind limb toes in
     adult mice after amputations performed at the age of two weeks. Can J Vet
     Res 1998;62:311–13.