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UC Davis
Institutional Animal Care and Use Committee (IACUC)

Title: Rodent Genotyping and Identification Methods

I. Purpose:

The purpose of this document is to provide guidelines to researchers regarding acceptable methods for marking animals to identify individuals (e.g., ear punching) and tissue collection for the purpose of rodent genotyping.

II. Background:

The IACUC must approve all methods for tissue collection prior to performing procedures on animals. Ear punching and tail and toe clipping are acceptable methods of tissue collection for the purpose of genotyping mice and rats. **However**, toe clipping, as a method of identification of small rodents, should be used ***only*** when ***all*** the following conditions are met:
(1) performed only on altricial neonates (mice ≤12 days and rats ≤7 days)
(2) the tissue is simultaneously used for genotyping.
Please note that the specific method(s) used must be described and approved in the Animal Care and Use Protocol.

III. Guideline:

IACUC approved methods for sample collection are as described below.

A. Tail Clipping:

This method involves amputating a very small segment of the distal tail. Perception of pain is assumed to be more likely in bone versus cartilaginous tissue. Thus, it is important to keep in mind that at <17 days of age, the degree of ossification of the coccygeal vertebrae in the distal 5 mm of the tail is much less than the ossification at 1 cm. After 17 days of age, the degree of ossification is similar at the distal 5 mm and 1 cm tail segments. Tail clipping on mice or rats **≤17 days of age** does not require anesthesia. Animals must be appropriately restrained during the procedure to minimize trauma. Sterile sharp scissors (must be disinfected between uses) or a sterile blade per animal can be used for the procedure. Only the distal 2-5 mm can be amputated. Hemostasis can be achieved by using a silver nitrate stick, Quick Stop powder, or by applying a clean gauze sponge over the site with gentle pressure until bleeding stops. Animals should be observed closely after returning them to their

cage to ensure hemostasis. **Heat cautery is not permitted for this procedure, nor should it be necessary if the correct amount of tail was clipped.** Campus Veterinary Services (CVS) must be contacted if the area shows necrosis, bone exposure, and/or the animal's activity level is not normal.

Age	Anesthesia	Analgesia	In Protocol
≤ to 17 days	No	No	Yes
>17 days or animals requiring more than one tail sample	Yes	Yes	Yes

Animals **>17 days of age** that require tail clipping must be under general anesthesia (i.e., ketamine/xylazine or isoflurane) during the procedures and administered a systemic analgesic (i.e., buprenorphine, meloxicam, carprofen) given at least once prior to the procedure. The use of these medications must be clearly detailed within the procedures in the approved Animal Care and Use protocol, including entries in the drug table. **If multiple tail clippings are required a maximum of 1 cm total tail length can be removed, with all tail clippings combined. <u>No more than 1 cm is permitted in total</u>.**

B. Ear Punching/Notching:

This method involves punching a hole or making a notch in the ear pinna. Investigators must use a commercial ear punch device if this method is used. Ear notching using a 0.5 to 2 mm diameter ear punch is a permanent form of identification. **The maximum number of punches/notches per ear is two.** Ear notch remnants can usually provide sufficient tissue for DNA sampling during the initial PCR screening. Ear punch samples collected on animals do not require the use of anesthesia or analgesics. However, for identification purposes the animal must be appropriately restrained to ensure proper technique. Avoid the area of the ear closest to the head where the cartilage is thicker and more vascularized as it may be painful and bleed. The ear punch device used must be disinfected between cages of animals. These devices can be autoclaved.

Animals may only be genotyped by this method after their ear pinna has fully developed and sits away from the head. In most mouse strains, this occurs at around 14 days of age.

C. Toe Clipping:

As stated above, toe clipping as a method of identification of small rodents, must only be performed on altricial neonates, and only be performed when combined with genotyping, and included in an approved Animal Care and Use protocol.

This method involves removal of the distal phalangeal (coffin) bone of one or more digits. Toe clipping has the potential to result in pain and distress and alter the

animal's gait and ability to feed. Only one toe per foot may be removed. If possible, it is preferable to remove digits from a hind paw rather than a forepaw, especially if the animals will be used in studies that include grip strength testing. If the forepaw must be used, it is preferable to not cut the hallux ("dew claw" or "little toe" of the forepaw) as this may decrease the rodent's grasping ability Clean the foot with alcohol prior to clipping. Disinfected sharp scissors should be used for this procedure (**must be disinfected between animals**). Hemostasis can be achieved by using a silver nitrate stick, Quick Stop powder, or by placing a clean gauze sponge over the site and applying gentle pressure until bleeding has stopped. Toe clipping can **only** be performed in **mice ≤12 days of age and rats ≤7 days of age** and must be appropriately and scientifically justified in the approved IACUC protocol.

D. Other Identification Methods:

1. Microchips: Injecting a small microchip transponder subcutaneously between the scapulae of a rodent is permissible. The microchip is detected by use of a microchip reader.
2. Micro-tattooing: A permanent mark made using a needle and ink can be applied to the tail, toes, or foot pads. Forcep style micro-tattoo tools should be considered to reduce the chance of needlestick injuries to staff, such as https://www.ketchummfg.com/aramis-laboratory-animal-mircotattoo-system.
3. Ear tagging: A metal tag with a unique identification number may be attached to one ear of a rodent. Either ear may be used, but no more than one tag per ear should be on an individual rodent.
4. Non-toxic dye/markers: Sharpies can be used to mark the tail or fur of rodents. However, the mark may need to be applied as often as every 24 hours to ensure the mark is still visible. Animal Marker is an example of another product available which can be used on rodent's fur. Animal Markers can last between 6-12 weeks. Non-toxic hair dye may also be used.

   All methods noted above are techniques that require training. For additional training, please contact the IACUC office iacuc-staff@ucdavis.edu.

IV. Resources:

1. ILAR, Guide for the Care and Use of Laboratory Animals http://nap.edu/12910
2. Hankenson FC Laire, Garzel LM, Fischer DD, Nolan B, and Hankenson KD. Evaluation of tail biopsy collection in laboratory mice (*Mus musculus*): Vertebral 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.

4. Identification Methods for Mice https://research.ucdavis.edu/policiescompliance/animal-care-use/training-classes/identification-methods-for-mice/
5. NIH Office of Intermural Research. (2022). Guidelines for Tissue Collection for Genotyping of Mice and Rats https://oacu.oir.nih.gov/system/files/media/file/2024-12/b3-rodent-genotyping.pdf
6. Aramis Laboratory Animal Microtattoo System https://www.ketchummfg.com/aramis-laboratory-animal-mircotattoo-system