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, 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) no other individual identification method is feasible, 2) performed only on altricial neonates (mice ≤12 days and rats ≤7 days), and 3) the tissue is simultaneously used for genotyping. Please note that the specific method(s) 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. At <17 days of age, the degree of ossification of the coccygeal vertebrae in the distal 5 mm is much less than that at 1 cm. After 17 days of age, the degree of ossification is similar at the distal 5 mm and 1 cm tail segments. Perception of pain is assumed to be more likely in bone versus cartilaginous tissue. 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. Campus Veterinary Services
(CVS) must be contacted if the area shows necrosis, bone exposure, and/or the animal’s activity level is not normal.

<table>
<thead>
<tr>
<th>Age</th>
<th>Anesthesia</th>
<th>Analgesia</th>
<th>In Protocol</th>
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<tbody>
<tr>
<td>≤ to 17 days</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>&gt;17 days or animals</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>requiring more than one</td>
<td></td>
<td></td>
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<tr>
<td>tail sample</td>
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Animals >17 days of age that require tail clipping must be under general anesthesia using 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 outline in the approved IACUC 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. No more than 1 cm is permitted in total.

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. 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.

C. Toe Clipping:
As stated above, toe clipping as a method of identification of small rodents, must be used only when no other individual identification method is feasible, must only be performed on altricial neonates, and only be performed when combined with genotyping.

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. 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 only and must be appropriately scientifically justified in the approved IACUC protocol.

D. Other Identification Methods:
1. Microchips: Injecting a small microchip transponder subcutaneously between the scapulae of the 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.
3. Ear tagging: A metal tag with a unique identification number may be attached to one ear of the 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
4. Identification Methods for Mice
   https://research.ucdavis.edu/policiescompliance/animal-care-use/training-classes/identification-methods-for-mice/