Rodent Genotyping & Identification Methods

Collecting Tissue for Genotyping and Identifying Purpose Bred Mice and Rats

**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.

**Background**
The Institutional Animal Care and Use Committee (IACUC) must approve all methods for tissue collection prior to performing procedures on animals. Tail clipping, ear punching, 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 no other individual identification method is feasible and should be performed only on altricial neonates (mice 12 days and rats 7 days) and when combined with genotyping. The specific method(s) must be described and approved in the Animal Care and Use Protocol. Under all circumstances, aseptic method should be followed. Reference to this document is sufficient description, provided the guidelines are adhered to as described below.

**Guidelines**
IACUC recommendations for sampling are as follows:

- **Tail Clipping**

  This method involves amputating a very small segment of the distal tail. At <21 days of age, the degree of ossification of the coccygeal vertebrae in the distal 0.5 cm is much less than that at 1 cm. After 21 days of age, the degree of ossification is similar at the distal 0.5 cm and 1 cm tail segments. Perception of pain is assumed to be more likely in bony versus cartilaginous tissue. Tail clipping on mice or rats 21 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 0.5 cm should be amputated. 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. Animals >21 days of age or animals requiring a second tail sample must be appropriately anesthetized using ketamine/xylazine or isoflurane or local anesthetic. Animals >35 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 following the procedure. TRACS Veterinary Services must be consulted regarding the appropriate analgesics. **If multiple tail clippings are required a maximum of 1 cm total tail length can be amputated,**
with all tail clippings combined.

- **Ear Punch**

This method involves punching a hole or making a notch in the ear pinna. Commercial ear punches are available and inexpensive. Ear notching using an ear punch is a permanent form of identification. Ear notch remnants can usually provide enough 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. The ear punch device used must be disinfected between cages of animals. These devices can be autoclaved.

- **Toe Clipping**

As stated above, 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.

This method involves removal of the distal phalangeal (coffin) 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. Only one toe per foot may be removed. Sterile sharp scissors can be used for this procedure (must be disinfected between uses). Hemostasis can be achieved by using a silver nitrate stick, Quick Stop powder, or by placing a gauze sponge over the site and applying gentle pressure until bleeding has stopped. Toe clipping can be performed in mice 12 days and rats 7 days of age only. Toe clipping required in mice/rats above these ages must be appropriately justified in the IACUC protocol for approval.

- **Other Identification Methods**

1. Micro Chipping: Injecting a small microchip transponder subcutaneously between the scapulae of the rodent. The microchip is detected by use of a reader.

2. Micro-tattooing: A permanent mark made using a needle and ink which is applied to the tail, toes, and foot pads.

3. Ear tagging: A metal tag with a unique identification number is attached to one ear of the rodent.

4. Non-toxic markers: Sharpies can be used to mark the tail or fur of rodents, however the mark must be reapplied every 24 hours to ensure the mark is still visible. Animal Marker is another product available which can be used on rodent’s fur. Animal Markers can last between 6-12 weeks.

The e-learning course “Identification Methods for Mice” is available for viewing on the lms.ucdavis.edu website. For additional training, please contact the IACUC office iacuc-staff@ucdavis.edu [1].

**References**

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

