

March 11, 2019

IDENTIFICATION AND GENOTYPING

Tail Snip, Ear Punch, and Toe Clip

1. PURPOSE: Proper identification of research animals is an essential component of research design. It allows for easy tracking of animal throughout a research project and assists animal care staff in providing appropriate care to individual animals.

2. POLICY:

a. Procedures described within this standard operating procedure provide guidance on the various methods of identifying and genotyping individual or groups of mice and rats on VA IACUC-approved research protocols.

3. PROCEDURES:

a. **CAGE CARDS** - Cage card information includes: species, strain or stock, source of animal, gender, names and contact numbers of responsible investigators, date of birth/arrival and protocol number. Cage card can be used as the only method of identification for animals on protocols where individual identification is not necessary.

b. **TEMPORARY MARKINGS** - Use an indelible marker to write numbers, bars, or other distinguishable markings on the tail or the ears. Temporary marking can be used for short-term individual identification; this marking may last up to 3-4 days.

c. **EAR TAGS** - Mice should be ear tagged at weaning age or older. Use tags that are about 5 mm long for mice. Rinse tag in 70% alcohol before use to help prevent ear infection. Position the tag in the applicator so that the end with the hole is positioned over the notched area of the applicator; the pointed end should be opposite the hole. Make ears easily accessible by scruffing. Place the ear between the point and the hole of the tag; the numbers should be in an upward configuration so that they can be more easily read. The tag should be positioned at the lateral base of the ear, approximately 3 mm from the edge of the ear pinna. Once the tag is positioned correctly, firmly squeeze the applicator to apply. Monitor the tag implantation intermittently for signs of local infection

d. **GENOTYPING METHODS** - Tail snip, ear punch, and toe clip are methods used to obtain tissues for genotyping small rodents.

(1) All genotyping methods needs to be included in an IACUC-approved protocol. A scientific justification to use toe-clip method must be included in the research protocol for IACUC approval. Clearly indicate why alternate methods of identification are not possible. Justification cannot be based solely on the number of animals requiring identification.

(2) DNA Analysis and/or Genotyping - must be described in an approved protocol.

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(3) The proper identification of genetically engineered animals in a litter is critical to the efficient pursuit of research and in reducing the number of animals involved in a research project. Most often the genotype is determined by analysis of DNA extracted from tissues of young rodents. Analysis by the Polymerase Chain Reaction (PCR) requires the least amount of DNA.

(4) Tail snip/biopsy:

(a) Obtaining tissue from a mouse or rat for DNA analysis via tail biopsy is a safe, effective and humane procedure. Tail clipping will not distinguish between animals but will serve as a source of DNA genotyping.

(b) When preformed properly it causes only minimal or transient pain and distress, and induces no more “physiological impact” (change in heart rate, body temperature, or activity level) than just restraining the animal for the procedure.

(c) Ideally, mice and rats should be 10-21 days old. At this age, the tail tissue is soft (vertebra are not yet calcified) and the yield of DNA is highest. In addition, prompt analysis of tail tissue allows the desired mice and rats to be identified prior to weaning which can facilitate more efficient use of cage space.

(d) Because pain sensory development may be complete, and to further minimize any transient pain or distress, investigators are strongly encouraged to apply local anesthesia to the tail, as recommended by the attending veterinarian.

(e) For mice and rats greater than 21 days of age: The use of a local or general anesthetic (e.g. isoflurane) is required prior to collection of tissue. Recent studies have proven that tail snips can cause hypersensitivity even six months after the tail has been snipped.

(f) Tail snip/ biopsy procedure: (i) Manually restrain the mouse or rat between thumb and forefinger. This is a convenient time to identify the animals using the appropriate method (i.e. ear punch, ear tag, transponder etc.) (ii) With sterile scalpel, razor blade, or scissors cleanly excise the distal 2mm (maximum 5 mm) of the tail. If the proper procedures are followed, the yield of DNA from 5 mm of tail should exceed 50 micrograms, enough for multiple analyses. The yield of DNA does not proportionally increase as tail fragments larger than 5mm are used. It is good practice to divide the tissue into several pieces after collection, label and freeze the extra samples at -20C in case you have to verify your samples. (iii) If small amounts of DNA are required, investigators should take only 2 mm of tail. (iv) If the analysis of the DNA is to be performed by PCR, great care should be taken to remove all tissue from the scissors or scalpel and disinfect after each animal. (v) If a scalpel is used, also disinfect the work surface on which the tail is placed. (vi) The investigator must monitor the animals to assure hemostasis after the animals are returned to the cage. Apply digital pressure and silver nitrate, as needed.

5. Ear notching/punching:

(a) This method involves collecting a sample of tissue from the ear using a punch designed for this task. The method is often used for identification purposes and so is ideal to fulfill

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both needs at the same time. The procedure is quick, easy, and should not cause bleeding if done properly. If bleeding does occur take proper measures to ensure the bleeding has stopped before returning the animal to its cage. The procedure is best performed at 10-21 days of age but is humane and acceptable at all rodent ages without anesthesia or analgesia.

(b) Procedural guidance: (i) Restrain the mouse by the scruff and using the ear punch to create holes and/or notches in the ears, following an identification chart. (ii) Whenever possible, use a simple code to limit the number of notches/punches. (iii) Have the identification chart readily available in the animal room to allow prompt identification of individuals. (iv) Use the excised tissue as a sample for genotyping, replacing the need for a tail biopsy. (v) Ear punch devices and scissors should be disinfected between animals. This can be done by wiping with 70% ethyl alcohol. (vi) Instruments used for ear notching can become dull after use and should be replaced often, as dull instruments can cause trauma to the notch site.

6. Toe clipping:

(1) Note: According to *The Guide for the Care and Use of Laboratory Animals*, "toe-clipping, as a method of identification for small rodents, should be used only when no other individual identification method is feasible and should be performed only on altricial neonates." Altricial, meaning "requiring nourishment", refers to a pattern of growth and development in organisms which are incapable of moving around on their own soon after hatching or being born.

(2) Toe clipping can only be performed in rodents in their first week of life. The ideal time is between postnatal day five and seven when the toes are large enough to work with yet the bones are not calcified.

(3) Toe Clipping procedure: (a) Digit removal is limited to two toes per foot, two feet per animal. (b) Whenever it is feasible, amputating half a digit is sufficient. (c) Instruments (surgical scissors, scalpel blades, etc.) must be sterilized before use and cleaned and disinfected between animals. (d) Confirm bleeding has stopped prior to returning animals to their cage. (e) Apply a local anesthetic on the site of amputation. (f) The removed tissue should be used for genotyping. (g) Currently, it is generally accepted that anesthesia is not required for this procedure in the first week of life. Investigators are advised to consult with VA Veterinary Medical Consult on the use of anesthesia in older animals.

4. REFERENCES:

- USDA library. DNA collection for genotyping. <https://www.nal.usda.gov/awic/dna-collection-genotyping>
- National Institutes of Health, ARAC, Guidelines for Toe Clipping of Rodents, Revised 6/13/07 (<http://oacu.od.nih.gov/ARAC/index.htm>)
- Guide for the Care and Use of Laboratory Animals, 8th ed, National Research Council, National Academy Press, 2011, page 75.

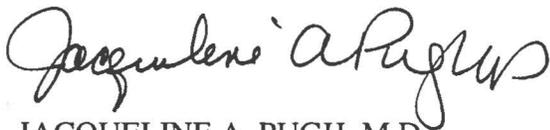
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- Federation of European Laboratory Animal Science Associations Working Group, FELASA Guidelines for the Refinement of Methods for Genotyping Genetically-modified Rodents, 2013. Laboratory Animals 47(3) 134-145.

5. RESPONSIBILITY: ACOS for Research and Development (151)

6. RECESSION: Research Service Policy Memorandum 11-55, January 18, 2011

7. RECERTIFICATION: March 11, 2024

A handwritten signature in black ink that reads "Jacqueline A. Pugh". The signature is written in a cursive, flowing style.

JACQUELINE A. PUGH, M.D.
ACOS for Research and Development