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CAR5: GUIDELINE ON RODENT IDENTIFICATION

The Animal Care Program has developed the following guideline to provide information regarding commonly used rodent identification techniques to the research community. It is the responsibility of the Principal Investigator (PI) to review their individual IACUC Animal Use Protocols to determine which method of identification is approved for use.

Definitions

- A) **Ear-punching:** Application of a specific combination of small hole punches or notches to the outside edges of a rodent's ear (see Appendix, Figure 1).
- B) **Ear-tagging:** Attachment of a metal or plastic tag with a unique identification number or code to the base of a rodent's ear (see Appendix, Figure 2).
- C) **Microchipping:** Injection of a small microchip transponder subcutaneously between the shoulder blades of the rodent. The microchip is read by use of a scanner.
- D) **Microtattooing:** A permanent mark made using needle and ink, which is applied to the tail, toes, ears, or foot pads. Manual or electric equipment can be used.
- E) **Toe-clipping:** Procedure in which the most distal bone of the toe (3rd phalanx) is removed (see Appendix, Figure 3).
 - i. As a method of identification of small rodents (mice and rats) toe clipping must only be performed on neonates 5-7 days of age.^{1,2,3,4,5}
 - ii. Written justification for toe clipping must be approved by the IACUC before the procedure can be performed. No more than 2 toes per foot should be removed, with a maximum of 6 total clipped toes per animal.
- F) **Non-permanent methods of identification:** These methods of identification include fur clipping or the application of non-toxic fur dyes or marker marks. While non-invasive, these techniques are temporary and are only appropriate for short term identification purposes.

Training

Only trained personnel can perform identification techniques. ***We strongly suggest that animals undergoing permanent identification procedures be anesthetized until the individual performing the procedure is proficient enough to perform the technique on conscious animals.***

Specific training sessions for rodent identification methods can be arranged through the CAR Training Department; please send requests for training by email to cartrain@msu.edu for further information.

References

1. National Research Council, Guide for the Care and Use of Laboratory Animals, 8th ed, pg 75
2. Wang L. A primer on rodent identification methods. *Lab Anim (NY)* 2005: 34, 64–7
3. Castelhana-Carlos, MJ, Sousa N, Ohl, F, and Baumans, V. Identification methods in newborn C57BL/6 mice: a developmental and behavioural evaluation. *Lab Anim* 2010: 1-16.
4. Schaefer, D, Asner, IN, Seifert, B, Burki, K, and Cinelli, P. Analysis of physiological and behavioural parameters in mice after toe clipping as newborns. *Lab Anim* 2009: 1-7.
5. Dahlborn, K, Bugnon, P, Nevalainen, T, Raspa, M, Verbost, P, Spangenberg, E. Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification. *Laboratory Animals*. 2013: 47, 2-11.

Other helpful resources

- Waalkes, MP, Rehm S, Kasprzak, KS, and Issaq, HJ. Inflammatory, proliferative, and neoplastic lesions at the site of metallic identification ear tags in Wistar [CrI:(WI)BR] rats. *Cancer Research*. 1987: 47, 2445-2450.
- Kitagaki, M and Hirota, M. Auricular chondritis caused by metal ear tagging in C57BL/6 mice. *Vet Pathol* 2007: 44, 458-466.
- Kitagaki, M and Shibuya, K. Nylon ear tags for individual identification of guinea pigs. *Contemp Top Lab Anim Sci* 2004: 43, 16-20.
- Stewart, K and Schroeder, VA. *JoVE* 2021.
Rodent Identification 1:
<http://ezproxy.msu.edu/login?url=https://www.jove.com/v/10189/rodent-identification-i>
Rodent Identification 2:
<http://ezproxy.msu.edu/login?url=https://www.jove.com/v/10182/rodent-identification-ii>

PERMANENT RODENT IDENTIFICATION TECHNIQUES

METHOD	AGE	ANESTHESIA REQUIREMENTS	ADDITIONAL INFORMATION
Ear-punching	14 days or older, due to ears being large enough for a biopsy	No anesthesia is required when performed by trained personnel	<ul style="list-style-type: none"> • Punch devices should be disinfected between animals • Tissue can also be used for genotyping • Punched tissue may re-seal; must be re-checked periodically and punching may need to be repeated
Ear-tagging	14 days or older, due to ears being large enough to sustain the affixed tag	No anesthesia is required when performed by trained personnel	<ul style="list-style-type: none"> • Tags are to be placed so they do not cause a bend in the ear or interfere with animal’s mobility • Position ear tag deeply in the concha of the ear; if the tag is placed too closely to the edge of the ear it can easily be torn • Ear tagging can lead to pressure necrosis, ulcerations, inflammation, neoplasia, and infection. These conditions can be exacerbated by improper placement • Site should be disinfected prior to placement to minimize potential for infection. • Ear tag should be monitored regularly after placement • May not be compatible with protocols involving advanced imaging (MRI, CT)

Microchipping	Should be performed on animals >7 days old, preferably performed after weaning.	No anesthesia is required when performed by trained personnel	<ul style="list-style-type: none"> • Similar to that of any other injection with a large bore needle • Increase in discomfort if performed in neonates less than 7 days of age • Site should be disinfected before injection to minimize potential for infection • Some microchips can also measure physiologic data (e.g., mouse body temperature) • May not be compatible with protocols involving advanced imaging (MRI, CT) • May lead to tissue response or neoplasia with prolonged placement
Microtattooing	Neonates	No anesthesia is required when performed by trained personnel	<ul style="list-style-type: none"> • Manual or electrical equipment is commercially available • Site should be disinfected before injection to minimize risk of infection
	Adults	Anesthesia is recommended but not required	
Toe-clipping	Neonatal mice and rats only; preferably between 5-7 days when toes have separated from the paw	No anesthesia is required; topical vapocoolant is not permitted	<ul style="list-style-type: none"> • Ensure only distal bone (P3) and nail bed are removed • May impair grip strength • Tissue can also be used for genotyping • No more than 2 toes per foot (up to 6 toes total) can be removed

APPENDIX

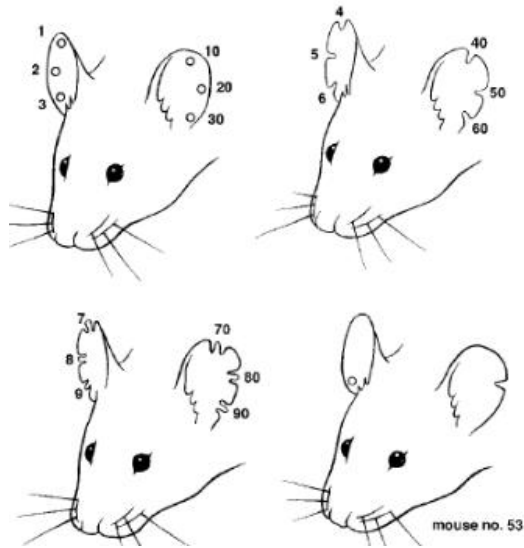


Figure 1. Commonly used numbering scheme for mouse ear punches. From AALAS Learning Library <http://www.aalaslearninglibrary.org>.

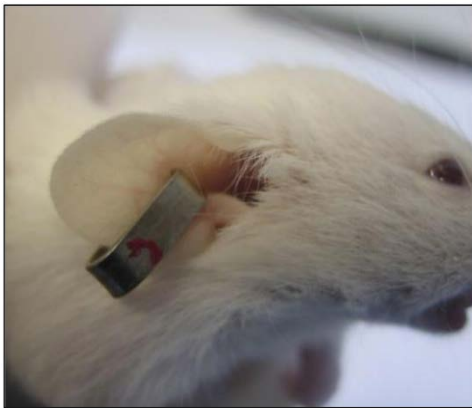


Figure 2. Correct placement of a metal ear tag. (Brown University. Rodent Identification Policy. 2020).

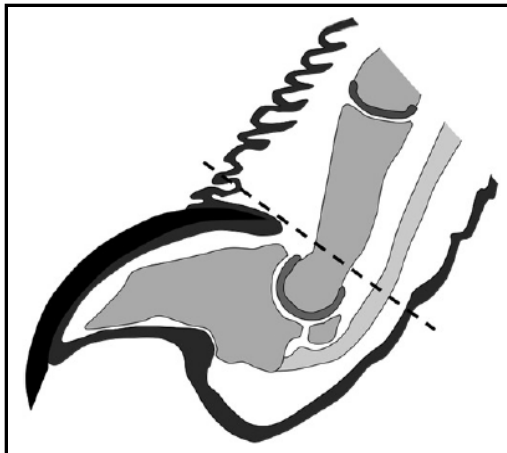


Figure 3. Proper technique for toe clipping requires removal of the entire most distal toe bone (3rd phalanx) and nailbed, which often requires removal of a small portion of the 2nd phalanx. (Adapted from Dahlberg, et al, 2013).