



INDIANA UNIVERSITY

Office of the Vice President for Research
Office of Research Compliance

Institutional Animal Care and Use Committee (IACUC) Office of Research Compliance (ORC)

Identification of Mice and Rats

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Policy Statement

This policy is designed to outline the methods available for identifying rodents. 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. All identification procedures must be approved by the Institutional Animal Care and Use Committee (IACUC). Please contact the LAR office (ext. 52356; Lar@indiana.edu) if training is needed on these techniques.

Reason for Policy

In the conduct of studies utilizing laboratory animals, animals must be identified using the following techniques to ensure their appropriate documentation under a specific protocol, study, housing type, or treatment. Individual identification of animals is essential when animals are co-housed in a single cage or enclosure. Animals experiencing medical issues need to be identified for diagnostic and treatment purposes.

Animals used on a study may need to be individually identified when they are in various phases of a study, used for breeding, treated for a health issue, or being euthanized.

Methods of Identification

1. As outlined in the current *Guide for the Care and Use of Laboratory Animals (Guide)*, every animal within the facility should be identified via a **cage card**. The cage card should include:
 - **names** and **contact information** for the responsible investigator(s)
 - the **strain** or **stock**
 - the **source** of the animal
 - **pertinent dates** (e.g., arrival date, birth date, etc.)
 - **protocol number**
 - **Genotype information**, when applicable, should also be included, and consistent, unambiguous abbreviations should be used when the full genotype nomenclature is too lengthy.

2. **Ear-punching:** Application of a specific combination of small hole punches or notches to the outside edges of a rodent's ear (see Table 1).
 - a. Ear punch or notching instrument is disinfected with alcohol or a hot bead sterilizer between animals to avoid sample contamination.
 - b. Ear notching/punching can also provide tissue for genotyping (*Guidelines for Genotyping*;
<https://research.iu.edu/doc/compliance/animal-care/bloomington/iub-biacuc-guidelines-genotyping-laboratory-mice-rats.pdf>)
 - c. The procedure should be performed **after 2 weeks of age**, when the pinnae (ears) are generally large and thin enough to punch/notch.
 - d. Mouse Ear-punching (see Figure 1a for examples)
 - i. The mouse is restrained by the scruff (see Fig. 1b) and an ear puncher (see Fig. 1c) is used to make holes and/or notches in the ears following an identification chart.
 - ii. Hemostasis of the ear punch/notch site can be achieved by compression.
 - e. Rat Ear-punching (see Fig. 2)
 - i. Firmly restrain the animal. Restraint methods include:
 - The two-handed technique: First place the rat on your arm, holding the base of the tail with your hand. Hug the rat at the shoulders and push gently at the elbows to cross the front legs. Still maintaining your hold at the base of the tail, stretch out the abdomen. Grasp the rear legs and immobilize (see Fig. 2a).
 - If more restraint is needed, the rat may be scruffed by grasping the loose skin behind the neck.
 - A restraining device, which allows access to the head while still immobilizing the animal, may also be used. An ear puncher is then used to make holes and/or notches in the ears (a second person will be needed to do this).
 - ii. Ear puncher (see Fig. 2b) is used to make holes and/or notches in the ears following an identification chart.
 - iii. Hemostasis of the ear notch/punch site can be achieved by compression.

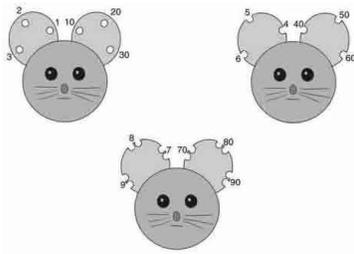


Fig. 1a Mouse ear punch



Fig. 1b Mouse restraint



Fig. 1c Mouse ear punches



Fig. 2a Rat restraint

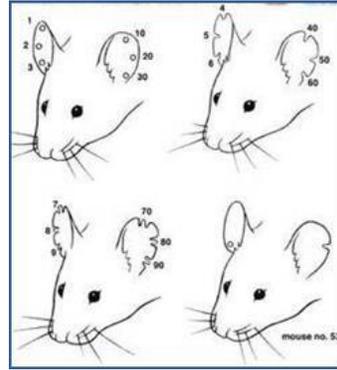


Fig. 2b Rat ear punches

3. **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 Table 1)
 - a. Ear tags should be washed in alcohol or disinfectant to help prevent infection to the ear. Disinfect the ear before placement of the tag.
 - b. Place the ear tag in the ear tag applicator, keeping the "hole" side with the identification number flat against the jaw of the ear tag applicator (Fig. 3a).
 - c. Locate the proper position for the tag on the ear, applying within the ring of cartilage (Fig. 3b).
 - d. After crimping, be sure the tag point has come through the hole and is bent over, securing the ear tag properly (Fig. 3c).



Fig. 3a- Tag applicator



Fig 3b- Placing tag



Fig. 3c- checking tag



Fig. 3d- tags

4. **Microchipping:** Injection of a small microchip transponder subcutaneously (sc) between the shoulder blades of the rodent. The microchip is read by the use of a scanner. (See Table 1)
 - a. Microchips are implanted sc between the scapula for permanent identification.
 - b. Each microchip is encrypted with a unique, non-replicable number.

- c. Should be performed in **animals > 7 days old or after weaning** since it can be painful in neonatal rodents.
- d. The chips are read with a portable, hand-held scanner.
- e. To implant these chips, the mouse may be briefly anesthetized to allow for more accurate chip placement. The hair is removed from the insertion site by shaving (Fig 4a).
- f. The area is prepped with an antiseptic (iodophor, chlorhexidine) followed by alcohol (Fig 4a).
- g. The implantation needle, with the syringe attached, is purchased in a sterile package. Make a tent from the loose skin at the implant site (Fig 4b).
- h. Insert the needle subcutaneously, with the bevel up, and depress the plunger.
- i. Pinch the skin as the needle is removed. The injection site should be observed for bleeding. If bleeding is noted, digital pressure with a sterile gauze pad should be applied for several minutes. If necessary, a drop of surgical glue can be applied to the needle entry site (Fig 4c).



Fig. 4a Clipping fur and cleaning

Fig. 4b Injecting chip

Fig. 4c Sealing with glue

5. **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. (See Table 1)
 - a. Micro-tattooing can be used on both neonates and adults as a permanent method of identification.
 - b. Anesthesia is not required, but can be used to immobilize the animal.
 - c. There are several types of animal micro-tattooing equipment. The Aramis® tattoo system, <http://www.braintreesci.com/prodinfo.asp?number=MTK>, <https://www.youtube.com/watch?v=UFoClcINieI>, is a mechanical device that can be used to write numbers or other characters on the tails of adult mice or tattoo the footpads of adult or neonatal mice. Training should be obtained on the specific micro-tattooing system.
 - d. Another micro-tattooing system is made by AIMS. (<https://animalid.com/specialized-lab-equipment/aims-tattoo-machine-platform>) AIMS provides a course and certification program. Consult a LAR veterinarian or equipment manufacturer for further instructions on performing micro-tattooing.
 - e. It is important to prevent potential cross contamination associated with the use of this equipment.
 - f. With the AIMS system, needles should be disinfected prior to use.
 - g. For the Aramis system, needles should be discarded after use. For both systems, excess ink should be discarded after use.
 - h. The micro-tattooing apparatus and other supplies should be ethylene oxide sterilized, cold sterilized, or steam sterilized before use between groups of animals with the same pathogen status.



Fig. 5a. Tail tattoos



Fig. 5b. Micro-tattoo equipment

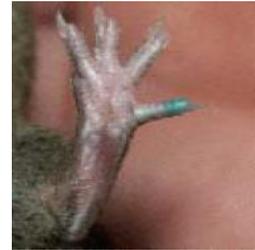


Fig. 5c. Toe tattoo

6. **Toe-clipping or toe amputation:** Procedure in which the most distal bone of the toe (3rd phalanx) is removed.
- As a method of identification of small rodents (mice and rats), toe clipping is best performed on neonates 5-7 days of age. Mice (not rats) may be toe-clipped up to 13 days of age.
 - Written justification for toe-clipping must be approved by the IACUC before the procedure can be performed. The number of toes that will be clipped per animal must be included in the justification.
 - Day 5-7 (mice and rats): No more than 2 toes per foot should be removed, with a maximum of 6 total clipped toes per animal.
 - Day 8-17 (mice): No more than 2 toes (total) can be removed.
 - (Guidelines for Genotyping;*
<https://research.iu.edu/doc/compliance/animal-care/bloomington/iub-biacuc-guidelines-genotyping-laboratory-mice-rats.pdf>)
7. **Non-permanent methods of identification:** These methods of identification include fur-clipping or the application of non-toxic fur dyes (please anesthetize the animal before dyeing) or permanent marker. While non-invasive, these techniques are temporary and are only appropriate for short term identification purposes.

Table 1. Methods Used to Identify Rodents

Method	Age	Anesthesia Requirements	Additional Information
Ear-punching	14 days or older	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 rechecked periodically and punching may need to be repeated after 2 weeks of age.
Ear-tagging	14 days or older	No anesthesia is required when performed by trained personnel	<ul style="list-style-type: none"> Ear tagging can lead to pressure necrosis, ulcerations, inflammation, neoplasia and infection. These conditions can be exacerbated by improper placement. Ear and tags 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	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. Increased in discomfort if performed in neonates less than 7 days of age. Site should be shaved and disinfected before

	after weaning		<ul style="list-style-type: none"> injection to minimize potential for infection. Some microchips can also measure physiologic data (i.e., Mouse body temperature). May not be compatible with protocols involving advanced imaging (MRI, CT). May lead to tissue response or neoplasia with prolonged placement.
Micro-tattooing	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. Can be performed on tail, toes, and footpads. Disinfect or use new needle between animals.
	Adults	Anesthesia is recommended but not required	
Toe-clipping	Rats from 5-7 days old Mice from 5-13 days old	No anesthesia is required when performed by trained personnel	<ul style="list-style-type: none"> Equipment and site should be disinfected prior to clipping to minimize risk of infection. Ensure only distal bone (P3) and nail bed is removed. May impair grip strength. Tissue can also be used for genotyping. Day 5-7 (mice and rats): No more than 2 toes/foot (up to 6 toes total) can be removed. Day 8-17 (mice): No more than 2 toes total can be removed.
Non-permanent marking	Any age	Only for dyeing	<ul style="list-style-type: none"> Inexpensive but not permanent. Use nontoxic dyes or markers.

Sanctions

Failure to comply with IACUC policies may result in noncompliance reports to the Institutional Official, the Office of Laboratory Animal Welfare (OLAW), the U. S. Department of Agriculture (USDA), and/or the suspension of animal use privileges. In addition, the availability of sponsored research funds may be affected when an investigator is found to be in violation of these policies.

Contacts

Subject	Contact	Phone	Email
Veterinary Concerns	LAR Veterinarians	855-2356	lar@indiana.edu
Policy	IACUC Administrator	855-5138	biacuc@indiana.edu

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