

Science Education Collection

Rodent Identification II

URL: <https://www.jove.com/science-education/10182>

Overview

Source: Kay Stewart, RVT, RLATG, CMAR; Valerie A. Schroeder, RVT, RLATG. University of Notre Dame, IN

Animal records must be accurately maintained to ensure that data collection is correct. Records range from maintaining information on cage cards to having a detailed database with all of the relevant information on each animal. The primary component of recordkeeping is the individual identification of research animals. There are a variety of methods suitable for identifying mice and rats. This video describes the procedural techniques for tattooing, microchip placement, and temporary identification methods, and also explores the benefits of each.

Principles

The use of tail tattooing is beneficial in many research protocols. Tail tattoos are ideal for an animal that is to be imaged in an MRI machine, or one that is genetically predisposed to ulcerative dermatitis. For quick identification of an animal in a cage, without having to restrain it, the tail tattoo is easily visualized. However, this technique requires skill, practice, and a steady hand; specialized equipment is also required.¹

Experimental protocols may require the genotyping of neonates as early as day one. As a result, it is essential that these pups be permanently identified. At this young age, the ear pinnae are not developed enough to insert an ear tag, and an ear punch cannot yet be used on them. In the past, it was common for a researcher to use a toe removal code to identify neonates. However, this was stressful to both to the young animal and the dam, and thus has been considered inhumane.² The use of toe tattooing allows the animals to be permanently identified until they are large enough for ear tagging or ear punching.³

Although the process for implantation of a microchip is relatively easy, there are several factors that limit its usefulness for identification in mice. The sterile microchips are the size of a large grain of rice, and require a 10 to 12 gauge needle for delivery. Thus, they are very large for a mouse. The cost of microchips and the reading device can also be prohibitive, as the life span of the mouse is short and the number of animals is usually extensive. Animals that are destined to be imaged in an MRI machine cannot have implants in them. However, if there is a group of valuable animals that must be permanently identified, microchips can be used.⁴

Acute studies that require animals to be identified for hours, or a few days, do not necessitate the use of permanent identification. Nontoxic markers are made specifically for coloring the fur of animals, and are available in several colors. These marker dyes can remain visible for several weeks. The markers can also be used in conjunction with other methods to easily spot specific animals in a group.

Procedure

1. Tail tattoo

Tail tattoos are easily read without the need to handle the animal.



Figure 1. Tail tattooing on adult mice

1. Prepare the tattoo equipment according to the manufacturer's instructions. A small amount of ink is required. Black dye should be used for albino mice or rats, and green dye for pigmented mice or rats.
2. Prepare the animals for identification.

NOTE: To minimize apprehension or distress, tail tattooing should be performed in a procedure room rather than in an animal housing area.

1. Appropriately restrain the animal for tattooing so that movement is minimal.
 2. Weanling rats are restrained using a plastic-notched cup, with the lip of the cup secured under the bolts on the tattoo platform, and the tail extending through the notch.
 3. Adult mice can be restrained by means of a cup restrainer, with the tail pulled taut from the opening and the flange of the cup held in place via a platform or other device.
 4. Clean the tail with a small amount of diluted tissue cleaner applied with a cotton-tipped swab. Adult rats generally have a buildup of scale and debris on the tail that must be cleaned prior to applying the tattoo. A soap and water scrub of the tail will remove the majority of the dirt and debris. Dry the tail well before proceeding.
 5. Apply tissue oil to the skin with a cotton-tipped swab just prior to tattooing. The tissue oil is necessary to minimize tissue damage by softening the skin and lubricating the tattoo needle. In addition, the oil dissolves any dirt not removed by the cleaning process and prevents ink staining of nontattooed skin.
3. Apply the tattoo.
 1. Dip the tip of the needle into the pigment with the machine off.
 2. Bring the tip of the needle to the tattoo site with the gun activated by the foot pedal.
 3. Make short, even strokes in one direction. Numerals and letters should be tattooed in such a way so that they are constructed in segments with minimal angles and curves (when possible).
 4. Penetrate deeply enough into the dermis with the needle for permanent pigment deposition. A change in the sound of the activated needle can help discern the depth.
 5. Approach the skin with the needle at a 90° angle for adequate deposition of pigment.
 4. Post-tattoo cleanup and care
 1. Blot (do not wipe) the completed tattoo of excess pigment with a paper towel.
 2. The presence of blood on the tattoo or the paper towel indicates that the tattoo was made too deep, and is therefore unlikely to be permanent.
 3. Reinforce thin areas of tattoo characters with additional pigment. Characters are reinforced with pigment deposited parallel to, but not on top of, already-deposited pigment.
 4. To add additional pigment, dip the tip of the needle into the dye reservoir for every two characters for mice and every one character for rats.
 5. Reasons for fading tattoos include inadequate amounts of pigment, tattoos made too shallow, tattoos made too deeply, an inappropriate angle of the needle to the skin, blunt or hooked needles being used, or the tattoo being made too quickly.
 6. Thoroughly clean the tattoo equipment after each use according to the manufacturer's instructions.

2. Toe tattooing of neonates

Experimental protocols can require the genotyping of neonates as early as day one, which makes it essential that these pups be permanently identified. The use of toe tattooing allows the animals to be identified until they are large enough for ear tagging or ear punching.

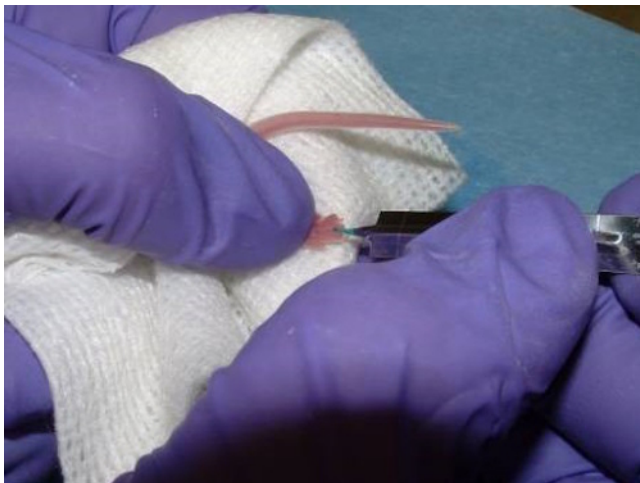


Figure 2. The proper restraint technique for tattooing a neonate mouse. The lancet has the green dye on the tip.

1. Restrain preweaned pups.
 1. Cup the neonate in the hand on a gauze pad. Position the foot between the thumb and crook of the index finger to expose the chosen toe, or toes, for tattooing.
 2. Hold the foot close to the toes so that the toe has a solid surface behind it, preventing it from bending away.
 3. Do not overtwist the leg when positioning the foot. Pups that are restrained may wiggle, but no vocalization is necessarily heard.
2. Prepare the equipment.
 1. Select a 4.5 mm Goldenrod animal lancet.
 2. Place a small spot of green tattoo paste on a nonporous surface; aluminum foil works well.

3. Dip only the tip of the lancet in the tattoo paste.
4. Use only a small amount of paste.
3. Apply the tattoo.
 1. Poke the desired toe with the Goldenrod lancet according to an identification code.
 2. Puncture the skin to introduce the paste into the skin, leaving a mark.
 3. Poke the same spot three times to insure proper penetration.
 4. Avoid poking so deeply that the toe bleeds; this can result in a fainter tattoo.
4. Post-tattoo cleanup and care
 1. Gently blot the spot with an absorbent wipe/towel to remove excess paste.
 2. Do not attempt to clean excess paste from the foot or body.
 3. Return the animal to its cage.
 4. When nursing pups are tattooed, it is advisable to also tattoo the dam. This may prevent her from overreacting to the paste on the pups, which could lead to excessive grooming of the tattoo.
 5. Use a new lancet for each litter.
 6. The tattoos should be verified on the day after the procedure. Redo the tattoo if the dots are faint or not visible. If the dots are visible the day after tattooing, they should remain visible for the lifetime of the animal.

3. Toe tattooing of adult rats and mice

When a litter of neonates is tattooed, it is suggested that the mother also be tattooed so that the paste on her pups is not foreign to her.

1. Restraint of the animal.
 1. Restrain the animal using either a plastic cone or a Plexiglass restrainer that allows access to the hind feet.
 2. Extend the foot from the restraint device.
 3. Position the foot between the thumb and crook of the index finger to expose the chosen toe, or toes, for tattooing
 4. Hold the foot close to the toes so that the toe has a solid surface behind it, preventing the toe from bending away.
 5. Do not overtwist the leg when positioning the foot. Adults that are restrained may try to withdraw the foot.
2. Prepare the equipment.
 1. Select a 5 mm Goldenrod animal lancet.
 2. Place a small spot of green tattoo paste on a nonporous surface; aluminum foil works well.
 3. Dip only the tip of the lancet in the tattoo paste.
 4. Use only a small amount of paste.
3. Apply the tattoo.
 1. Poke the desired toe with the Goldenrod lancet according to an identification code.
 2. Puncture the skin to introduce the paste into the skin, leaving a mark.
 3. Poke the same spot three times to insure proper penetration.
 4. Avoid poking so deeply that the toe bleeds; this can result in a fainter tattoo.
4. Post-tattoo cleanup and care
 1. Gently blot the spot on an absorbent wipe/towel to remove excess paste.
 2. Do not attempt to clean excess paste from the foot or body.
 3. Return the animal to its cage.
 4. Use a new lancet for each animal.
 5. The tattoos should be verified on the day after the procedure. Redo the tattoo if the dots are faint or not visible. If the dots are visible the day after tattooing, they should remain visible for the lifetime of the animal.

4. Microchipping

The implantation of an RFID chip is a commonly used method for identifying animals.

1. Load the microchip into the applier, or select a preloaded syringe.
2. Restrain the animal using a Plexiglass restraint tube that allows grasping of the skin over the shoulders.
3. Tent the skin, creating a pocket.
4. Place the needle through the skin, bevel up and parallel to the spine, and directed it toward the tail.
5. Eject the microchip subcutaneously.
6. Withdraw the needle and pinch the skin closed at the point of injection. This prevents the microchip from following the needle out of the skin. Continue to apply pressure to provide hemostasis for any skin bleeding.
7. Remove the animal from the restraint device and scan the chip to confirm the identification code.



Figure 3. Microchip placement in adult rats.

5. Temporary identification for mice

Nontoxic dyes and animal markers can be used for temporary identification of mice.

1. Restrain the mouse such that the area between the scapulae is accessible for markings on the fur.
2. Place a marker, spot, or streak of dye on the fur in this area. This region is used to prevent the animal from removing the marking while grooming.
3. Check the marking periodically if using it as the sole identifier for more than a few days.
4. The tail can also be used for short durations. However, the animal may remove a mark from it while grooming.

6. Temporary identification for rats

1. Restrain the rat so that the area for dye or marker application is easily accessible. In some cases, when only a stripe or dot is needed, the animal will not need to be restrained but is allowed to move freely in the cage.
2. Manual restraint by grasping the tail and holding it taut while applying the identification is commonly done.
3. Temporary immobilization using inhalant anesthetics can be done for fractious animals.
4. Check the marking periodically if using this method as the sole identifier for more than a few days.

Summary

When choosing the appropriate identification method, many factors must be deliberated. Each technique has advantages and disadvantages that must be considered in relation to experimental needs. While the costs must be weighed along with other factors, the ease of the technique and the level of discomfort to the animals should be the primary considerations.^{4,5}

References

1. Robinson, V., Morton, D.B., Anderson, D., Carver, J.F.A., Francis, R.J., Hubrecht, R., Jenkins, E., Mathers, K.E., Raymond, R., Rosewell, I., Wallace, J., and Wells, D.J. 2003. Refinement and reduction in production of genetically modified mice. *Laboratory Animals*. 37:S1-S50.
2. Schaefer, D.C., Asner, I.N., Seifert, B., Bürki, K., and Cinelli, P. 2010. Analysis of physiological and behavioural parameters in mice after toe clipping as newborns. *Laboratory Animals*. 44:7-13
3. Castelhana-Carlos, M.J., Sousa, N., Ohl, F., and Baumans, V. 2010. Identification methods in newborn C57BL/6 mice: a developmental and behavioural evaluation. *Laboratory Animals*. 44: 88-103.
4. Danneman, P.J., Suckow, M.A., and Brayton, C.F. 2013. The laboratory mouse. Second edition. New York, NY: CRC Press.
5. Institute for the Laboratory Animal Research. 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.