
Rodent Identification

The intent of this standard operating procedure (SOP) is to describe methods that can be used to identify rodents- mice, rats, guinea pigs. This SOP is intended for use by Principal Investigators (PIs), research staff, NUS Institutional Animal Care and Use Committee (IACUC) and Comparative Medicine (CM). This SOP is approved by the NUS IACUC. Any deviation must be approved by the IACUC in advance.

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1. INTRODUCTION

The National Advisory Committee on Laboratory Animal Research (NACLAR) Guidelines requires research animals to be adequately and appropriately identified.

Animal identification is a necessary component of a research project. It allows monitoring of an animal throughout the course of a study and also assists animal care staff in providing appropriate care to individual animals.

The ideal identification method should provide reliable individual identification, has no adverse effects on the animal or the animal model and be technically easy to apply.

2. MATERIALS

- a. Cage cards
- b. Identification specific materials (e.g., ear tags, ear punch, microchips, tattoo machine)
- c. Anesthesia and analgesia
- d. Disinfectant (e.g alcohol, povidone iodine)

3. PROCEDURES

See Appendix A for recommended choice of identification method depending on age of animals.

a. Non-invasive Methods

i. Cage Cards – Required for all rodents

1. Cage card information must include: species, strain or stock, source of animal, date of birth of animal, contact number of responsible person to be notified in case of a problem, PI name, and protocol number
2. The cage card is a sufficient method of identification for:
 - a. Individually housed or a breeding pair
 - b. Groups of rodents on protocols where individual identification is not necessary

ii. Temporary Markings

1. Use an indelible (permanent) non-toxic marker to write numbers, bars, or other distinguishable markings on the ears or tails
2. Temporary markings last up to 3-4 days.
3. Repeat markings can be made every 3-4 days as necessary.

iii Shaving or cutting the fur

1. Use a fur shaving machine or a pair of scissors.
2. An area on the body, mainly on the back (for visibility without handling), is shaved or haircut.
3. Use this method when pups have acquired a full coat of fur, after approximately two weeks of age.

b. Invasive Methods

Invasive identification methods below are required to be described in the research protocol and should only be done by trained personnel.

i. Ear Tags

1. Use tags that are about 5 mm long marked with a predetermined code.
2. Rinse tag in 70% alcohol before use to help prevent ear infection.
3. Place the tag in the applicator so that the holed end of the tag is positioned over the notched area of the applicator. The pointed end should be opposite the hole.
4. Scruff the animal so that the ears are easily accessible.
5. Place the ear pinna between the point and the hole of the tag. The numbers should be in an upward configuration so that they can be easily read without restraining the animal.
6. The tag should be positioned on the lower edge of the pinna. See Appendix B Figure 1.
7. Once the tag is positioned correctly, squeeze the applicator firmly to apply the tag.
8. Monitor the tag implantation site intermittently for signs of local infection or inflammation (redness, swelling, bleeding).

ii. Ear Notch/Ear Punch

1. Restrain the animal by the scruff and use the ear punch to create holes and/or notches in the ears, following an identification chart. See Appendix B Figure 2 for example
2. Whenever possible, use a simple code to limit the number of notches/punches.
3. If possible, use the excised tissue as a sample for genotyping to replace the need for a tail biopsy.

iii. Microchips

1. Use appropriate anesthesia and analgesia during implantation. See CM SOP 101: Anesthetic Regimens for Mice, Rats, and Guinea pigs and CM SOP 104: Rodent Analgesia, for further details.
2. Test the microchip before implanting it into the animal by scanning it with a compatible reader while it is still in the package. If the chip fails to read do not implant it.
3. Clip the fur and apply disinfectant to the area (e.g., chlorhexidine, povidone iodine).
4. Implant microchips subcutaneously in the dorsal neck area. Chips vary in size, the larger chip 12x2mm is most suitable for animals ≥ 50 g and should not be used before adulthood in mice. Smaller chips 6x1mm are more suitable for mice. See Appendix B Figure 3.
5. Using the reader, test the chip again after implanting to confirm proper function. It should match the number in the initial scan.

iv. Tattoos

This procedure is easier to perform under general anesthesia. If not using general anesthesia, a local anesthetic such as EMLA cream or a local anesthetic spray should be applied before tattooing. See CM SOP 101: Anesthetic Regimens for Mice, Rats, and Guinea pigs and CM SOP 104: Rodent Analgesia, for further details.

1. Toe tattoo
 - a. Mice less than 14days old- cup in hand with sterile gauze or some of the nesting materials from the cage
 - b. Mice 14days or older- use a sterilized restrainer or anesthetic so the foot can be immobilized.
 - c. Place the foot, pad side up on a stable sterile surface, be careful not to over twist the leg
 - d. Load a small amount of tattoo paste on to the tip of the lancet/needle .
 - e. Pierce the pad of the toe three times in the same spot
 - f. Using alcohol wipe gently wipe off any excess paste
 - g. Do not wipe vigorously

h. Follow a toe numbering map to uniquely identify each animal

2. Tail Tattoo

Tattooing on the tail can be performed in two different ways: the micro-tattoo system or the electric tattoo equipment (similar to that used in humans).

- a. Micro-tattoo system should only be applied in young animals before the ossification of the tail (tail ossification occurs between 2 and 3 weeks of age) since it is inserted completely through the tail.
- b. Tail tattooing with an electric machine can be performed on adult rodents of all ages if one wishes to write digits or letters. It can also be used in young mice to imprint dots or stripes on the tail.
 - i. applied ink on the skin and the tattoo needle
 - ii. transfer the ink into the skin layers
 - iii. remaining ink is gently wipe off

An alternative to the electric tattoo machine is the use of a lancet/needle. It is done manually by making puncture/s, followed by rubbing tattoo ink into the area. Whenever possible, use a simple identification code to limit the number of tattooed areas.

Note: Toe amputation/ clipping is not acceptable method of animal identification.

4. REFERENCES

- Report of the FELASA working group on animal identification (2013): *Laboratory Animals* 2013; 47: 2–11. DOI: 10.1177/002367712473290
<http://lan.sagepub.com/content/47/1/2.full.pdf+html?sid=96cdcfee-80b2-4a62-9c87-ed786b72d890>
- *Cornell University Institutional Animal Care and Use Committee. ACUP 552: Mouse Identification* <https://www.research.cornell.edu/care/documents/ACUPs/ACUP552.pdf>
- CM SOP 101 Anesthetic Regimens for Mice, Rats, and Guinea pigs
- CM SOP 104 Rodent Analgesia
- Identification of GM Mice. *Lab Anim* July 2, 2003 37: 33-35, doi:10.1258/002367703766452886
http://lan.sagepub.com/content/37/suppl_1/33.full.pdf+html
- Ear Punching: <http://www.bu.edu/orc/policies-procedures/animal-care/ear-punching/>
- John Hopkins University. Toe Tattooing Procedure by Kinta Diven

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.01	Anna Acuna	15 June 2015	570.01

5. APPENDIX

Appendix A

Choice of identification method depending on age of mice

	Less than 2 weeks	3–4 weeks	Over 4 weeks
Non Invasive methods	√	√	√
Ear Notching		√	√
Ear tags		√	√
Microchips		√	√
Tattoos	√	√	√

Reference: Laboratory Animals (2003) 37 (Suppl. 1)

Appendix B

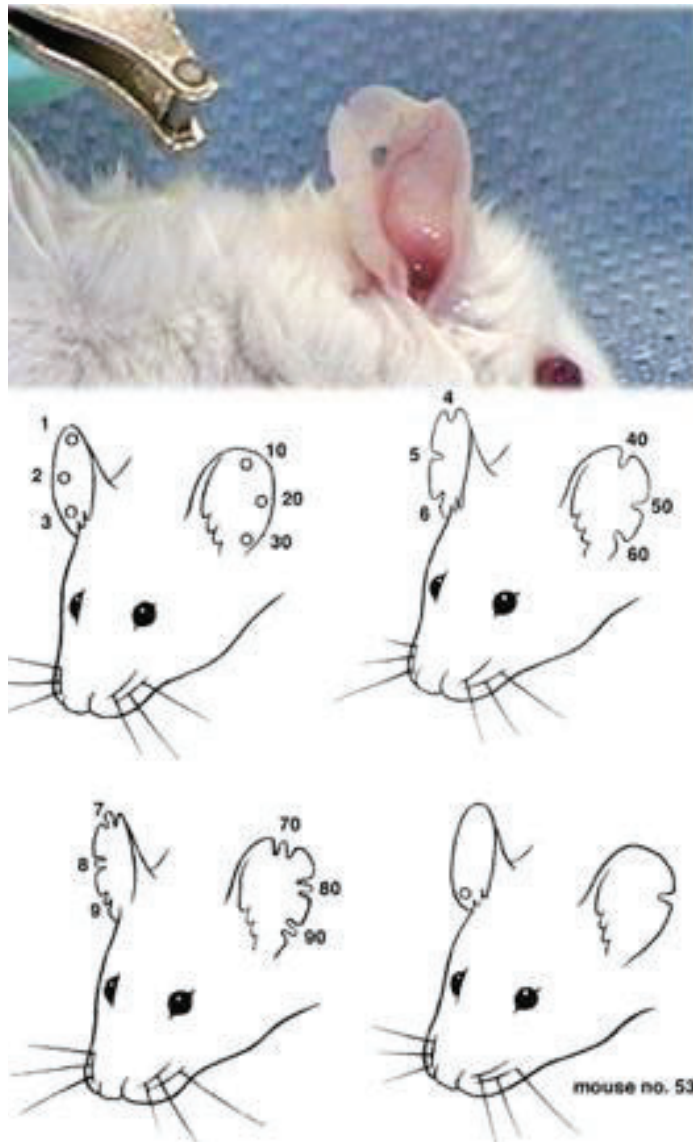
Figure1



Tag placed correctly on the lower edge of the pinna

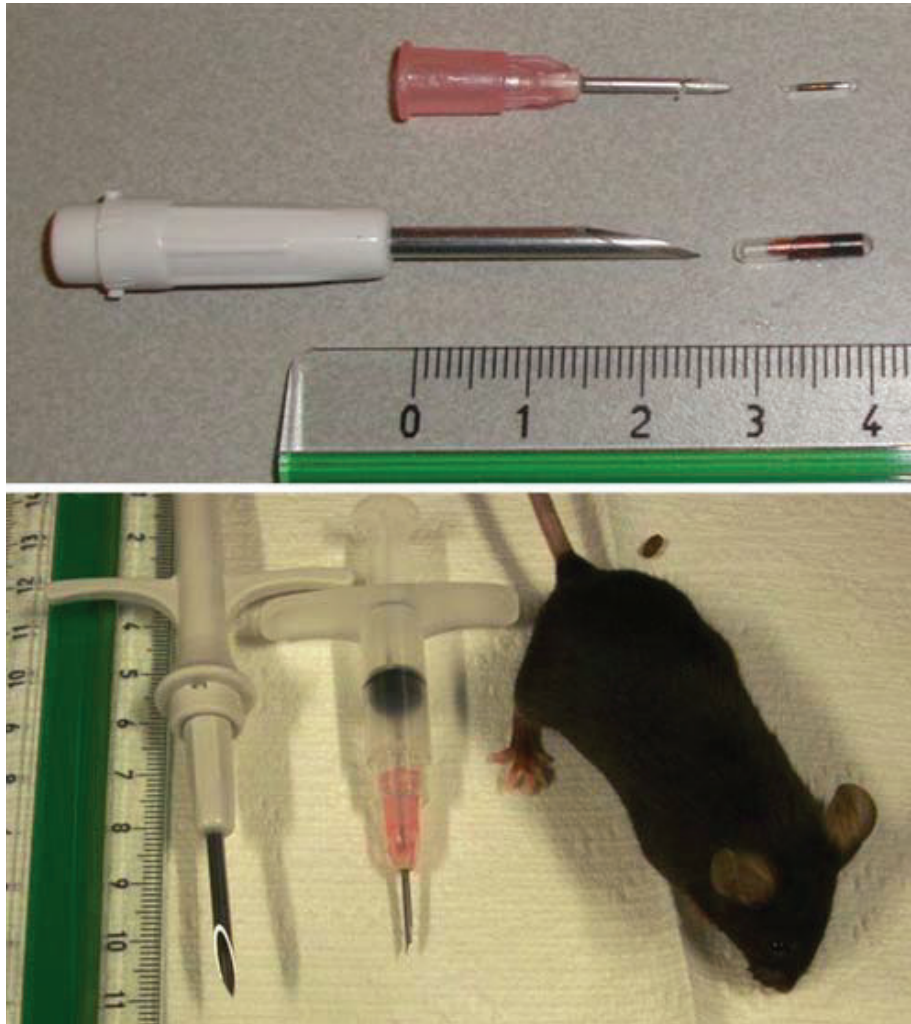
Source: Laboratory Animals Volume 4,7 January 2013

Figure 2



Source: <http://www.bu.edu/orc/policies-procedures/animal-care/ear-punching/>

Figure3



Size of microchips suited to mice (1 X 6 mm) and rats (2 x 12 mm)
(top picture) and the size of the needles (12G, to the left and 18G, to the right)
in relation to a fully grown C57BL mouse (bottom picture)

Source: Laboratory Animals Volume 4,7 January 2013