

SOP: Rodent Identification

These SOPs were developed by the Office of the University Veterinarian and reviewed by Virginia Tech IACUC to provide a reference and guidance to investigators during protocol preparation and IACUC reviewers during protocol review. They can be used as referenced descriptions for procedures on IACUC protocols. However, it is the sole responsibility of the Principal Investigator to ensure that the referenced SOPs adequately cover and accurately represent procedures to be undertaken in any research project. Any modification to procedure as described in the SOP must be outlined in each IACUC protocol application (e.g. if the Principal Investigator plans to use a needle size that is not referenced in the SOP, simply state that alteration in the IACUC protocol itself).

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I. Procedure Summary and Goal

Describes procedures for individual identification of rodents.

Considerations

- a. Individual identification of rodents plays a critical role in accurate record keeping, tracking an animal throughout a project, and also assists the animal care and veterinary staff in providing appropriate care to the correct animal or animals.
- b. Temporary and permanent methods of animal identification can be used to individually identify animals within a cage.
- c. Selection of an appropriate identification method depends on the age of the animal, duration of studies, and number of animals to be included.
- d. Ear punch and toe clip can also provide tissue to be used for genotyping.
- e. The method of identification must be described in the IACUC protocol.

II. Personal Protective Equipment and Hygiene

- a. Ensure appropriate PPE is used to protect technician from accidental exposure to blood and other body fluids, such as:
 - i. Gloves
 - ii. Eye protection
 - iii. Mask
 - iv. Other PPE as required by protocol/facility
- b. Hands should be washed and/or gloves changed between animals.
- c. Promptly dispose of used sharps in the provided leak-proof, puncture resistant sharps container.

III. Supply List

- a. Dependent on procedure; see individual procedure

IV. Detailed Procedures

- a. **Cage Cards**
 - i. *Every cage* must have a cage card with the following information: Principal Investigator, IACUC approved protocol number, date of birth, sex of animals, species and strain, and the source of the animals.
- b. **Indelible Ink/Permanent Marker**
 - i. Used as a temporary method of identification (24 – 72 hours maximum)
 - ii. Use only non-toxic markers
 - iii. Restrain mouse with gloved hands by scruffing.
 - iv. Place markings at the base of the tail in a pre-determined pattern.
- c. **Hair Clipping**
 - i. Used as a temporary method of identification (3-10 days maximum)
 - ii. Restrain mouse with gloved hands by scruffing.

d. Ear Punch or Notch

- i. Simple, inexpensive and permanent method of identification.
- ii. Ears must be big enough for the punch
 - This is generally 14 days old.
- iii. Numbering system may be difficult to read and discern.
- iv. Ear notch or punch tissue remnants may provide enough DNA for genotyping.

Punch Procedure

- i. Gently restrain mouse with gloved hands by scruffing the dorsum high on the neck to control both the body and the head.
- ii. Quickly depress a sterile ear punch instrument to entirely punch through the pinna (external ear).
 - Punches should be made on the outer 1/3 of the pinna.
 - Do not punch too close to the head where the cartilage is thicker and more blood vessels are present because it is painful and is more likely to bleed.
 - The ear punch instrument should be sterile when beginning and re-sterilized after every 5 animals or between each animal if genotyping is to be performed.
- iii. Remove the instrument and repeat until the identification pattern is complete.
- iv. Any bleeding should be immediately controlled by gentle fingertip pressure.
- v. Punches may be placed on both ears in a pattern that correlates to a numbering system.

Notch Procedure

- i. Gently restrain mouse with gloved hands by scruffing the dorsum high on the neck to control both the body and the head.
- ii. A small wedge-shaped notch can be created on the outer edge of the pinna (external ear) using sterile, very sharp scissors.
- iii. The amount of tissue removed should be kept to a minimum. A general guideline is a small wedge of tissue not wider than 2-3mm at the ear edge and 2-3 mm in length.
 - Scissors and forceps should be sterilized and re-sterilized after every 5 animals or between each animal if genotyping is to be performed.
- iv. Bleeding should be immediately controlled by gentle fingertip pressure.
- v. Notches may be placed on both ears in a pattern that correlates to a numbering system.

Potential Adverse Events, Mitigation, or Treatment

- i. Ear punches may regrow or be torn
- ii. Control bleeding by applying light pressure to the pinna

e. Tattoos

Toe/foot tattoo

- i. Can be done at any age without anesthesia.
- ii. Extensive numbering system may be achieved.
- iii. Identification is permanent.
- iv. May be difficult to read.
- v. Procedure
 - Take a small gauge (25-27g) needle and dip it in tattoo ink [India ink will work] and make small dot(s) in their tails, feet and /or ears by introducing needle tip into skin along with ink.
 - Using tattoo ink of various colors enables you to make up a legend based on colors of dots, not just numbers/location of dots.

Tail tattoo

- i. Can be done at any age without anesthesia.
- ii. When done properly, the tattoo is permanent.
 - May be difficult to read.
- iii. Procedure
 - A tattoo machine with a fresh needle is used according to manufacturer's instructions.
 - Needle is replaced when dull or about every 50 animals.
 - The needle is dipped into tattoo ink then applied with firm pressure to the surface of the tail.

f. Metal Ear Tags

- i. Inexpensive
- ii. Anesthesia not required
- iii. Extensive numbering system available
- iv. Procedure
 - Soak ear tags in alcohol or other disinfectant prior to application.
 - Place tag appropriately into applicator device.
 - Gently restrain mouse with gloved hands by scruffing the dorsum high on the neck to control both the body and the head.
 - Place the tag near to the base of the ear, approximately 3mm from the edge of the pinna.
 - Apply tag
 - Be sure that the tag point has come through the hole and bent over to secure the tag properly.
- v. *Potential Adverse Events, Mitigation, or Treatment*
 - Apply at ≥ 21 days
 - Due to the weight of the tags, animals are typically tagged at weaning or older.
 - May be ripped out by mouse
 - Generally only last ~ 6 months
 - Bleeding should be controlled by gentle pressure to the pinna.

g. Microchips

- i. Extensive numbering system
- ii. Permanent identification
- iii. Expensive and must have chip reader
- iv. Implant at > 21 days of age
- v. Procedure
 - Test chip with reader, record chip number.
 - The animal must be restrained.
 - Anesthesia is recommended but not required.
 - Place sterile microchip into sterile trocar/dispenser and introduce subcutaneously in the scruff area (see SOPs for subcutaneous injections).
 - Depress the plunger
 - Observe injection site for bleeding; apply digital pressure with sterile gauze pad until bleeding stops.
 - Test that the chip is still detectable by the reader.

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- Return the animal to its cage and observe for bleeding, or signs of pain or distress.

h. Toe Clipping

- i. May be difficult to perform due to small size of toes.
- ii. Must be done ≤ 7 days of age.
- iii. Guidelines below are specifically for identification of mice (*Mus musculus*) in the laboratory setting.
- iv. May be used as a method of identification and toe tissue can be used for genotyping under the following conditions:
 - Only acceptable when the study requires that mice be identified at a very young age.
 - Must provide scientific justification.
 - Only performed on mice between 5 - 7 days of age.
 - Remove only the most distal bone of digit.
 - Do not remove the dew claw.
- v. Procedure
 - No more than 2 digits per extremity may be amputated - include numbering scheme in protocol documents.
 - Aseptic technique must be used – clean foot with 70% ethanol or betadine prior to amputating digit.
 - Using sharp, sterile scissors, cut the joint between the middle and distal phalanx. Only the distal phalanx of the digit is to be removed.
 - After removing the digit apply gentle pressure until hemostasis occurs.
 - Use silver nitrate or quick stop powder if bleeding continues.
 - Monitor mice with amputated toes at 24 hours and 72 hours post-amputation.