| Institutional Animal Care & Use Program - UTEP | |
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| **Title**: Rodent Identification and Genotyping | |
| **Policy#**: 002 | **Date in Effect**: 24 October 2014 |
| **Version #:** B | **Rev Date**: 18 November 2019<br>24 February 2025 |
| **In Effect** ☒ **Rescinded** ☐ | **Date Rescinded**: |

A) RESPONSIBILITIES

It is the responsibility of all personnel using animals at The University of Texas at El Paso (UTEP) to abide by this policy.

B) APPLICATION

This policy applies to all mice and rats used in research and teaching at UTEP.

C) BACKGROUND

Individual animal identification is important for colony management (to track breeding crosses and genotype, etc.), for surgical and medical records, and for tracking individualized data of animals on chronic studies. There are several methods available for identifying rodents, including ear punches/notches, ear tags, tattoos, and microchip transponders. Ear punching can also provide tissue for genotyping.

Genotyping of genetically modified rodents is critical for research reproducibility and reduction of animal numbers. Genotype is most often determined by polymerase chain reaction (PCR) analysis of DNA extracted from tissues of young rodents[5]. DNA is most commonly obtained from a tail biopsy, but may also be collected from ear punches, blood[2], hair[3], buccal swabs[4], and feces.

D) IDENTIFICATION METHODS

1) Markers (if this is the ONLY animal identification method used by the PI, it is allowable and does not need to be described on the protocol)

| Advantages | Disadvantages |
|---|---|
| • Relatively easy and inexpensive to perform<br>• Can be applied without anesthesia<br>• Extensive identification system achieved with multiple colors, numbers, letters, or symbols<br>• Can be used on rodents of all ages | • Identification is temporary and may be removed during the grooming process or skin antiseptics (e.g., ethanol)<br>• Red marker may be mistaken for blood and is not recommended |

a) Procedure

- An indelible (permanent) marker is used to write on the tail, skin of hairless strains or neonates, or light-colored haircoats.
- Reapplication is recommended every 2-3 days, as needed

b) Recommendations

- While ethanol-based inks in permanent markers have bactericidal properties[6,7], markers may be a source of cross-contamination. A colony-dedicated marker should be used for identification only.
- Makers should be replaced when soiled or dried-out as they can harbor pathogens.[6,]
- Products include: Sharpie permanent marker (ear, tail, coat, feet; lasts 2-3 d), regular and fine tip animal markers (coat only; lasts 6-12 wks);

2) Ear Tag

| Advantages | Disadvantages |
|---|---|
| • Can be applied without anesthesia<br>• Can be performed on rodents ≥ 14 d of age<br>• Ear tags can be sterilized<br>• Extensive identification system achieved with up to on average 6 characters | • Require restraint and can be difficult to read[14]<br>• Tag may be removed or fall out if placed incorrectly<br>• Adverse effects include inflammation[8] or infection[10], ulceration, necrosis, neoplasia[9], and/or torn ears.<br>• Tags may be incompatible with advanced imaging (MRI, CT, etc.) |

a) Procedure

- Application tools are tag-specific and cannot be used interchangeably
- Load ear tag in the applicator
- Manually restrain the rodent so that the ear is accessible
- Apply the tag to the lower/ventral 1/3 of the pinna, next to the cartilage ring
- Quickly apply firm pressure to the applicator so that the tag pierces the ear and tag and folds on the other size
- Incorrectly placed tags must be removed before replacing on the opposite ear

b) Recommendations

- Either ear may be tagged, but no more than one tag should be on a rodent
- Use a 1 mm rodent ear punch to create a guide hole for application of metal ear tags
- Standard rodent metal ear tags: such as National Band & Tag Company

- Alternative ear tags to metal bands: such as RapID Tags® and Stoelting© Mouse & Rat Ear Tags

- Tools for removing ear tags:
  - Wire Cutting Scissors
  - Wire Cutters

3) Tattoo

| Advantages | Disadvantages |
|---|---|
| • Can be applied to the tail, toes/foot, or ears<br>• Most applications do not require anesthesia<br>• Extensive identification system achieved using dots, dashes, or characters<br>• Identification is permanent | • Some methods technically challenging<br>• Some methods require anesthesia<br>• May be difficult to read<br>• Ink may stain draining lymph node<br>• Adverse effects include infection and necrosis |

a) Micro-tattoo (ear(s), toe(s)/foot, tail)
   - Can be performed without anesthesia at any age
   - Swab the location to be tattooed with 70% ethanol
   - Dip a sterile, small gauge needle (25-30g) in tattoo ink (see recommendations below) and pierce the skin to make small dots on the ear(s), toe(s), or tail.[11]
     - A new needle should be used for each animal
   - Tattoo ink may also be injected intradermally using a small gauge needle and syringe (28-31 g insulin syringe + needle recommended)
   - Remove excess ink with 70% ethanol
   - Commercial micro-tattooing forceps can be purchased, and tattoos are applied following the manufacturer's instructions

b) Electric Tattoo Machine (ear, tail)
   - Cannot be performed on neonates; complexity of identification scheme limited by age and size of the ear or tail
   - Anesthesia is required
   - Tattoo needles must be sterile and sharp, and should be changed regularly in accordance with the manufacturer’s recommendations, or once the needle becomes dull
   - Equipment should be disinfected between each use
   - Procedure:
     - Anesthetize the rodent
     - Swab the ear or tail with 70% ethanol

- Using an electric tattoo machine, free-hand tattoo identification symbols on the tail or ear
- Remove excess ink with 70% ethanol
- Recover the animal and return it to its cage

c) Somark® Labstamp™ Tattoo Machine (tail only):

- Available for rental through DLAR VTS with purchase of ink cartridges
- For mice older than 3 weeks of age
- Does not require anesthesia

E) IDENTIFICATION AND GENOTYPING METHODS

1) Ear Punch

| Advantages | Disadvantages |
|---|---|
| • Relatively easy to perform and read<br>• Inexpensive<br>• Can be performed on rodents ~14 d of age and older<br>• Does not require anesthesia<br>• Tissue can be used for genotyping | • Tissue may grow back<br>• Ears may tear<br>• Limited identification scheme compared to other methods |

a) Procedure

- Restrain rodents by gently scruffing
- Use an ear punch instrument to remove punches of tissue on one or both ears
- Punches may be used on outermost edge of the pinna to create or notch or within the center to create a hole
- Avoid the area of the ear closest to the head where the cartilage is thicker and more vascularized as it may be painful and bleed
- The ear punch instrument should be disinfected between cages or individual animals if the tissue is to be used for genotyping to prevent DNA contamination

b) Recommendations

- Each animal is allowed to have no more than 3 punches per ear.
- Ear punch instruments are available in different sizes, 0.5 to 2 mm diameter punches.
  - 2 mm diameter punches are recommended to reduce hole closure and healing as well as to ensure sufficient sample size for DNA analysis

- 1 mm diameter punches are recommended for generating holes for ear tagging
- Ear punch tools may dull with regular use and should be replaced as need
- There are a variety of punch instruments available such as:
  - Fine Science Tools (Plier-Style, Finger Loop, Ear Punch with Replaceable Tip)
  - Kent Scientific (Nail-Clipper Style, Scissor Style)
  - VWR®Animal Ear Punches (Plier-Style, Scissor Style, Thumb-Style)
  - World Precision Instruments (Simple Ear Punch, Scissor Style)
  - Stoelting (Scissor Style, Thumb-Style)

F) GENOTYPING METHODS

1) Tail Biopsy

- Anesthesia is required for mice and rats 21 days of age and older and must be described in your IACUC protocol
- Tail biopsy length should be limited to the smallest amount possible
  - ~2 mm yields sufficient DNA for multiple PCR reactions
  - If larger samples are required, it should be described with a justification in the IACUC protocol
- Procedure
  - Sterile surgical scissors, scalpel or straight blades may be used to biopsy tails
  - Surgical scissors must be disinfected after each animal or use a new blade for each animal. This helps ensure correct genotyping.
  - 2-3 mm of the tail tip is removed
  - Bleeding should be controlled with gentle fingertip pressure
  - The use of styptic powder with benzocaine (Kwik-Stop) is recommended for hemostasis and analgesia.
  - Cessation of bleeding must be confirmed prior to returning animals to their cage
- Recommendations
  - Best practice is to use a new sterile scalpel or straight blade for every animal; they must be replaced after 5 uses or sooner if they become dull
  - Analgesics should be considered and described in the protocol for biopsy of rodents > 21 d age

G) REFERENCES


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