THE UNIVERSITY OF TOLEDO
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

SUBJECT: Rodent Identification and Genotyping

DATE: November 18, 2026

Rodent Identification and Genotyping Guideline

Rodents often need to be individually identified for experimental or genotyping purposes. Genotyping of animals is critical to the efficient pursuit of research and for reducing the number of animals involved in a research project. Genotype is most often determined by analysis of DNA extracted from tissues of young rodents. Analysis by the Polymerase Chain Reaction (PCR) requires the least amount of DNA. DNA for PCR analysis can be obtained from ear punches, tail biopsies, hair, blood, fecal, or oral specimens. For genotyping, the UToledo IACUC recommends the ear punch procedure as it can also be used for identification purposes as well. Tail biopsies should be considered only as a last option, and with justification for why an alternative procedure cannot be used.

Identification options
Rodent Identification

a. **Temporary Markings**
    i. A temporary marking may be used for short-term individual identification, and must be repeated or re-applied as often as necessary to maintain visible markings.
    ii. There are no restrictions on age of animals.
    iii. Methods of temporary marking include:
        1. Clipping or shaving fur.
        2. Non-toxic dyes or stains.. A small area of fur that is deeply stained is the best method.
            a. Note that certain stains wear off quickly and reapplication is necessary.
        3. A non-toxic “permanent” marker may be used to write numbers, bars, or other distinguishable markings on the tail, fur, or ears.

b. **Implanted Microchips**
    i. Microchips provide a permanent method of individual identification. They are easy to implant, and the animal does not need to be restrained to read the microchip.
    ii. Rodents should be at least 3 weeks old for microchips to be implanted.
        1. Refer to each product manufacturer’s specific recommendations for implantation.

iii. The cost of each microchip can be high, making microchips one of the more expensive methods of rodent identification.
    1. Different chips may require specific readers, depending on the manufacturer. A compatible reader is necessary in order to accurately read the chip once it is implanted.
    2. The appropriate reader needs to be readily available to confirm successful implantation of the microchip and to identify the rodents.

iv. Read the manufacturer's instructions and follow them appropriately and accurately prior to performing the procedure.
    1. Microchips may fall out if not properly placed.
    2. Inject subcutaneously on the dorsal aspect of the neck with use of the appropriate injection apparatus provided by the manufacturer.
    3. Read chips before and after implantation to ensure that they are working properly.
    4. Microchips can be reused after proper cleaning and sterilization, following the manufacturer's recommendation.

c. **Tattoo**

i. Tattoo application provides a permanent method of identification, and can be performed with an electric tattoo machine or microtattoo equipment.
    1. An electric tattoo machine can be used to write numbers or symbols on the tail, ear, or footpads.
    2. Microtattooing can be used to quickly and easily mark animals, especially neonates.

ii. Anesthesia is recommended, as it may aid in the ease of this procedure. Describe anesthetics in the appropriate protocol.

iii. Reapplication of the tattoo as the animal ages may be needed if performed on neonates as the ink may fade due to the small amount originally applied.

iv. Poor technique can render the identification difficult to read.

v. Although the original cost of the tattoo gun may be expensive, the cost associated with applying tattoos is low.

vi. All tattoo equipment must be disinfected between cages of animals.
    1. Follow manufacturer recommendations for cleaning electric tattoo equipment including the needle.

vii. Apply a local anesthetic, such as lidocaine cream, which takes 15 minutes to be effective, to the skin prior to the procedure.

viii. Needles must be sterile and sharp. Clean needles between animals and change needles when they become blunted.

ix. Follow manufacturer's guidelines for procedures and recommended age for both electric tattooing and microtattooing. Electric tattooing may require special training.

d. **Ear Tags**

i. Ear tags provide a quick and easy method of identification. Ear tags can fall out if not applied properly or can be lost if ears become ripped or torn.

ii. The tags are easy to apply, but handling the animal and mild restraint may be necessary to read the ear tag number.

iii. Placement of ear tags are recommended when rodents are approximately 2-4 weeks of age. The ears may not be able to support the weight of the ear tags if the animals are too young, causing the ear to rip. The eyes or face may also be injured if the tag rubs in this area. Older animals receiving ear tags may experience increased discomfort in placement that could be avoided by placing the ear tags at this earlier age.

iv. The cost of the applicator and tags are relatively inexpensive.

v. Ear tags are available in metal and plastic. Metal ear tags are commonly made from a nickel-copper alloy, and can be associated with inflammatory and

proliferative reactions of the surrounding skin. Neoplasia, specifically squamous cell carcinomas, can also be associated with metal ear tags. Cleaning the tags by spraying with isopropyl alcohol prior to use may help reduce reactions.

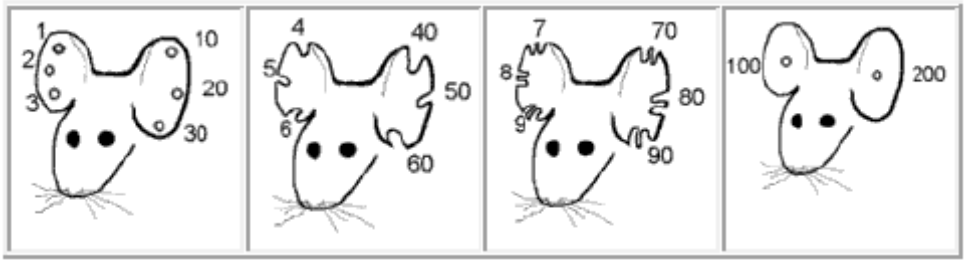
vi. In rats, ear tags may be applied to the skin over the shoulders, a.k.a., skin tags. This application must be described in the protocol.

e. **Ear Notch or Punch**
   i. Ear notching or punching is a quick and easy method to identification, although the ID pattern may be lost if the ear is injured or mutilated. Animals with similar markings may become indistinguishable if animals are grouped between cages.
   ii. Performing ear notching is recommended after 2 weeks of age, which is when the pinnae (ears) are generally large enough to notch or punch.
   iii. Disinfect equipment with disinfectant before use and between cages of animals.
   iv. If equipment becomes dull, it should be sharpened or replaced.
   v. Use a simple ear notching scheme to limit the number of notches or punches if possible. Combining animals from different cages should be avoided to decrease the chance of two animals with the same ear notch to be placed in the same cage.
   vi. The excised tissue can be used for genotyping, thus, eliminating the need for a tail biopsy.

f. **Toe Amputation (Toe Clipping)**
   i. This method is NOT recommended and must not be done without explicit approval from the IACUC. Contact the IACUC for more information.

Genotype sample options

1. Ear Punch
   a. Ear punching does not require anesthesia in rodents when performed by a skilled individual. Ear punches are generally obtained at approximately 15-17 days of age, after the ear has "thinned." Several tissue samples can be obtained using a commercially available rodent ear-punch. (https://www.finescience.com/en-US/Products/Animal-Accessories/Animal-Identification/Ear-Punch) and the punch pattern can be used for animal identification.
   b. The ear punch procedure should be performed using clean gloves and a sterile ear-punch. Manually restrain the animal and place the punch device on the pinna of the ear (external ear) in a location where you want to mark the animal for identification. Press firmly to punch a circular hole through the ear. As you remove the punch, be careful not to rip the delicate membrane of the pinna. Gently separate the ear from the device and remove the tissue sample. Bleeding after ear punching is uncommon and the animal can be released directly into the cage.
   c. 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. If a small amount of bleeding does occur, it can be controlled by gentle constant pressure.
   d. If the analysis of the DNA is to be performed by PCR, care should be taken to remove all tissue from the ear punch and clean the instrument with a sterile alcohol pad after each animal. Sterilizing the ear punch between animals using a hot-bead sterilizer will minimize the potential of DNA cross contamination.
   e. Whenever possible, a simple code should be used to limit the number of notches/punches, examples are shown below.



2. Tail Clipping
   a. No more than 2mm of tail should be removed. If more than 3mm of tail biopsy is needed, justification within the protocol is required.
   b. Rodents should preferably be less than 17 days old, when the tail is less ossified.
   c. For rodents less than 21 days of age, dip the tail in ice cold ethanol for 10-15 seconds prior to clipping.
   d. For rodents 21 days of age and older, anesthesia and analgesia must be used. Isoflurane is the recommended anesthesia, as animals recover quickly. Meloxicam or buprenorphine are the recommended analgesics.
   e. Pressure must be applied after tissue removal to achieve hemostasis.
3. Buccal Swabs/Saliva
   a. The method is accurate and non-invasive, so can be performed without anesthesia on any age of rodent(5-6).
   b. Cotton swabs are used to retrieve cheek cells from the mouths to be used for the genotyping.
   c. A small amount of saliva is collected using a plastic pipette tip and applied to sample collection paper.
4. Blood
   a. Samples can be obtained through any standard blood collection method but must be stated in the IACUC protocol.
   b. Veterinary staff will determine if anesthesia is necessary for blood collection depending on the proposed method.
5. Hair bulbs
   a. This method is non-invasive and does not require anesthesia on any age of rodent.
   b. The method involves plucking a small amount of hair from the animal for use in genetic analysis.
6. Fecal Pellet
   a. Stool can be collected for use in genetic sampling and is non-invasive.

References

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