



**THE UNIVERSITY OF TOLEDO
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**

SUBJECT: Rodent Genotyping

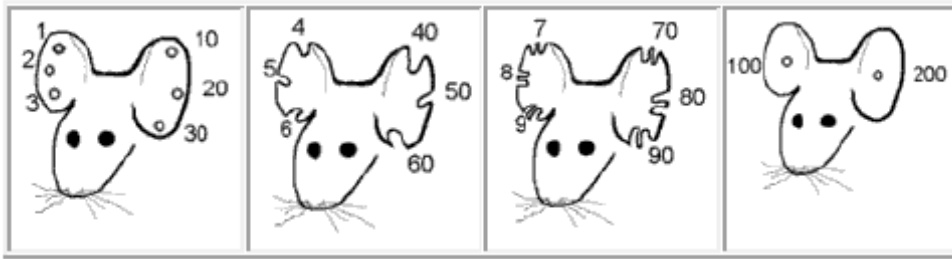
DATE: November 17, 2021

Rodent Genotyping Guideline

Genotyping of animals in a litter is critical to the efficient pursuit of research and in 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.

Procedures

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 sample for 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
 - d. If the analysis of the DNA is to be performed by PCR, great care should be taken to remove all tissue from the ear punch after each animal. Sterilizing the ear punch between animals using a hot-bead sterilizer will also minimize the potential of DNA cross contamination.
 - e. Whenever possible, a simple code should be used to limit the number of notches/punches



2. Tail Clipping/Biopsy
 - a. No more than 2mm of tail should be removed. If more than 3mm of tail is to be removed, 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 must be used. Isoflurane is the recommended anesthesia, as animals recover quickly.
 - e. Pressure must be applied after tissue removal to achieve hemostasis.
3. Buccal Swabs/Saliva
 - a. The method is non-invasive, so can be performed without anesthesia on any age of rodent.
 - b. Cotton swabs are used to retrieve cheek cells from the mouths to be used for the genotyping
4. Blood
 - a. Samples can be obtained through any standard blood collection method but must be stated in 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

1. Bonaparte D, Cinelli P, et al. FELASA guidelines for the refinement of methods for genotyping genetically-modified rodents. *Lab Anim* 2013; 47(3): 134-145.
2. Castelhana-Carlos MJ, Sousa N, Ohl F, et. al. Identification methods in newborn C57BL/6 mice: a developmental and behavioural evaluation. *Lab Anim* April 2010; 44(2)88-103.
3. *Guide for the Care and Use of Laboratory Animals*, 8th Edition. 2011. National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US).
4. *Guidelines for Genotyping of Mice and Rats*. National Institutes of Health ARAC Guidelines, September 2021. [b3-rodent_genotyping.pdf \(nih.gov\)](#)