THE UNIVERSITY OF TOLEDO
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

SUBJECT: Rodent Genotyping

DATE: November 20, 2024

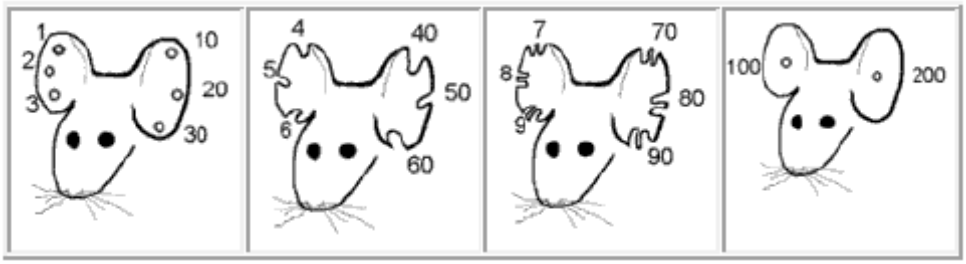
Rodent Genotyping Guideline

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.

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 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 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 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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4. *Guidelines for Genotyping of Mice and Rats*. National Institutes of Health ARAC Guidelines, September 2021. b3-rodent_genotyping.pdf (nih.gov).

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