Georgia State University  
Institutional Animal Care and Use Committee  
(IACUC)  
It is the responsibility of the Georgia State University (GSU) Institutional Animal Care and Use Committee (IACUC) to ensure judicious and humane use of animals used in its teaching and research programs that is consistent with federal requirements.*

Policy on Rodent Genotyping and Identification

Rodent Genotyping
Rodent genotyping is commonly performed using tail snipping or ear punch techniques, and less frequently using toe-clipping. Tail tipping involves cutting the tip of the tail of small rodents for the purpose of obtaining a tissue sample for genetic analysis. Ear punch tissue sampling is a less invasive technique. Further, genotyping via the use of a 2mm ear punch sample appears to yield results comparable to that produced via genotyping via tail snipping. In addition, the ear punch method can also be used for animal identification. Toe-clipping involves cutting the distal half of a digit to obtain tissue sampling for genotyping and/or for identification; it is generally considered to be the most invasive procedure of the 3 forms of genotyping, and therefore it is less frequently used. Accordingly, the use of the ear punch method is encouraged.

PROCEDURAL DESCRIPTIONS:

Tail Snip:

Pain perception of tail clamping in rats does not start to develop until 12 to 14 days of age\(^1\), so performing tail biopsy earlier in rodents may cause less pain. When performed properly in adult mice it causes only minimal or transient pain and distress, and induces no more “physiological impact” (change in heart rate, body temperature, or activity level) than restraint of the animal for the procedure\(^2\).

Guidelines for Tail Snip

For mice and rats 12 days of age and younger: Tail biopsies should preferably be taken from animals between the ages of 8 and 12 days of age because there is a lack of nervous system development, bleeding is minimal, and anesthesia is not required.

For mice and rats 13-21 days of age: Based on the physiological impact and rodent pain ontogeny studies, investigators are required to utilize an analgesic regimen such as immersion of the tail in ice cold ethanol for 10 seconds, topical application of ethyl chloride spray, by injection of an analgesic (e.g. carprofen, buprenorphine, etc) at least 20 minutes prior to the conduct of the procedure, etc.

For mice and rats greater than 21 days of age: The use of an analgesic or general anesthetic is required prior to collection of tissue.

For mice and rats greater than 35 days of age: The use of a general anesthetic is required.
To conduct the procedure, manually restrain the mouse or rat between thumb and forefinger. This is a convenient time to identify the animals using the appropriate method (i.e., ear punch, ear tag, transponder etc.). With sterile scalpel, razor blade, or scissors cleanly excise the distal 2mm (maximum 5 mm) of the tail. If the proper procedures are followed, the yield of DNA from 5 mm of tail should be enough for multiple analyses. The yield of DNA does not proportionally increase as tail fragments larger than 5mm are used. If small amounts of DNA are required, investigators should consider taking only 2 mm of tail. If the analysis of the DNA is to be performed by PCR, great care should be taken to remove all tissue from the scissors or scalpel after each animal. Disinfect the scalpel or scissors between animals. If a scalpel is used, also disinfect the work surface on which the tail is placed between animals.

The investigator must monitor the animals to assure hemostasis after the animals are returned to the cage. If needed, apply digital pressure, silver nitrate, or some other means of hemostasis. Each animal will typically only be genotyped once. However, if a second tail snip is needed (e.g., due to a technical error with the PCR, etc.) then only one additional tail snip is permissible and the total amount of tail removed will not exceed 5 mm (first and second snip combined).

**Ear punch:**
- Ear punches can be performed on any age of mouse beginning at about two weeks of age.
- Manually restrain the mouse.
- A 2 mm ear punch device is used to carefully punch the ear and allow for the removal of a sufficient sample of ear tissue for genotyping.
- The ear punch device will be sanitized (wipe with 70% alcohol) between mice.
- Bleeding from the site of the ear punch is uncommon. If bleeding is seen, hemostasis will be ensured by applying gentle pressure to the collection site for 1 minute. Blood cauterization (e.g., via application of a silver nitrate stick) may be used as indicated.

**Toe-clipping:**
- Toe clipping of neonatal mice provides permanent identification and genotyping material. The 8th edition of *The Guide for the Care and use of Laboratory Animals* states the use of toe-clipping as an identification method, should only be used when no other individual identification method is feasible. The Guide also states that “It may be the preferred method for neonatal mice up to 7 days of age as it appears to have few adverse effects on behavior and well-being at this age.”
- **Toe-clipping technique can be applied to animals up to 7 days of age without anesthesia, and should not be used on animals after 7 days of age.**
- Toe-clipping must be scientifically justified, and used as both an identification method and for genotyping material in order to obtain IACUC approval.
- The distal half of each toe is transected with a sterile scalpel blade or surgical scissors.
- A maximum of 2 digits per paw are permitted to be removed.
- Scissors or scalpel blade will be sanitized (wipe with 70% alcohol) between mice.
• Upon completion, hemostasis will be ensured by applying gentle pressure to the collection site for 1 minute. Blood cauterization (e.g. via application of a silver nitrate stick) may be used as indicated.

**Rodent Identification**
Rodent identification can be accomplished via several techniques including ear punching, ear tagging, ear snipping, tattooing, micro-chipping, permanent marker, or toe-clipping. All listed forms of identification are acceptable.

**PROCEDURAL DESCRIPTIONS:**

**Ear punching:**
• See above for description.

**Ear tagging:**
• Ear tagging can be performed on rodents weaning age or older animals.
• Disinfect (with 70% alcohol) or sterilize the metal ear tag.
• Manually restrain the rodent.
• An ear tag device is used to apply the ear tag to the pinna.
• Seldom are there complications with tagging such as feet getting caught in the ear tag, or the development of severe irritation around the ear tag site.

**Ear Snipping:**
• This practice of tissue collection requires the removal of a 2-3mm wedge of tissue from the ear pinna with sharp scissors.
• Ear snips should be taken from animals between the ages of 8 and 12 days of age because there is a lack of nervous system development, bleeding is minimal, and anesthesia is not required.
• After day 12, local anesthesia is required because of the developed vascular and nervous system.

**Tattooing:**
• Manually restrain the rodent.
• A permanent mark is made on the tail, toes, ears, or possibly foot pads by using a needle or appropriate micro-tattooing device.

**Micro-chipping:**
• Ear tagging can be performed on rodents weaning age or older animals.
• Manually restrain the rodent.
• Inject a small microchip transponder subcutaneously between the scapulae of the rodent.
• The microchip is detected by use of a reader.

**Permanent marker:**
• Permanent marker technique is used for temporary identification.
• Manually restrain the animal.
• Apply the marker to the appropriate location.
• This method is non-invasive.

Toe-clipping:
• See above for description.

Pertinent Regulations*
U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
Public Health Service Policy
Guide for the Care and Use of Laboratory Animals
Animal Welfare Act (AWA) and AWA Regulations

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Signature IACUC Chair: [Signature]
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References: