Tissue collection for genotyping mice and rats, whether performed by DACT personnel or investigators, will follow the protocol described here unless alternate methods are described in an IACUC-approved protocol. This protocol was developed from the NIH “Guidelines for the Genotyping of Mice and Rats” (revised 5/12/10).

**Purpose**
The specific genetic identification of genetically engineered animals in a litter is critical to the efficient pursuit of research and in reducing the number of animals involved in a research project. The genotype is most often determined by analysis of DNA extracted from tissues of young rodents. Analysis by Polymerase Chain Reaction (PCR) requires the least amount of DNA. DNA for PCR analysis can be obtained from tail biopsies, ear punches, hair, blood, fecal, or oral samples. Larger amounts of DNA are required for Southern Blot determination of the genotype.

Obtaining tissue from a mouse or rat for DNA analysis via tail biopsy is a safe, effective, and humane procedure. Pain perception in rats does not start to develop until 12 to 14 days of age, 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 just restraining the animal for the procedure. DNA prepared from tail biopsies is suitable for analysis by either Southern Blot or PCR. Depending on the requirements of the study, investigators are urged to consider noninvasive alternatives such as hair, fecal, or oral samples.

Protocols that include tissue collection for genotyping must include a statement that confirms that tissue collection will strictly follow these guidelines or it may include a detailed description of an alternate approach with scientific justification for deviating from the approach described here.

**Guidelines for Tail Biopsy**
1. Ideally, mice and rats should be **10-21 days old** at the time of tissue collection. At this age, the yield of DNA is highest. In addition, prompt analysis of tail tissue allows the desired mice and rats to be identified prior to weaning which can facilitate more efficient use of cage space.

   a. **For mice and rats 10-21 days of age**: Based on the physiological impact and rodent pain ontogeny studies, investigators are **strongly encouraged to apply local anesthetica** to the tail and to conduct tail biopsy as early as possible within this age range. Local anesthesia may be achieved by immersion of the tail in ice cold ethanol for 10 seconds, by application of ethyl chloride spray, by application of 2% lidocaine jelly, or by the use of another suitable anesthetic as recommended by the ASU veterinary team.

   b. **For mice greater than 21 days and rats 22-35 days of age**: The use of a **local or general anesthetic is required** prior to collection of tissue. Topically applied lidocaine with epinephrine is the preferred local anesthetic, since the epinephrine will constrict the blood vessels locally and will enhance the longevity of the lidocaine-induced local anesthesia. After applying the lidocaine, wait 1-2 minutes before cutting the tail. Isoflurane is the

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preferred general anesthetic, because it has a high degree of safety and provides rapid induction and recovery.

c. **For rats greater than 35 days of age:** The use of a general anesthetic is required.

3. Rodents can be restrained by hand between thumb and forefinger or in a rodent restraint device. Once restrained, it is a convenient time to identify the animals using the appropriate method described in the protocol (e.g., ear punch, ear tag, transponder, etc.).

4. With a sterile scalpel, scissors, or razor blade cleanly excise the distal 2 mm (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 5 mm 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 instruments between animals.** If a scalpel is used, also disinfect the work surface on which the tail is placed between animals.

5. The investigator must monitor the animals to ensure hemostasis after the animals are returned to the cage. If needed, apply digital pressure, silver nitrate, or Kwik Stop with benzocaine.

6. If additional DNA is needed for retesting, alternatives to a second tail biopsy should be considered\(^\text{11}\). **Repeat tail biopsies require anesthesia** and must be justified in the protocol. The use of post-procedural analgesia should be considered.

**Ear Punches as an Alternative Source of Tissue**

1. Ear punches provide an alternative source of tissue for genotyping.

2. Ear punching can be a superior source of tissue for animals over 21 days of age because:
   a. It does not require anesthesia.
   b. The ear punch can also serve to identify the individual.
   c. The sample size is consistent for every animal.

3. The awake mouse is restrained by firmly holding its scruff in one hand, and then, using a 2-3 mm commercial rodent ear punch in the other hand, collecting a small tissue sample from the pinna.

**References**


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