

**PORTLAND VETERANS AFFAIRS MEDICAL CENTER
SUBCOMMITTEE ON ANIMAL STUDIES (PVAMC SAS)
ANIMAL CARE AND USE GUIDELINES**

<p>CHECK ONE:</p> <p><input type="checkbox"/> I agree to comply with the following guidelines.</p> <p><input type="checkbox"/> I have attached a written justification for deviation from these guidelines.</p>	
<p>_____</p> <p>Principal Investigator</p>	<p>_____</p> <p>Date</p>

GUIDELINES FOR TISSUE COLLECTION FOR GENOTYPING

Background: It often is necessary to obtain tissue samples from mice to determine or confirm their genotype. A number of methods exist for tissue sampling, and the chosen technique should balance the research needs with the humane treatment of the animal. Procedures that are expected to cause more than momentary pain or distress in a human require that anesthetics or analgesics be administered when performed in animals (USDA Policy 11).

A number of methods exist for tissue samples (see Table 1), and the chosen technique should balance the research needs with the humane treatment of the animal. Most common is the removal of tail tissue (tail biopsy in Table 1) for DNA analysis by polymerase chain reaction (PCR) or Southern Blot. However, less invasive methods are available to obtain DNA, including ear punches, hair samples, saliva/oral swabs, and blood or fecal analysis (Table 1). Further, PCR is encouraged, since it may require smaller tissue samples when compared to Southern Blot. Other considerations to ensure that pain and distress are minimized include the age of the mouse when sampled, use of local or general anesthesia and analgesia, and proper training of personnel performing the procedure. General recommendations/guidelines are provided in Table 1. Consult the VMO for assistance with specific procedures.

Because the removal of tail tissue from mice for genotyping may be considered a painful procedure (and because it is the most common method for genotyping), additional guidelines are provided after Table 1 to assist investigators with the selection of methods to alleviate pain or distress and with the determination of the appropriate pain category.

TABLE 1

Method	< 2 Weeks of Age	3-4 Weeks of Age	> 4 Weeks of Age
Saliva or fecal samples	Acceptable	Acceptable	Acceptable
Tail biopsy	Acceptable with justification	Acceptable***	Acceptable with justification
Ear notching	Not recommended ¹	Acceptable	Acceptable
Blood	Not recommended	Acceptable	Acceptable
Toe amputation	Not recommended, but acceptable with justification ²	Not acceptable	Not acceptable

¹Ear notching can be performed at 14 days of age.

²Toe amputation as a method for genotyping or identification is not recommended, except in extraordinary circumstances where no other method is feasible. These conditions should be scientifically justified in the animal use protocol and should only be performed in neonatal animals that are less than 12 days of age (eyes should not be open).

*****Additional guidelines for tail tissue collection:**

1. In mice greater than 10 days of age, a maximum of 1 cm of tail (total) may be removed for genotyping. Anesthetics are not required. Analgesic agents are required if tail removal exceeds 0.4 cm, and these animals will be classified as Category D. If genotyping can be performed using a maximum of 0.4 cm of tail, then analgesics are not required for this procedure, and these animals will be classified as Category C. Appropriate analgesic agents include:
 - a. Morphine: 5-10 mg/kg body weight, SC or IP
 - b. Buprenorphine: 0.2 mg/kg body weight, SC or IP
 - c. Butorphanol: 0.05-0.1 mg/kg body weight, SC or IP
 - d. Isoflurane: to effect
 - e. EMLA cream: apply topically at least 15 min prior to procedure
2. If the investigator wishes to be exempted from the analgesia requirement when tail removal exceeds 0.4 cm, written scientific justification must be provided to the IACUC. If the IACUC approves the exemption, analgesics may be withheld, and the genotyped animals will be classified as Category E.
3. Mice younger than 10 days of age are difficult to safely anesthetize or sedate. In these animals, analgesic and anesthetic agents are not recommended. A maximum of 0.4 cm of tail may be removed for genotyping. These animals will be classified as Category C.
4. If additional tail tissue is required for subsequent genotyping, an additional 1 cm of tail may be removed once, for a total of 2 cm of tail collected. If additional tail collection beyond this 2 cm is required, scientific justification must be provided to the IACUC for discussion and approval. Appropriate analgesics must be administered, as described in Item 1.
5. In all cases, a sharp pair of scissors or blade must be used to perform the procedure. The instrument(s) must be disinfected between animals to prevent cross-contamination of animals. Heat sterilization (using alcohol and flame or a hot bead sterilizer) or "cold" sterilization (using a quarternary ammonia compound and rinsing with sterile water or saline) are acceptable methods. Care should be taken to ensure that the instruments remain sharp and sterile during collection.
6. After completing the tail snip, the tail tip should be sealed in warm wax (taking care not to burn the tail) to prevent cannibalism after return to the cage.