Guidelines for Mouse Genotyping Tissue Harvesting

**SOP**

Researchers must consider all sources of DNA to perform genotype analysis, including alternatives to invasive procedures such as tail biopsy. As with any procedure, the specific method of tissue collection must be detailed in the approved IACUC protocol. In general, a tail biopsy may be performed in rodents up to 12 days of age without analgesia or anesthesia, in rodents 13-21 days of age with local anesthesia, and in rodents over 21 days of age under general anesthesia and post-procedural analgesia. Tail samples should be no more than 2-5 mm in length. Aseptic practices and hemostasis must be assured.

**Reason for SOP**

Determining the genotype in a rodent litter that has been genetically engineered is critically important. The 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. Small amounts of DNA can be obtained from ear punches, tail biopsies, or various non-invasive alternatives. Larger amounts of DNA required for determination of genotype by Southern Blot are usually obtained from tail biopsies. Depending on the requirements of the study, investigators are urged to consider noninvasive alternatives such as hair, fecal, or oral samples.

**Ear Punching:** This method generally provides sufficient DNA for PCR analysis. Its advantages: it is less stressful than a tail biopsy; it does not require anesthesia; and it allows for permanent identification. This method is best performed on mice near or at the age of weaning (day 21). Prior to weaning age, the small size of ear flaps does not allow for identification by ear punch.

**Tail Biopsy:** Pain and distress associated with tail biopsies vary with an animal’s age and strain. Behavioral, physiological, and electroencephalographic evidence suggests that the ability to perceive pain develops gradually and begins as early as postnatal day 12 in mice. Responses to pain increase with age (Diesch et al. 2009, Hankenson et al. 2008). The development of mineralized bone in the mouse tail corresponds with the development of sensory and sympathetic neurons and associated pain pathways (Mach et al. 2002). Most common mouse strains have measurable, mature vertebrae in the distal 5 mm of tail by day 21 and in the distal 2 mm by day 31. Strains such as C57BL/6 and C3H have measurable, mature vertebrae in the distal 5 mm of tail by postnatal day 17 and in the distal 2 mm by day 21 (Hankenson et al. 2008).

Therefore, the pain and distress change with animal age:
- For most strains, before 12 days of age, mature vertebrae are not detectable and pain perception has not likely developed.
- Between 13 and 21 days of age, mature vertebrae are measurable and some pain perception has developed.
- Post 21 days of age, mature vertebrae have developed in the distal 5 mm of tail in all rodent strains and in the distal 2 mm of some strains. Pain perception is thought to be fully developed at this time.

The SOP below reflects these changes in pain and distress with age.

Regardless of animal age, sample sizes for tail biopsies should be as small as possible, particularly since the rodent tail is a principal thermoregulatory and proprioceptive appendage. DNA yield (μg DNA per mg tail weight) is significantly higher in 5 mm samples than in either 10 mm or 15 mm samples from animals aged 3 to 42 days (Hankenson et al. 2008). Thus, smaller samples should provide most efficient DNA yield.
Procedures

Ear Punching:
- A sterile ear punch must be used and appropriately disinfected between animals.
- A small amount of bleeding is expected and can be controlled by gentle, constant pressure. Animals should be checked several hours post-procedure to ensure proper hemostasis.

Tail Biopsy:
1. Tail biopsy should involve the minimal sample size possible (preferably 2-5 mm) and must not exceed 5 mm without first securing IACUC approval.

2. Sterile scalpel, razor blade, or sharp scissors must be used to collect the tissue.

3. Hemostasis must be assured using digital pressure, skin/styptic powder, or silver nitrate before returning the animal to a (clean) cage. Electrocautery for hemostasis is not appropriate. Animals must be monitored as long as necessary to assure that the bleeding has ceased.

4. For tail biopsy performed in rodents **on or before 12 days of age**, samples may be excised without anesthesia or analgesia, although local anesthesia is recommended.

5. For tail biopsy performed **between 13 and 21 days of age**, samples may be excised using local anesthesia. Local anesthesia may be achieved by immersing the tail in ice-cold ethanol for 10 seconds, by applying ethyl chloride spray, or by using another suitable agent as recommended by a veterinarian. The use of an analgesic agent such as Meloxicam or Buprenorphine is encouraged but not required.

6. For tail biopsy of **2 mm or less in rodents older than 21 days of age**, samples may be excised using local anesthesia. Local anesthesia may be achieved following number 5 above. The use of an analgesic agent such as Meloxicam or Buprenorphine is required for at least 24 hours. Closure of the skin wound with surgical glue is recommended.

7. For tail biopsy of **more than 2 mm in rodents older than 21 days of age**, samples must be excised using general anesthesia and post-procedural analgesics. General anesthesia may be achieved with a variety of agents that induce a surgical plane of anesthesia. The use of an analgesic agent such as Meloxicam or Buprenorphine is required for at least 24 hours. Closure of the skin wound with surgical glue is recommended.
8. Repeat sampling may only occur once. For all rodents less than 21 days of age, repeated sampling requires local anesthesia (as described in number 5 above). For all rodents over 21 days of age, repeated sampling must use general anesthesia and post-procedural analgesics (as described in numbers 6 & 7 above). IACUC approval is first required before any animal can be sampled more than twice.

9. Any deviations or exceptions must be scientifically justified and require prior IACUC approval. For example, weanlings from small or unthrifty strains may have tail biopsies performed between 21 and 28 days of age using only local anesthesia with specific justification and IACUC approval.

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**Related Information**

References:


