Tissue Harvesting for Rodent Genotyping

**Policy:** Principal investigators must consider all sources of DNA for performing 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 IACUC Proposal. In general, 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. Samples should be less than 5 mm. Aseptic practices and hemostasis must be assured.

**Rationale:** DNA for genotyping can be obtained from ear punches, blood, saliva, hair or fecal samples, oral or rectal mucosal swabs, or via tail biopsy. Each source may have distinct advantages; for example, some authors recommend tail or ear tissue for quantitative PCR analysis over fecal pellets, hair, or buccal swab samples (Garzel et al, 2010). Regardless, since extremely small sample sizes are generally required, non-invasive alternatives to invasive procedures such as tail and ear biopsy should be considered.

Pain and distress associated with tail biopsy are age- and strain-dependent. 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 and Hankenson et al. 2008). The development of mineralized bone in the mouse tail has been shown to correspond with the development of sensory and sympathetic neurons and associated pain pathways (Mach et al. 2002). Most common mouse strains have detectable mature vertebrae in the distal 5 mm of tail by day 21 and in the most distal 2 mm by day 31; strains such as C57BL/6 and C3H have detectable mature vertebrae in the distal 5 mm of tail by post-natal day 17 and even in the most distal 2 mm by day 21 (Hankenson et al. 2008). Therefore, before 12 days of age, mature vertebrae are not detectable and pain perception has not likely developed. Between 13-21 days of age, mature vertebrae are detectable and some pain perception has developed. Following 21 days of age, mature vertebrae have developed in the distal 5 mm of tail in all strains and in the distal 2 mm of some. Pain perception is considered to be fully developed.

Sample sizes should remain as small as possible. It is important to recognize that the tail in rodents is an important thermoregulatory and proprioceptive appendage. DNA yield (µg DNA per mg tail weight) is significantly higher in 5 mm samples over either 10 mm or 15 mm samples from animals ranging in age from 3 days to 42 days (Hankenson et al. 2008).

**Procedures, Guidelines, and Exceptions:**

1. Tail biopsy should involve the minimal sample possible and must not exceed 5 mm without specific IACUC approval.

2. Sterile scalpel, razor blade, or sharp scissors must be used.
3. Hemostasis must be assured using digital pressure, skin glue (e.g., “VetClose”), styptic powder, silver nitrate, or other established means before placing animal back into preferably a clean cage. Electrocautery for hemostasis is not appropriate. Animals must be monitored as long as necessary to assure hemostasis.

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; analgesia is encouraged but not required. Local anesthesia may be achieved by immersion of the tail in ice cold ethanol for 10 seconds, by an application of ethyl chloride spray, or by the use of another suitable agent as recommended by a veterinarian. The use of an analgesic agent such as ketoprofen or buprenorphine is encouraged.

6. For tail biopsy of 2 mm or less in rodents older than 21 days of age, samples may be excised using local anesthesia; analgesia is encouraged but not required. Local anesthesia may be achieved as described in number 5 above.

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 ketoprofen or buprenorphine is required for at least 48 hours.

8. Repeat sampling may only occur once and requires local anesthesia (as described in number 5 above) for all rodents under 21 days of age and using general anesthesia and post-procedural analgesics (as described in number 7 above) for all rodents over 21 days of age. More than two samples are not allowed without specific IACUC approval.

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

References: