University of Louisville
Institutional Animal Care and Use Committee
Policies and Procedures

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 remain as small as possible and must be less than 5 mm unless otherwise approved by the IACUC. 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. Toe clipping is not recommended, but may be used for identification and genotyping under certain circumstances (see IACUC Policy "Rodent Identification" for additional details).

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 and 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.

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2. Sterile scalpel, razor blade, or sharp scissors must be used.

3. Hemostasis must be assured using digital pressure, skin glue (e.g., "VetClose"), stypic powder, silver nitrate, or other established means before placing animal back into a clean cage. *Electrocautery for hemostasis is not appropriate*. Animals must be monitored as long as necessary to assure hemostasis.

4. Tail biopsies performed in rodents ***on or before 12 days of age*** may be performed *without* anesthesia or analgesia, although local anesthesia is *recommended*.

5. Tail biopsies performed ***between 13 and 21 days of age*** require *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 meloxicam or buprenorphine is encouraged.

6. Tail biopsies of ***2 mm or less in rodents older than 21 days of age*** require *local anesthesia*; analgesia is encouraged but not required. Local anesthesia may be achieved as described in number 5 above.

7. Tail biopsies of ***more than 2 mm in rodents older than 21 days of age*** require *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 48 hours.

8. Repeat tail biopsy 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 and total combined biopsies must not exceed 5mm.

9. *Any deviations or exceptions from these guidelines must be scientifically justified in the IACUC Proposal and requires prior IACUC approval.*

References:

Diesch TJ, DJ Mellor, CB Johnson, and RG Lentle. 2009. Electroencephalographic responses to tail clamping in anaesthetized rat pups**.** *Lab. Anim*. **43**(3): 224-31.

Garzel LM, Hankenson FC, Combs J, and KD Hankenson. 2010. Use of quantitative polymerase chain reaction analysis to compare quantity and stability of isolated murine DNA. *Lab Anim*. **39**(9): 283-289.

Hankenson, FC, LM Garzel, DD Fischer, B Nolan, and KD Hankenson. 2008. Evaluation of tail biopsy collection in laboratory mice (*Mus musculus*): vertebral ossification, DNA quantity, and acute behavioral responses. *J. Am. Assoc. Lab. Anim. Sci*. **47**(6): 10-18.

Mach DB, SD Rogers, MC Sabino, NM Luger, MJ Schwei, JD Pomonis, CP Keyser, DR Clohisy, DJ Adams, P O'Leary, and PW Mantyh. 2002. Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. *Neuroscience* **113**:155-166.Office of Animal Care and Use National Institutes of Health. *Guidelines for the Genotyping of Mice and Rats. ARAC Guidelines*. Sept. 2007. http://oacu.od.nih.gov/ARAC/index.htm.

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