

*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*. Samples should remain as small as possible and must be less than 2 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, but less invasive sampling is preferred if appropriate DNA samples can be collected. Modern DNA samples require very small sizes.

Pain and distress associated with tail biopsy are age- and strain-dependent. Therefore, anesthesia and analgesia are required for post-weaning rodents.

### **Procedures, Guidelines, and Exceptions:**

- A. Preferred tissue collection sites for rodents. Please see IACUC Policy “IACUC Standard Procedures for Rodents” for technical details.
  - a. Ear Pinna
  - b. Hair
  - c. Buccal swabs/saliva
  - d. Fecal pellets
- B. Conditionally acceptable tissue collection sites for rodents
  - a. Distal Tail
    - 1. The tail of a mouse contains a variety of tissues, including bone, cartilage, blood vessels and nerves. In a pre-weaned mouse, the distal 2mm tail does not contain mature vertebrae (bone). Therefore, removal of the very end of the tail (<2mm) is comparable to removal of a similar size of tissue from the mouse ear. The tail biopsy should be performed at as young of an age as is feasible. In most, if not all cases, the procedure can and should be performed prior to weaning. If re-sampling for repeat genotyping from the same mouse, no more than 2 mm cumulative of the distal tail should be harvested and multiple samplings must be approved in the IACUC proposal. In this situation, other tissue sources should be used for harvest (e.g. ear pinna).
    - 2. Procedures (Also see IACUC Policy “IACUC Standard Procedures for Rodents”:
      - i. For pre-weaned mice: Biopsy of tail tissue can be performed without general anesthesia in mice prior to weaning age. Ice cold ethanol as a topical anesthetic

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- should be considered in pre-weaned mice. Topical cetacaine or ethyl chloride sprays do not provide appropriate anesthesia/analgesia and should not be used on mice.
- ii. For weaned mice (>28 days): With increasing age, tail maturation includes mineralization of bone and increased vascularity; it has been demonstrated that tail biopsy sampling performed on older mice (> 28 days) can result in prolonged discomfort. Anesthesia (e.g., Isoflurane, Ketamine / Xylazine) and analgesia (e.g., meloxicam, carprofen) is required when tail biopsy is performed on animals older than 28 days of age. See IACUC document “Recommended Rodent Anesthetics and Analgesics”.
3. Any bleeding at the tail tip must be controlled (hemostasis) following the biopsy. If less than 2 mm is taken, hemostasis can usually be achieved by direct manual pressure with clean paper towel or gauze on the end of the tail. If direct pressure does not stop the bleeding, the use of hemostatic agents (e.g. styptic powder (Kwik-Stop ®) is recommended and should be readily available as a precautionary measure. Animals may not be left with actively bleeding collection sites. Animals should not be returned to the cage until bleeding is controlled.
  4. If general anesthesia has been administered, the mouse must be observed until it regains consciousness. See IACUC Policy “Rodent Anesthesia”.
  5. If you anticipate the possibility of needing an additional tissue sample from a mouse at a later date, other tissue sources (described above) are recommended so as to not remove excess tail tissue and must be approved in the IACUC proposal.

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

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