FSU
ANIMAL CARE & USE COMMITTEE
DIVISION OF RESEARCH

Guidelines for Rodent Tail Biopsy

Accurate identification of genetically modified rodents is essential for quality research and aids in the reduction of animal numbers. Genotype is frequently determined by Polymerase Chain Reaction (PCR) analysis of DNA extracted from tissues of young rodents, however other alternative genotyping procedures may include single-nucleotide polymorphism (SNIP) analysis or Southern Blotting. Tissue from tail biopsies and ear punches are the most common utilized sources of DNA.

While rodent tail biopsy is a safe and effective procedure, the FSU ACUC encourages investigators to consider less invasive methods for DNA collection such as ear punches (that can also be used for animal identification), hair, blood, feces, and oral and rectal samples.

- As less invasive alternative methods are available, a literature search to determine that tail biopsy is the most appropriate method must be documented in the Animal Use Protocol.
- Since animals must be individually identified at the time of tissue collection for genotyping, a method that provides a DNA sample at the same time as it identifies the animal, e.g., ear punching, should be prioritized. This minimizes the number of procedures carried out on the animals and hence minimizes pain and distress.
- The greatest yield of DNA in animals <21 days old is obtained from tissue collected from the tail, ear or rectal swabs and in adults from tail tissue and blood.
- The optimal age for tail biopsy is between 10-17 days of age prior to ossification of tail vertebrae and development of pain perception. By 21 days of age, mature vertebrae have developed in the distal 5 mm of the tail in all strains and in the distal 2 mm of some strains. At this age pain perception is considered to be fully developed.
- Investigators should limit the tissue collected to the smallest amount possible. In general, a biopsy of approximately 2 mm is sufficient to generate DNA for multiple PCR tests. No more than 5 mm may be collected, whether that be a single or total of two separate biopsies. If longer tail samples are required, scientific justification must be provided in the Animal Use Protocol.
- If required, animals may undergo a second tail biopsy, but this must be at least one week after the first tail biopsy and anesthesia and analgesia must be used regardless of the age of the animal. Any need for obtaining a second sample must be described in the ACUC protocol.

- It is recommended that, to avoid a second biopsy, investigators consider dividing the initial sample and freezing one-half of the sample at -80ºC. A study has shown that both tail and ear samples continue to yield adequate amounts of DNA for PCR analysis even after being frozen for up to 44 months.

1.0 Summary of Method

Genotyping details and collection techniques must be described and approved in the IACUC protocol.

- **Anesthesia and analgesia requirements**
  - For mice and rats < 21 days old: While tail biopsy may be performed without use of anesthesia or analgesia, investigators are encouraged to perform the tail biopsy as early as possible within this age range. Local anesthesia may be achieved by immersion of the tail in ice-cold ethanol for 10 seconds or use of other suitable anesthetics recommended by LAR veterinary personnel. Local analgesia may be achieved by dipping the cut end of the tail in 0.75% bupivacaine for 30 seconds post-biopsy or application of a single drop of local anesthetic (bupivacaine, lidocaine, or ropivacaine).

  - **For mice and rats ≥ 21 days old:** Use of a general anesthetic is required prior to tail biopsy, and choice of anesthetic agent should be made in consultation with LAR veterinary personnel. A minimum of one (1) dose of systemic pre-emptive analgesia (e.g. buprenorphine or meloxicam) must be administered.

  - Please note that the use of topical ethyl chloride is no longer recommended due to issues related to histologic tail tissue damage and necrosis.

- **Procedure**
  - Rodents must be manually/physically restrained or under general anesthesia for tail biopsy (age dependent, see above).
  - Snip 2-3mm off the tip of the tail with sharp, sanitized scissors or disposable scalpel or razor blade.
  - Place the tissue sample into an identified collection tube.
  - Sanitize the scissors or blade after each snipping (wipe with 70% alcohol or dip in glass bead sterilizer for at least 30 seconds) if collecting the tails from several mice. If a scalpel or razor blade is used, also disinfect the work surface on which the tail is placed between animals.
  - Following the biopsy procedure, bleeding should be controlled using local pressure and sterile gauze. The use of a cotton-tipped applicator with 1% lidocaine with epinephrine may enhance hemostasis as well as providing post-procedural local analgesia. It may be necessary to use styptic powder, silver nitrate, or surgical glue in cases where bleeding is not controlled with digital pressure. If silver nitrate is used, the tissue must be washed free of the chemical with saline following

hemostasis to neutralize the chemical reaction. After releasing the animal back into the cage, it should be observed to make certain that the bleeding has stopped.

- Animals that have been anesthetized must be continuously monitored until able to move around the cage on their own.

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Revision History

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