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WVU IACUC POLICY:
Tail Biopsy for DNA Extraction in Mice

Introduction

To determine the genotype of genetically engineered mice (GEM), it is necessary to obtain a tissue biopsy, isolate DNA from the biopsy, and subject the DNA to molecular biological testing such as polymerase chain reaction (PCR), Southern analysis, or dot/slot blot hybridization. This is commonly performed at the time of weaning by obtaining a small piece of the most distal portion of the tail or a small tissue punch from the ear (see below) and at the same time giving the mouse unique identification. The purpose of this policy is to establish standards for obtaining tail biopsy material while minimizing pain and distress to GEMs.

A recent publication compared five strains of inbred mice to determine the age of coccygeal vertebrae ossification, the order of coccygeal vertebrae ossification, and the behavioral response to tail biopsy[4]. This publication demonstrated that ossified vertebral body and growth plates in the distal 5 mm of tail were apparent in most strains using micro-computed tomography by 17 – 21 days of age (or 34-38 days when evaluated by micro-radiography). In addition, all strains showed an acute behavioral response during the initial 10 minutes after biopsy that diminished by 60 minutes post-biopsy (<10 percent in mice 17-28 days at 60 min), and that the distal 5 mm tail vertebrae ossified several days later than those more proximal to the body. This article concluded that a 5 mm tail biopsy in mice no older than 17 days of age could be performed without anesthesia whereas older mice required anesthesia. Moreover, pre-ossified tissue yielded more DNA per length. Similar studies in mice using telemetry of heart rate concluded that tail tip biopsy effects were short-lived even in adult mice.[5]

Regulations and Guidelines

The *Guide for the Care and Use of Laboratory Animals* (8th edition, 2011)[6] does not address this issue.

Alternatives to Tail Biopsy

There are several sources of tissue that can be used to obtain DNA for the purpose of genotyping mice. Such sources include buccal cells obtained by oral swab or fecal material containing sloughed gastrointestinal cells; however, in studies published using this method the low DNA yield requires a nested PCR, which may lead to false positives due to contamination of the laboratory with amplified DNA.[1, 3] Another source of DNA is an ear punch biopsy. When this method of identification is used, care must be taken to adequately remove residual ear tissue from the ear punch as this can confound results of subsequent biopsies.[2]

Biopsy Policy

The following WVU IACUC policy is based upon: post-biopsy behavioral and telemetry data in the literature, data on age of ossification of distal tail vertebrae, routine GEM colony management practices, and years of GEM tail biopsy observation:

Note: all animals undergoing biopsy, no matter the method, should be listed in Category C, unless further procedures warrant Category D or E.

1. The biopsy procedure must be included in an approved protocol.

2. If one of the alternatives to tail biopsy above is suitable for the laboratory, it can be used without anesthesia (local or general) in mice of any age.

3. If a tail biopsy is required, mice should be no older than 17 days. A piece of tail 5 mm from the distal end should yield a sufficient amount of DNA for PCR, dot blot, or slot blot analysis. If mice older than 17 days are biopsied, they must receive anesthesia either topically or systemically. Recommended analgesics are listed in this policy.

4. If more than 5 mm of tail is to be biopsied, or a single mouse needs to be biopsied more than one time, then the use of a local or general anesthetic is required.
   Options for local anesthesia include: EMLA cream (OLAR has this), or topical 1% lidocaine in 50% dimethylsulfoxide.

5. In all cases, tails should be disinfected prior to the biopsy and monitored for hemostasis after the biopsy. In some cases, bleeding may need to be controlled; provide hemostasis by digital pressure and/or application of a styptic agent (e.g., Clotisol® or silver nitrate).

6. Amputate 5 mm or less of the distal tail tip using a sterile scalpel or razor blade or sharp scissors. Place tissue into a tube labeled with the identification of the mouse.

7. Control bleeding if necessary by digital pressure or styptic (Clotisol) application and stabilize if anesthetic is used.

8. Return mouse back into a clean cage, once stable.

Tissue Collection Bibliography

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