Washington State University
Institutional Animal Care and Use Committee

Standard Operating Procedure #2
Title: Guidelines for Rodent Tissue Sampling for Genotyping attachment

A. Scope

This SOP must be followed by all WSU researchers and technicians who collect tissue samples from rodents for genetic identification.

B. Introduction

Biopsy of the distal tail is often performed as a means of obtaining tissue or blood for biochemical analysis or genetic monitoring in rodents. For genetic monitoring, Polymerase Chain Reaction (PCR) techniques may require less tissue. Principal investigators are strongly encouraged to utilize a less invasive procedure for obtaining tissue. Alternatives to tail snipping include the use of auricular flap tissue obtained during the ear punch identification procedure, hair bulb or fecal samples or the saliva swab method. Southern Blot testing may require more material and need tissue from the tail. Distal phalanx biopsy is another method of obtaining tissue for genotyping. While this method is often considered equally as invasive as amputation of the distal tail, it carries the added benefit of being able to be used for identification purposes. When performed appropriately in neonatal rodents up to 7 days of age, there appears to be few adverse effects and when combined for both genotyping and identification purposes, may be the preferred method for neonatal mice (Guide pg. 75)

C. Guidelines

1. Tail biopsy

In accordance with NIH Guidelines, tail biopsies should not be performed before 10 to 14 days of age.

Anesthesia is not required in rodents before weaning (21 days of age) if less than 5 mm of length is taken. The skin can be pushed toward the tip of the tail so that the vertebrae are avoided. Innervation of the tip of the tail is minimal at this age.

Tail tip samples greater than 5 mm in length will probably damage the coccygeal vertebrae and will require anesthesia in rodents of any age. Anesthesia is required for any tail biopsy if animals are greater than 21 days old or if multiple tail biopsies are required. These procedures must be described in the ASAF for approval by the IACUC.

a. Anesthesia
   Local: topical lidocaine.
   Injectable: Ketamine/xylazine, ketamine/dexmedetomidine
   Inhalant: Inhalant anesthetics (isoflurane)

b. Standard Technique
   - Gloves should be worn when handling laboratory animals
Anesthetize rodent (if required).
Gently, but securely, restrain rodent.
Swab tail with alcohol (providone iodine or chlorhexidine solutions may interfere with the DNA identification tests).
Push skin toward tip of tail.
Snip skin sample with sterile instrument[s].
Apply gentle compression until bleeding stops.
Apply surgical glue or silver nitrate powder (Kwik-stop) to tail tip if bleeding continues.
Release rodent.
Observe mouse for bleeding or abnormal behavior.
Check tail daily to ensure tip is healing.

2. Ear Tissue Biopsy

As an alternative to tail biopsy, tissue can be collected from the ears of rodents. These biopsies may be collected by using a standard rodent ear punch instrument. The instrument punctures a small hole in the pinna of the ear and provides the researcher with a small amount of tissue for analysis. This procedure does not require the use of anesthetics or analgesics as long as the procedure is performed by a trained individual. The other advantage to utilizing this procedure is that the hole that remains in the ear of the rodent may be used for individual animal identification. Unlike ear tags, ear punch identification systems have not been associated with ear infection or wound induced carcinomas.

3. Fecal Sampling

Stool samples from rodents can easily be obtained either by collecting samples from the cage bottom or by collection of a fresh sample directly from the animal (rodents routinely defecate when gently handled). Procedures for genotyping rodents using stool samples are described in the following articles:


4. Hair Bulb Sampling
This sampling involves the use of hair bulbs from rodents for genetic analysis. Hair bulbs can be directly used for PCR analysis after alkaline lysis. This procedure allows for a large number of animals to be tested in minimal time and is non-invasive. Procedures for genotyping rodents using hair bulb samples are described in the following articles:


5. Saliva Sampling

Studies have shown that a small amount of saliva contains enough oral epithelial cells and lymphocytes to yield sufficient DNA for PCR analysis. This is a non-surgical technique that involves oral washing of weanling rodents with a plastic pipet tip. Procedures for genotyping rodents using saliva samples are described in the following articles:


6. Distal Phalanx Biopsy

Distal phalanx biopsy consists of the removal of a distal phalanx of a newborn rodent, that is then used as both a source of DNA and a means of identification. This method replaces a tail biopsy as a sample for genotyping. Distal phalanx biopsy should be performed in rodents 7 days of age or younger and can be done without the use of anesthesia. For rodents >7 days old, justification must be described in the ASAF and anesthesia must be used.

a. Anesthesia for mice > 7 days old
   Local: topical lidocaine.
   Injectable: Ketamine/xylazine, ketamine/dexmedetomidine
   Inhalant: Inhalant anesthetics (isoflurane)

b. Standard Technique
   - No more than 1 toe per paw may be removed
     o Removal of the distal phalanx of the hind paws is preferred over the fore paws
     o DO NOT remove the first digit/toe (i.e. thumb) on either fore paw
• Only remove the last bone of the digit (3rd phalanx)

• Prepare the digit by wiping with alcohol (providone iodine or chlorhexidine solutions may interfere with the DNA identification tests).

• Only use sharp sterile scissors

• Control bleeding with a gauze pad and gentle pressure

• Release rodent

• Observe for bleeding or abnormal behavior and contact OCV staff if noted

References:


Cover CE, Keenan CM, Bettinger GE. Ear tag induced Staphylococcus infection in mice. Lab Anim. 1989 Jul;23(3):229-33. PMID:2761227


Approved by WSU IACUC on: 4/25/18