Mouse Tissue Collection for Genetic Analysis

All Animal Program Personnel

Veterinary Staff, Facility Manager, Animal Care and Research Staff

To Outline the Proper Procedures for the Collection of Mouse Tissue for Genetic Analysis

I. PURPOSE

1. Provide guidelines for techniques and procedures of obtaining tissue to determine the genetic background of newborn, weaned, or adult mice.

II. RESPONSIBILITIES

1. The Veterinarians oversee all aspects of animal health, and are assisted by all program staff.

2. Facility Managers ensure implementation of all procedures.

3. Research staff is required to follow these guidelines.

III. GENERAL CONSIDERATIONS

1. The methods of tissue collection should be described in the IACUC approved protocol under which the animals are held.

2. Polymerase Chain Reaction (PCR) techniques may require less tissue and allow for the use of auricular (ear) flap tissue obtained during ear punch identification procedures. Southern Blot testing usually requires more tissue and makes it necessary to collect tissue from the tail tip.

3. Ear Punching for auricular tissue does not require anesthesia in mice. Several tissue samples, approximately 0.5 mm in diameter, can be obtained using the mouse ear punch (Fischer Scientific – Catalog #01-337B or Kent Scientific – Catalog # INS500075).

4. Samples are preferably taken from mice through 21 days of age. Anesthesia is not required for tail snipping in mice through 21 days of age, if less than 0.5 cm of skin is taken. Innervation of the tip of the tail is minimal at this age. Skin should be pushed down toward the tip of the tail so that the vertebrae are avoided.

5. Anesthesia and analgesia is required for tail tip collections when mice are greater than 21 days old.
IV. ANESTHESIA AND ANALGESIA

1. General anesthesia is provided using a suitable inhalant anesthetic agent (e.g., isoflurane) with appropriate scavenging of the waste anesthetic gas.

2. Analgesia is provided using a suitable systemic anesthetic agent (e.g., carprofen, 5-10 mg/kg SQ).

V. TISSUE HARVEST

1. Prior to collecting tissue(s) for a PI ensure that procedures for handling the collected samples and methods of identifying both samples and animals are clearly understood.

2. Anesthetize the mouse if required, or gently but securely restrain the mouse.

3. Swab the tissue with alcohol (povidone iodine or chlorhexidine solutions may interfere with the DNA identification tests).

4. When collecting auricular tissue, ensure that the sampling location does not interfere with animal identification, or if using ear punch as a method of identification that the code is clearly understood. Disinfect ear-punch instrument between animals using the bead sterilizer followed by cooling with saline.

5. When collecting tail tissue, push the skin toward the tip of the tail and using sterile scalpel, or razor blade, cleanly excise the distal 0.5 cm of tail. The blade as well as the surface the tail is placed should be sterile prior to use and between animals. A bead sterilizer can provide complete sterilization and accomplish hemostasis by cauterization. A disposable blade is recommended to prevent cross-contamination when collecting tail tissue for genetic analysis.

6. If the proper procedures are followed, the yield of DNA from 0.5 cm of tail should exceed 50 micrograms, enough for multiple analyses. The yield of DNA does not proportionally increase as tail fragments larger than 0.5 cm are used. If small amounts of DNA are required, investigators should consider taking only 0.2 cm of tail.

7. For more detailed information on tissue collection techniques a PowerPoint presentation entitled Tail Snips, Tattoos and Identification Procedures - Training Presentation can be viewed at: http://www3.research.usf.edu/cm/docs/powerpoint/Tails_n_Tatts_Training_files/frame.htm

8. Place tissues into a labeled micro-centrifuge tube, and handle according to PI’s instructions.

9. Following tissue collection ensure hemostasis using digital pressure, silver nitrate sticks, Clotisol, or a styptic powder with benzocaine.

10. Ensure that the animal has sufficiently recovered from anesthesia prior to returning it to the housing room.

11. Check tail daily for 7 days to ensure tip is healing.