RODENT IDENTIFICATION

I. Purpose

A conventional procedure for genotyping transgenic animals entails cutting off the distal portion of the animal's tail (tail biopsy). From this tissue, template DNA may be extracted to ascertain the presence or absence of a specific transgene using PCR or other DNA analysis techniques. PCR techniques may require less tissue and allow use of auricular flap tissue obtained during the ear punch identification procedure. Southern Blot testing may require more material and need tissue from the tail. Ideally, mice should be 10-21 days old. At this age, the tail tissue is soft (vertebra are not yet calcified) and the yield of DNA is highest.

II. Responsibility

This procedure applies to colony breeders, researchers, and technicians who collect tissue samples from mice for transgene identification.

III. Identification Procedure: Tail Snipping

A. Standard Techniques
   1. Tail snipping: Anesthesia is not required in mice through 21 days of age if less than 1 cm of skin is taken (skin can be pushed down toward the tip of the tail so that the vertebrae are avoided). Innervation of the tip of the tail is minimal at this age. This procedure must be limited to a maximum of <u>two times</u>, since the tail is important in body temperature control and balance in rodents.
   2. Tail samples greater than 1 cm in length will damage the coccygeal vertebrae and will require anesthesia in mice of any age. Anesthesia is required for any tail snipping if animals are greater than 21 days old.

B. Procedures
   1. Gloves should be worn when handling laboratory mice.
   2. Prepare the mice by sexing and weaning from the cage.
   3. Determine the ID numbers for the mice and record on their cage cards and on the sample collection vials.
   4. Record the procedure in the Breeding Book for the group – include the date/day, parents, generation, ID# with sex and coat color.
   5. Place warmed towel on the counter to prevent loss of body heat if mice are to be anesthetized.
   6. For mice 10-21 days old, a local anesthetic is not required but encouraged. For mice greater than 21 days of age, the use of a local (Emla cream) or general anesthetic (isoflurane) is required prior to collection of tissue.

7. Gently, but securely, restrain the mouse between thumb and forefinger.
8. Swab tail with 70% alcohol (povidone iodine or chlorhexidine solutions may interfere with the DNA identification tests).
9. Push skin toward the tip of tail.
10. Snip skin sample with sterile scissors or sterile #15 scalpel blade.
11. Apply gentle compression with sterile gauze until hemostasis occurs. When hemostasis has been achieved, the gauze will be removed and a small portion of triple antibiotic ointment will be applied to the amputation site as a prophylactic measure.
12. Replace the mouse in the cage. Observe mouse for bleeding or abnormal behavior. Check tail daily to ensure tip is healing.
13. The tail snip is placed in the labeled vial. The scissor blades or scalpel blade are wiped with an alcohol-soaked gauze sponge to remove any tissue and blood.
14. The instruments and equipment are washed in Nolvasan Surgical scrub and then autoclaved. The countertop is cleaned using the disinfectant spray provided in the room. All paper waste is placed in the room trash can.

IV. Identification Procedure: Ear Punches

A. Standard Techniques
   1. Ear punching: Anesthesia is not required in mice through 21 days of age. Several tissue samples approximately 0.5 mm in diameter can be obtained.

B. Procedures
   1. Select the mice for identification
   2. Gloves should be worn when handling laboratory mice.
   3. Prepare the mice by sexing and weaning from the cage.
   4. Determine the location and number of punches necessary to correspond to the numerical identification required. Record number on their cage cards and on the sample vials if collecting tissues for DNA analysis.
   5. Record the procedure in the Breeding Book for the group – include the date/day, parents, generation, ID# with sex and coat color.
   6. Place warmed towel on the counter to prevent loss of body heat if mice are to be anesthetized.
   7. Scruff the mouse and restrain so that the ears are easily accessible.
   8. Place the ear margin in the ear punch so that the hole side of the punch is under the pinna in the desired location.
   9. Squeeze the punch closed quickly and firmly. The punches can be either notches or holes based on the number needed.
   10. Replace the mouse in the cage. Observe mouse for bleeding or abnormal behavior. Check ears daily to ensure healing.

11. The tissue removed in this process may be collected for DNA analysis, is placed in the labeled vial.
12. To prevent rusting and to sanitize between animals, the punch is then thoroughly cleaned with alcohol-soaked gauze sponge and dried before using.
13. The countertop is cleaned using the disinfectant spray provided in the room. All paper waste is placed in the room trash can.

V. Identification Procedure: Ear Tags

A. Standard Techniques
   1. Ear tags: Anesthesia is not required in mice for identification with ear tags.

B. Procedures
   1. Select the mice for identification
   2. Gloves should be worn when handling laboratory mice.
   3. Prepare the mice by sexing and weaning from the cage.
   4. Record the procedure in the Breeding Book for the group – include the date/day, parents, generation, ID# with sex and coat color.
   5. Use tags that are about 5 mm long. Rinse the tags in 70% alcohol before use.
   6. Orient the tag into the tag applier so that the end with the hole is positioned over the notched area of the applier. The pointed end should be opposite the hole.
   7. Scruff the mouse and restrain so that the ears are easily accessible.
   8. Place the ear between the point of the tag and the hole of the tag being careful to position the tag well into the ear as far from the margins as possible. Firmly squeeze the applier closed. The ear tag will pierce the ear and lock together. Release the applier and the tagged ear will fall out of the applier.