ANTEMORTEM TISSUE COLLECTION FOR GENOTYPING/IDENTIFICATION

Table of Contents
1. Background
2. Methods
   a. Tail Tip Collection
   b. Ear Notching or Punching
   c. Toe Clipping
   d. Caudal Fin Clip
3. Summary Table
4. References

Background
To uniquely identify and differentiate individual genotypes of a specific animal, it may be necessary to collect tissue antemortem. These procedures must be outlined in the Animal Use Protocol (AUP) and approved by Animal Care and Use Committee (ACUC). Any proposed deviations from these guidelines require additional justification and approval from the ACUC.

Following AUP approval, individuals must receive training by the Office for Laboratory Animal Care (OLAC) veterinary staff (643-9232) prior to initiation of these procedures. Depending on the procedure, the OLAC veterinary staff may allow training to be conducted by certified lab members or OLAC staff may have to observe and certify individuals prior to independently performing the procedure.

Tail Tip Collection
   A. Rodents
      a. For mice prior to weaning or at weaning (up to 21 days of age), general anesthesia is not required. Instead, the tail tip should be submerged in ice cold water as a local anesthetic.
      b. General anesthesia is required for mice older than weaning age (>21 days of age). Investigators should consider giving a post-biopsy analgesic following general anesthesia.
      c. Sharp, disinfected scissors or sterile blade can be used to cut the tip. No more than 5mm of tail tissue may be removed per rodent per tissue collecting procedure. Repeat tail biopsies require anesthesia and must be justified in the AUP.
      d. Bleeding should be minimal. If bleeding occurs, it can be stopped by applying with gentle pressure over the site.
B. Reptiles
   a. Many reptiles (e.g., lizards) have the ability to autotomize their own tail when seized by a predator. Because this event naturally happens, the tail tip may break off very easily without using surgical instruments.
   b. Anesthetic is not required for this procedure.

Ear Notching or Punching
A. Although only a small piece of the ear is removed, the ear notch or punch remnants can provide enough DNA for an initial PCR screening.
B. Ear punch samples collected on animals under 3 weeks of age do not require the use of anesthesia or analgesics. Anesthesia is required if the animal is greater than 3 weeks of age.
C. The ear punch must be disinfected between animals. These devices can be autoclaved.

Toe Clipping
A. Because of the potential to induce pain and distress in addition to altering the animals’ gait and ability to feed, the need for toe clipping must approved by the ACUC. As per the *Guide for the Care and Use of Laboratory Animals* (*Guide*; Chapter 3; p. 75), this method can be considered by the ACUC for all animals under the following conditions:
   a. Alternative methods of identification are first considered and scientific justification is provided.
   b. The first, most distal bone of one digit per extremity can be removed using this procedure.
   c. The foot should be cleaned with aseptic technique. Sharp sterile scissors should be used and disinfected between uses.
B. Rodents
   a. Toe clipping should only be performed when rodents are between 5-10 days of age and general anesthesia is required for rodents over 7 days of age.
   b. Hemostasis can be achieved by applying gentle pressure over the site until bleeding has stopped.
C. Amphibians
   a. Anesthesia is required.
   b. Hemostasis can be achieved by applying gentle pressure over the site until bleeding has stopped.
Caudal Fin Clip

A. Prior to this procedure, fish must be anesthetized via immersion in anesthetic (e.g., MS-222).

B. The fin is clipped with the sterile blade or scissors at a point not greater than halfway between the tip of the fin and the body. If done correctly, the caudal fin regenerates within two weeks.

Summary Table

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Age</th>
<th>Tissue Amount</th>
<th>Anesthetic Required?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail Tip Collection</td>
<td>&lt; 21 days</td>
<td>&lt; 5 mm</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>&gt; 21 days</td>
<td>&lt; 5 mm</td>
<td>Yes</td>
</tr>
<tr>
<td>Ear Notch/Punch</td>
<td>&lt; 21 days</td>
<td>N/A</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>&gt; 21 days</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Toe Clip</td>
<td>&gt;7 days of age</td>
<td>Distal bone and tissue of 1 digit per extremity</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>(rodents only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudal Fin Clip</td>
<td>N/A</td>
<td>Less than half of the fin</td>
<td>Yes</td>
</tr>
</tbody>
</table>

References