ANTEMORTEM TISSUE COLLECTION FOR GENOTYPING

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Background

To uniquely identify and to characterize the genotypes of individual animals, it may be necessary to collect tissue antemortem. These collection 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 justification and approval from the ACUC. The OLAC staff offers training for in these procedures if needed.

Methods

1. Tail Tip Collection in Mice

a. General anesthesia is required for mice older than weaning age (>21 days of age). General anesthesia is not required for mice prior to the age of weaning (i.e., up to 21 days of age) but it is recommended for mice 14-21 days of age.

b. Investigators should consider giving an analgesic (e.g., buprenorphine 0.05 mg/kg SQ, carprofen 5 mg/kg SQ, or meloxicam 5 mg/kg SQ) to adult mice following general anesthesia.

c. Sharp, sterile scissors or sterile blades are used to remove the tip of the tail. Scissors/blades should be sterilized in between cages of animals. Tools should be sterilized by wiping off organic material with alcohol, followed by the application of heat (hot bead sterilizer). Scissors/blades and work surfaces should be cleaned of all organic material between animals to prevent DNA contamination.

d. 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.

e. Bleeding should be minimal. If bleeding occurs, it should be stopped by applying gentle pressure or a hemostatic agent over the site.

2. Ear Notching or Punching in Rodents

a. Anesthesia is not required for mice at any age, but is required for rats over 3 weeks of age.

b. The ear punch should be cleaned of all organic material between animals to prevent DNA contamination. In addition, the ear punch should be sterilized in between cages of animals by wiping off organic material with alcohol followed by the application of heat (hot bead sterilizer).

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3. Toe Clipping

a. Because of the potential to induce pain and distress in addition to altering an 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:

   i. Alternative methods of identification are first considered and scientific justification for rejecting these methods is provided.

   ii. The first, most distal bone of one digit per extremity can be removed using this procedure.

   iii. The foot should be cleaned with aseptic technique.

   iv. Sharp, sterile scissors or sterile blades are used to cut the toe. Scissors/blades should be sterilized in between cages of animals by wiping off organic material with alcohol followed by the application of heat (hot bead sterilizer). Scissors/blades and the work surface should be cleaned of all organic material between animals to prevent DNA contamination.

b. <u>Toe Clipping in Rodents</u>

   i. Toe clipping should only be performed on rodents up to 7 days of age.

   ii. Care should be taken to remove the least number of digits possible. Only one digit per extremity may be removed, with preference for removing digits from hind paws over forepaws. If removing digits from forepaw, the first digit (i.e., pollex or dewclaw) should not be removed as this may decrease the rodent's grasping ability. Toe clipping may be the preferred method for marking and genotyping neonatal mice up to seven days of age.

iii. Hemostasis can be achieved by applying gentle pressure or a hemostatic agent over the site until bleeding has stopped.

b. Toe Clipping in Amphibians

i. Anesthesia is required.

ii. Sharp, sterile scissors or sterile blade can be used. Scissors/blades should be sterilized in between animals using heat (e.g., hot bead sterilizer, flame) or chemical methods (e.g., 2% glutaraldehde, Cidex with 12 minute contact time, or a 1:10 dilution of bleach) and rinsed free of chemicals before use on animals.

iii. Hemostasis can be achieved by applying gentle pressure or a hemostatic agent over the site until bleeding has stopped.

4. Caudal Fin Clip in Fish

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. Sharp, sterile scissors or sterile blade can be used. Scissors/blades should be sterilized in between animals using heat (e.g., hot bead sterilizer, flame) or chemical methods (e.g., 2% glutaraldehde, Cidex with 12 minute contact time, or a 1:10 dilution of bleach) and rinsed free of chemicals before use in animals.

c. The caudal fin will regenerate within two weeks if the procedure was performed correctly.

Summary Table

Procedure	Age	Tissue Amount	Anesthetic Required?
Tail Tip Collection in Mice	< 21 days > 21 days	< 5 mm < 5 mm	No Yes
Ear Notch/Punch in Mice	< 21 days > 21 days	N/A	No No
Ear Notch/Punch in Rats	< 21 days > 21 days	N/A	No Yes
Toe Clip in Rodents	<7 days of age (rodents only)	Distal bone and tissue of 1 digit per extremity	No
Caudal Fin Clip in Fish	N/A	Less than half of the fin	Yes

References

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