

Standard Operating Procedure

SOP Number: **02-18-5827**

Service: **Research**

Operating Section: **IACUC**

Unit: **LUCHSD**

Title: **Rodent Genotyping**

Purpose:

To define the appropriate procedures for obtaining tissues from mice and rats for genotyping.

Non-invasive methods include hair, fecal or blood samples, and oral or rectal swabs. Depending on the requirements of the study, investigators are urged to consider noninvasive alternatives. For information on these procedures, please contact the CMF Laboratory Coordinator.

Procedure for Ear Punch:

- 1) Procedures for ear punch for genotyping must be described in an approved animal protocol.
- 2) This method involves collecting a sample of tissue from the ear using a punch designed for this task. The method is often used for identification purposes and so is ideal to fulfill both needs at the same time.
- 3) This procedure is best performed at 14-21 days of age but is humane and acceptable at all rodent ages. This procedure does not require the use of anesthetics or analgesics as long as the procedure is performed by a trained individual. Do not use this method in mice under 2 weeks of age.
- 4) Punch a hole 1-2 mm from the ear pinnae margin. If using iris scissors, notch the pinnae margin. Ear punch devices or scissors should be disinfected between animals.
- 5) Instruments used for ear notching can become dull after use and should be replaced often, as dull instruments can cause trauma to the notch site.
- 6) The procedure should not cause bleeding if done properly. If bleeding does occur take proper measures to ensure the bleeding has stopped before returning the animal to its cage.



Procedure for Tail Biopsy:

- 1) Procedures for tail biopsy for genotyping must be described in an approved animal protocol.
- 2) Tail biopsy involves cutting a piece of tissue from the distal end of the tail for the purpose of obtaining a small tissue sample for genetic analysis.
- 3) Tail biopsy of mice and rats is permitted if investigators demonstrate that less invasive alternatives (i.e. ear punch, buccal mucosa, etc.) are unsuitable, and if IACUC approval is obtained. This procedure must not be used as a method of bleeding mice and rats.
- 4) Ideally, mice and rats should be 10-15 days old. At this age, the tail tissue is soft (vertebra are not yet calcified) and the yield of DNA is highest. In addition, prompt analysis of tail tissue allows the desired mice and rats to be identified prior to weaning which can facilitate more efficient use of cage space.

- a. For mice and rats 10-21 days of age, because pain sensory development may be complete, and to further minimize any transient pain or distress, investigators are strongly encouraged to apply local anesthesia to the tail.
 - b. For mice and rats greater than 21 days of age, the use of a local or general anesthetic is required prior to collection of tissue.
 - c. For mice and rats greater than 35 days of age or removal of more than 5 mm of tissue, the use of a general anesthetic is required.
- 5) The tail must be disinfected prior to clipping. With sterile scalpel, razor blade or sharp scissors cleanly excise the distal end of the tail. The total amount of tail tissue clipped and removed should be the minimum necessary (1-2 mm ideally), but no more than 5 mm. For multiple litter samplings, the blade or scissors must be sanitized with an appropriate disinfectant between animals.
 - 6) Repeated tail clips on a single mouse are discouraged. If additional tail clips are required, the rationale must be justified to the IACUC. Additional clips must not be performed at an interval of less than one week from the first clip and the use of anesthesia is mandatory regardless of age or amount of sample taken.
 - 7) Hemostasis must be achieved by observing the animal until no bleeding is observed for 2 minutes before returning the animal to its cage. Surgical glue, silver nitrate or direct pressure with a sterile gauze pad can be used for this purpose. The animal must also be observed until it recovers from general anesthesia, if used. The procedure for controlling bleeding must be described in an approved animal protocol.

Procedure for Toe Clipping:

- 1) Toe clipping is discouraged by the IACUC and requires scientific justification. This procedure has the potential to induce pain and distress, alter the animal's gait and ability to feed. Only one toe per foot may be removed. This method should be used only when no other method is feasible and should be performed only on altricial (hairless) neonates (< 7 days of age). An advantage of toe clipping is that it can be used both as a source of DNA and a method of identification.
- 2) This method involves removal of the distal phalangeal (coffin) bone of one limb. Only the minimum amount of tissue necessary for the objective should be taken.
- 3) A pair of sterile sharp scissors can be used for this procedure. The scissors must be disinfected between each animal.
- 4) Hemostasis must be achieved by observing the animal until no bleeding is observed for 2 minutes before returning the animal to its cage. Surgical glue, silver nitrate or direct pressure with a sterile gauze pad can be used for this purpose. The procedure for controlling bleeding must be described in an approved animal protocol.

Responsible Official Signature		Date	
		07/19/22	
QA Signature		Date	
		07/19/2022	
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