FLORIDA INTERNATIONAL UNIVERSITY

SOP #: 704.02

Title: SOP - Methods for Rodent Genotyping

Approvals:

Attending Veterinarian _____ **Date:** 03/03/2019

1. Purpose

1.1 The intent of this standard operating procedure (SOP) is to describe methods for genotyping in rodents as approved by Florida International University Institutional Animal Care and Use Committee (IACUC).

2. Responsibility

2.1 This SOP is intended for use by Principal Investigators and Animal Care Facility personnel that genotype mice. Any exemptions to this SOP must be approved by the IACUC prior to their application.

3. Definitions

3.1 ACF - Animal Care Facility

3.2 Genotyping: is the process of determining differences in the genetic make-up (genotype) of an individual by examining the individual's DNA sequence using biological assays and comparing it to another individual's sequence or a reference sequence

3.3 PI – Principal Investigators

4. Guidelines

4.1 Genotyping is important when working with transgenic animals. Analysis by Polymerase Chain Reaction (PCR) requires small amounts of DNA which can be obtained from ear tissue, buccal or saliva swabs and hair bulbs.

4.2 The IACUC strongly encourages researchers to use the least invasive procedures for obtaining samples for genotyping.

4.3 Tail Clipping Method

4.3.1 Information: The tail of a mouse contains a variety of tissues, including bone, cartilage, blood vessels and nervous tissues. In a young mouse (<21 days) the tissue near the tip of the tail is soft and the bones have not completely mineralized. Therefore, removing of the tail tip of a young mouse probably amounts to momentary pain for the animal. As the animal ages, tissue maturation includes mineralization of the bone and increased vascularity. Tail tip sampling performed on an older animal (>21 days) is likely to involve more than momentary pain and distress as well as the potential for significant hemorrhage.

4.3.2 Procedures: The IACUC believes that tail tip removal should be performed at as young of an age as is feasible. In most, if not all, cases the procedure can be performed prior to weaning and there is nothing to be gained by genotyping at an older age. Therefore, the IACUC has adopted the following guidelines for tail tip removal.

4.3.2.1 Clipping of tail tissue can be performed without general anesthesia in mice less than 3 weeks (<21 days) of age.

4.3.2.2 General anesthesia is required when tail clipping any mouse that is older than 3 weeks of age.

4.3.3 The total amount of tail tissue clipped and removed should be the minimum necessary (1-2 mm ideally), but not more than 5 mm. If taking more than 5 mm, it is not acceptable at any age without the use of anesthesia.4

4.3.4 Repeated tail clips on a single mouse are discouraged. If additional tail clips are required; the rationale must be justified to the IACUC in the animal use protocol and the use of anesthesia is mandatory regardless of age or amount of sample taken. If you anticipate the possibility of needing an additional sample from a mouse at a later date, cut the original sample in half and preserve the extra piece at -20°C or -80°C. 5

4.3.5 Regardless of age or amount of sample, the tail must be disinfected prior to clipping, any bleeding must be controlled, and the mouse observed until it recovers from any anesthesia provided. If less than 2 mm is taken, then hemostasis can usually be achieved by direct pressure on the end of the tail. If greater than 2 mm is taken or if direct pressure does not work, the use of chemical cauterizing agents is required, and these should always be on hand as a precautionary measure. Styptic powder and silver nitrate are two very effective cauterizing agents commonly used for these procedures.

Rodent Age	< 5 mm Tissue Sample	> 5 mm Tissue Sample
< 21 Days Old	NO Anesthesia Required	Anesthesia Required

> 21 Days Old	Anesthesia Required	Anesthesia Required
ANY AGE	**Always Control Bleeding**	**Always Control Bleeding**

4.4 Ear Clipping Method

4.4.1 Information: This method is less invasive than tail clipping. Ear tissue can be gathered either by ear punching or ear snipping. Special ear punching tools or sharp scissors can be utilized. The procedure is quick, easy, and 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.

4.4.2 Procedures:

4.4.2.1 Ear Punch: Ear punching is also a method of identification used on rodents. This method does not require anesthesia. After performing the identification procedure, the tissue that is "punched" out can be used for genetic analysis.

4.4.2.2 Ear Snip: A small portion (2-3 mm) of the ear pinna is cut off with sharp scissors to obtain tissue. This can be done on mice at any age and does not require anesthesia.

4.4.2.3 Ear punch device and scissors should be disinfected between animals. This can be done by autoclaving or soaking in Peroxiguard for at least 5 minutes or inserting it into a Steris bead sterilizer for 10 seconds.

4.5 Alternative Genotyping Methods

4.5.1 Buccal Swabs/Saliva: This is a non-invasive procedure that can be performed on rodents of any age and does not require anesthesia. Cotton swabs are used to retrieve cheek cells from the mouths of mice. Samples are processed and subjected to genetic analysis via PCR.

4.5.2 Blood: Blood samples can be obtained using any standard blood collection method. The sample can be used for PCR analysis.

4.5.3 Hair Bulbs: This method is a non-invasive procedure in which hair is plucked from the animal and for use in genetic analysis.

4.5.4 Fecal Pellets: Collecting stool is a non-invasive procedure. Stool can be collected directly from the animal or from the cage.10

5. References

5.1 Kansas State University, IACUC Guide #5;
http://www.ksu.edu/research/animal/iacuc/iacuc05.html

5.2 IACUC at the University of Washington; _SOP 403.01- Methods for Rodent Genotyping
http://www.hscer.washington.edu/iacuc/policies/tailing.html

5.3 Penn State IACUC Guidelines;
http://www.research.psu.edu/orp/areas/animals/policies/guide2.asp

5.4 University of Iowa, IACUC Guides; http://research.uiowa.edu/animal/?get=tbiopsy

5.5 Meldgaard, M., P.J.A. Bollen, and B. Finsen. Non-invasive method for sampling and extraction of mouse DNA for PCR. Laboratory Animals (2004) 38, 413-417

5.6 Zimmermann, K., H.P. Schwarz, and P.L. Turecek. Deoxyribonucieic Acid Preparation in Polymerase Chain Reaction Genotyping of Transgenic Mice. Comparative Medicine (2000) 50(3), 314-316

5.7 NIH Guidelines for the Genotyping of Rodents;
http://oacu.od.nih.gov/ARAC/FinalGenotyping0602.pdf

5.8 Campbell, D.B., Hess, E.J. Rapid genotyping of mutant mice using dried blood spots for polymerase chain reaction (PCR) analysis. Brain Research Protocols, 1:117-123, 1997.

5.9 Schmitteckert EM, Prokop CM, Hedrich HJ. DNA detection in hair oftransgenic mice – a simple technique minimizing the distress on the animals. Lab Animal. October 1999. 33(4): 385-9.

5.10 Broom, RL, Broome RL, Feng L, Zhou Q, Smith A, Hahn N, Matsui SM, Omary MB. Non-invasive transgenic mouse genotyping using stool analysis. FEBS Letters. November 26, 1999. 462(1-2): 159-160.

6. References

6.1 Rev 02 (March 2019) – change on the sterilization method for the ear punch.