Collection of Tissues For Genotyping

IACUC Standard Procedure
Effective Date: February 2024

UCSF Office of Research
Institutional Animal
Care and Use Program

Description of Procedure:

Collection of tissues for genotyping is a common procedure when using genetically modified mice. Researchers should remove the least amount of tissue necessary to perform genotyping and consider using genotyping techniques that do not permanently alter the animal (ex: collection of hair, fecal pellets, buccal swabs) when scientifically appropriate. The use of rusted or dull equipment is unacceptable. Scissors and reusable punches should be sharpened or replaced at appropriate intervals based on use. Blades should be discarded after each session.

Tail Snip: The most common method for gathering tissue is tail snipping. Refer to these steps when performing tail snipping:

1. Restrain or anesthetize the rodent.
2. Starting with sanitized scissors, cut the defined length of distal tail.
3. Monitor the animals to assure hemostasis after the rodents are returned to the cage. If needed, apply digital pressure, heat cautery (briefly), or silver nitrate to stop bleeding.
4. Take great care to remove all tissue from the scissors or scalpel after each animal. Disinfect scissors/ scalpel with 70% ethanol between animals to reduce risk of infection and prevent sample contamination.
5. Take no more than 1-2 mm of tissue as described below.

Other analytical and confirmatory techniques may require more tissue, however, the amount taken may not exceed 2mm. If additional samples are needed from the same animal, the PI must first secure IACUC approval of the request in the protocol and include justification for why additional tail sample must be collected and why alternative sample collection sites cannot be used. Analgesic administration may also be required depending on the request.

Refer to Table 1 below for anesthesia requirements by age. Administer analgesics per LARC veterinarian prescription. Refer to UCSF Anesthesia Guidelines when using anesthesia.

Table 1.

Age	Length of Tail	Anesthesia required
<P21	<2 mm	no
≥P21	2mm maximum	yes

Ear Notching: If ear notching mice for identification, the piece of tissue punched out should be used for genotyping. Refer to IACUC Standard Procedure, Rodent Identification for a description of this procedure. Does not require anesthesia.

Ear Snip: A small portion (2-3 mm) of the edge of the pinna is cut off with sharp scissors to obtain tissue. This can be done on mice once the ears have developed (> 8 days of age) and does not require anesthesia.

Toe Clip: Toe clipping is not a standard procedure and must be justified in the IACUC protocol. If justified, toe clipping tissue may be used for genotyping. Refer to IACUC Standard Procedure, Rodent Identification for a description of this procedure.

Hair: Tufts of hair (n=2 tufts per mouse, > 20 follicles) are plucked from the animal using tweezers or hemostats to obtain samples. Samples can be collected at the neck line between the shoulder blades. Animals should not have exposed patches of skin following sampling, as only small tufts are needed. This method does not require anesthesia. Care should be taken to avoid contamination with fomites and with hair from cage mates of the animal to be assessed.

Fecal Pellets: Samples of feces (n=3 pellets) can be collected directly from the animal at the time of defecation, or from the cage floor of individually housed animals within 24 hours of defecation. Epithelial cells shed in the feces are the target tissue type for processing and analysis. This method does not require anesthesia.

Buccal Swabs/Saliva: Salivary samples to harvest epithelial cells from the mouth can be performed on rodents once they are a few days old; this method does not require anesthesia. Individual sterile mini-cotton swabs (rubbed against both inner cheeks per swab) should be used to sample cells. Care should be taken within the mouths of animals to ensure gentle swabbing.

Agents:

For procedures that require anesthesia, all agents administered to animals should be listed in the “Agents” section of the IACUC protocol .

Potential adverse effects to be considered:

Hemorrhage, infection.

References:

Animal Research Advisory Committee, NIH, Office of Intramural Research. 2018. Guidelines for Tissue Collection for Genotyping Mice and Rats.

Braden-Weiss, G.C., Brice, A.K., Hankenson, F.C. 2011. Minimizing the impact of tail biopsy in preweanling laboratory mice: inhaled isoflurane compared with topical anesthetics. J Am Assoc Lab Anim Sci **50**: 736-737.

Braden , G.C., Brice, A.K., Hankenson, F.C. 2015. Adverse effects of vapocoolant and topical anesthesia for tail biopsy in preweanling mice. J Am Assoc Lab Anim Sci **54**: 291-298.

Broome, R. L., L. Feng, Q. Zhou, A. Smith, N. Hahn, S. M. Matsui, and M. B. Omary. 1999. Non-invasive transgenic mouse genotyping using stool analysis. FEBS Letters **462:**159-60.
Cinelli, P., A. Rettich, B. Seifert, K. Burki, and M. Arras. 2007. Comparative analysis and physiological impact of different tissue biopsy methodologies used for the genotyping of laboratory mice. Laboratory Animals **41:**174-84.

Dudley E.S., Johnson R.A., French D.C., Boivin G.P. 2016. Effects of Topical Anesthetics on Behavior, Plasma Corticosterone, and Blood Glucose Levels after Tail Biopsy of C57BL/6NHSD Mice (Mus musculus). JAALAS 55(4):442-450.

Garzel LM , Hankenson FC, Combs J, Hankenson KD. 2010. Use of quantitative polymerase chain reaction analysis to compare quantity and stability of isolated murine DNA**.** *Lab Anim (NY),* 39(9): 283-289.

Hankenson, F.C., Braden-Weiss, G., Blendy, J.A. 2011. Behavioral and activity assessment of laboratory mice (Mus musculus) after tail biopsy under isoflurane anesthesia. J Am Assoc Lab Anim Sci **50:**686-94.

Hankenson, F. C., L. M. Garzel, D. D. Fischer, B. Nolan, and K. D. Hankenson. 2008. Evaluation of tail biopsy collection in laboratory mice (Mus musculus): vertebral ossification, DNA quantity, and acute behavioral responses. J Am Assoc Lab Anim Sci **47:**10-8.

Irwin, M. H., R. J. Moffatt, and C. A. Pinkert. 1996. Identification of transgenic mice by PCR analysis of saliva. Nature Biotechnology **14:**1146-8.

Picazo, M.G., García-Olmo, D.C. 2015. DNA from tissues of young mice is optimal for genotyping. Electronic Journal of Biotechnology. 18: 83-87.

Schmitteckert, E. M., C. M. Prokop, and H. J. Hedrich. 1999. DNA detection in hair of transgenic mice--a simple technique minimizing the distress on the animals. Laboratory Animals **33:**385-9.