Rodent Genotyping Guidelines

The University of Maryland School of Medicine Institutional Animal Care and Use Committee (IACUC) provides the following guidelines to assist the research community by setting and clarifying the minimum standards for genotyping techniques that are consistent with federal regulations and/or guidance. Carefully designed breeding strategies and accurate genotype assessment can help to minimize the generation of animals with unwanted genotypes (Guide 2011).

Genotyping is the process through which the genotype of an animal is determined using a tissue sample for extraction and purification of deoxyribonucleic acid (DNA). To determine if mice are of the proper genotype for research studies, DNA must be isolated from viable tissue sources and analyzed using polymerase chain reaction (PCR), single-nucleotide polymorphism analysis, or Southern blotting. Once the genotype has been determined, animal colony populations can be maintained at reduced animal numbers necessary for experimental efficiency. This document provides guidance in various approved methods of genotyping.

KEY CONCEPTS

- Researchers should ***remove the least amount of tissue necessary*** to perform genotyping. Minimal amounts of tissue, documented to result in genotyping by PCR, are listed below by tissue type.
- If animal identification is being performed through the removal of a piece of tissue—that same sample of removed tissue should be used for genotyping purposes.
- Methods that do not permanently alter the animal or produce slight momentary pain should be prioritized, when scientifically applicable, i.e. if hair, fecal pellets, and buccal swabs can produce consistent genotyping results with your animal model and housing method, then those techniques should be considered.
- Most of the tissue-harvesting procedures require equipment (scissors, punches, tweezers, razor blades, etc). All instruments should be disinfected (e.g., steam sterilization, wiped with ethanol) prior to use. If multiple animals are to be genotyped in a single session, it is recommended that instruments should be disinfected (e.g., wiped with 70% ethanol) between animals.
- To prevent DNA contamination, it is recommended to use a fresh, sterile scalpel for *each* animal. Alternatively, a glass bead sterilizer (230 ˚C) may be used between animals. Use water (+/- soap if needed) to remove organic debris then place the distal 1/3 of the instrument (that will contact the animal) in the glass bead unit for 10 seconds (or longer dependent on manufactures requirements). After removal, the entire instrument should be placed on a sterile field to air cool 5 minutes minimum prior to use on the next animal or may be rinsed with sterile water or saline to cool the tips if immediate re-use is required.
- Rusted or dull equipment is unacceptable for use when genotyping animals. Scissors should be sharpened or replaced at appropriate intervals (based on use). Blades should be discarded after each session (discarded at least each day).

PREFERRED TISSUE COLLECTION SITES

Ear Pinna

Ear tissue can be harvested either by ear punching of a circle of tissue or ear snipping of the edge of the pinna. The procedure should not cause bleeding if done properly. If bleeding does occur, ensure the bleeding has stopped before returning the animal to its cage.

Procedures:

Ear Punch: Ear punching (2 mm diameter) taken from the middle of the pinna is the preferred sampling site. Care should be taken to not accidentally lose track of the small piece of tissue following the punch. This method does not require anesthesia, but should be performed on mice close to weaning age or older to ensure that the pinnae are large enough for the punch size. Sharp commercial punch devices should be used for this procedure.

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.

Hair

Tufts of hair (5 – 10 follicles) are plucked from the animal using flat forceps to obtain samples. *Note: The hairs usually are loose and not much effort is needed to dislodge them. If pulled too strongly, the hairs stick to the forceps due to static electricity and will be hard to transfer to the eppendorf tube.* 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. Spray down the forceps with 70% ethanol and clean with a paper towel between subsequent collections. 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.

CONDITIONALLY ACCEPTABLE **TISSUE COLLECTION SITES** *(performed by experienced staff)*

Distal Tail

The tail of a mouse contains a variety of tissues, including bone, cartilage, blood vessels and nerves. In a pre-weanling mouse, the **distal 2mm** tail does not contain mature vertebrae (bone). Therefore, removal of the very end of the tail (≤2mm) is comparable to removal of a similar size of tissue from the mouse ear. The tail biopsy should be performed at as young of an age as is feasible. In most, if not all cases, the procedure can and should be performed prior to weaning.

If re-sampling for repeat genotyping from the same mouse, **no more than 5 mm cumulative** of the distal tail should be harvested. In this situation, other tissue sources should be used for harvest (*e.g. ear pinna*).

Procedures:

1. ***For pre-weaned mice:*** Biopsy of tail tissue can be performed without general anesthesia in mice prior to weaning age.
2. ***For weaned mice (>28 days):*** With increasing age, tail maturation includes mineralization of bone and increased vascularity; it has been demonstrated that tail biopsy sampling performed on older mice (> 28 days) can result in prolonged discomfort. **Anesthesia (*e.g., Isoflurane, Ketamine / Xylazine*) is required when tail biopsy is performed on animals older than 28 days of age. Topical cetacaine or ethyl chloride sprays *do not* provide appropriate anesthesia/analgesia and should not be used on mice.**
3. Any bleeding at the tail tip must be controlled (hemostasis) following the biopsy. If less than 2 mm is taken, hemostasis can usually be achieved by direct manual pressure with clean paper towel or gauze on the end of the tail. If direct pressure does not stop the bleeding, the use of hemostatic agents (e.g. styptic powder (Kwik-Stop ®) is recommended and should be readily available as a precautionary measure. Animals may not be left with

actively bleeding collection sites. Animals should not be returned to the cage until bleeding is controlled.
4. If general anesthesia has been administered, the mouse must be observed until it regains consciousness.
5. If you anticipate the possibility of needing an additional tissue sample from a mouse at a later date, other tissue sources (described above) are recommended so as to not remove excess tail tissue.

ADDITIONAL RESOURCES

Boivin, GP, et al. 2013. Genotyping DNA Isolated Using Cross-Linked Iminodiacetate Sytrene Divinylbenzene Copolymer Beads. J Am Assoc Lab Anim Sci **52:**682.

Bonaparte D, Cinelli P, Douni E, Hérault Y, Maas M, Pakarinen P, Poutanen M, Lafuente MS, Scavizzi F. 2013. FELASA guidelines for the refinement of methods for genotyping genetically-modified rodents: a report of the Federation of European Laboratory Animal Science Associations Working Group. Lab Anim. Jul;**47**(3):134-45.

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.

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.

Burkhart, C. A., M. D. Norris, and M. Haber. 2002. A simple method for the isolation of genomic DNA from mouse tail free of real-time PCR inhibitors. Journal of Biochemical and Biophysical Methods **52:**145-9.

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.

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.

Institute of Laboratory Animal Resources, N. R. C. 2010. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C.

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.

Jones CP, Carver S, Kendall LV. 2012. Evaluation of common anesthetic and analgesic techniques for tail biopsy in mice. J Am Assoc Lab Anim Sci. Nov;**51**(6):808-14.

Paluch, LR, Lieggii, CC, Dumont, M, Monette, S, Riedel, E, Lipman, NS. 2014. Developmental and Behavioral Effects of Toe Clipping on Neonatal and Preweanling Mice with and without Vapocoolant Anesthesia. JAALAS Vol 53(2): 132-140.

Petkov, P. M., M. A. Cassell, E. E. Sargent, C. J. Donnelly, P. Robinson, V. Crew, S. Asquith, R. V. Haar, and M. V. Wiles. 2004. Development of a SNP genotyping panel for genetic monitoring of the laboratory mouse. Genomics **83:**902-11.

Pinkert, C. A. 2003. Transgenic animal technology: alternatives in genotyping and phenotyping. Comparative Medicine **53:**126-39.

Ren, S., M. Li, H. Cai, S. Hudgins, and P. A. Furth. 2001. A simplified method to prepare PCR template DNA for screening of transgenic and knockout mice. Contemporary Topics in Laboratory Animal Science **40:**27-30.

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.