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CAR4: GUIDELINE ON RODENT GENOTYPING

Genotyping is the process through which the genetic makeup 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. Carefully designed breeding strategies and accurate genotype assessment can help to minimize the generation of animals with unwanted genotypes (Guide 2011).

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 rectal 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, specialized sterile swabs, scalpel blades, etc). If multiple animals are to be genotyped in a single session, instruments should be disinfected (e.g., wiped with 70% ethanol) between animals to prevent DNA contamination. Other methods to prevent contamination by instruments used between animals include hot bead sterilizers, use of sterilized equipment or new individual blades.
- 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).

TISSUE COLLECTION SITES

Distal Tail

The tail of a mouse contains a variety of tissues, including bone, cartilage, blood vessels and nerves. In a preweaned 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.

Procedures:

- Biopsy of tail tissue can be performed without general anesthesia in mice prior to weaning age. ***Topical cetacaine or ethyl chloride sprays do not provide appropriate anesthesia/analgesia and should not be used on mice.*** 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. ***General anesthesia is required when tail biopsy is performed on animals older than 28 days of age.***

- 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.
- If general anesthesia has been administered, the mouse must be observed until it regains consciousness.
- **If you anticipate the possibility of needing an additional tissue sample from an animal at a later date, *other tissue sources (described below) are recommended so as to not remove excess tail tissue.***

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 animals 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 animals once the ears have developed (> 8 days of age) and does not require anesthesia.

Rectal Swabs

With appropriately sized swab tips (Puritan PureFloc Ultra Swab Micro Ultra-fine Tip (25-3318-U); tip diameter 0.14" (3.556 mm)), rectal swabs have recently been shown in adult mice and rats to serve as a less invasive and robust source of cellular DNA for genotyping. For mice and rats, the rectal swab provided the highest quality results, comparable with the ear punch control, followed in quality by the oral swab. The recommended approach for sampling is taken with swab inserted 50-100% into rectum and circled 3-4 times while pressed against the mucosa. It is recommended that animals be sedated for this collection method, as there may be some slight mucosal bleeding after sampling. *This method should not be used for neonatal/pre-weaning animals.*

Skin Swabs

Sterile swabbing of the ventrum of mice (at least 3 strokes of about 3 cm length, against direction of hair growth) has been shown to be successful; however, others have shown this to be a challenging method to obtain enough sloughed skin cells on the swab from which cellular DNA can be detected for either rats or mice.

Buccal Swabs/Saliva Sampling

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 but can be invasive and distressful to young mice and rats. 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. It may be challenging to obtain appropriate volumes of DNA to achieve positive genotyping results.

Toes

Removal of the distal end of a toe can be performed for genotyping purposes in **animals between 5 to 7 days of age** when the toes have developed and become well-separated. It is preferable to remove digits from a hind paw rather than a forepaw, especially if the animals will be used in studies that include grip strength testing. If the forepaw must be used, it is preferable to not cut the hallux ("dew claw" or "little toe" of the forepaw) as this may decrease the rodent's grasping ability. ***The primary use of this procedure is for identification purposes***; however, the toe sample should serve a dual-purpose if genotyping is to also be performed. *Please see the Rodent Identification Guideline for more information on this procedure.*

Hair

Tufts of hair (2 tufts per mouse, > 20 follicles) are plucked from the animal using tweezers or hemostats to obtain samples. Samples can be collected at the neckline 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

NOTE: Recent review of this methodology (Dysko, Hankenson et al, 2020) found that fecal samples from rats and mice tend to be insufficient in epithelial cell count and fecal samples from mice did not yield detectable quality DNA compared to other sample types tested. Therefore, this method is no longer recommended when compared to other sampling options described above.

ADDITIONAL RESOURCES

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

Bonaparte D, et al. 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. **47**(3):134-45.

Braden GC, et al. 2015. Adverse Effects of Vapocoolant and Topical Anesthesia for Tail Biopsy of Preweanling Mice. J Am Assoc Lab Animal Sci **54**(3): 291-298.

Broome RL, et al. 1999. Non-invasive transgenic mouse genotyping using stool analysis. FEBS Letters **462:**159-60.

Burkhart CA, et al. 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, et al. 2007. Comparative analysis and physiological impact of different tissue biopsy methodologies used for the genotyping of laboratory mice. Laboratory Animals **41:**174-84.

Dysko RC, Hankenson FC, et al. 2020.Assessment of Existing and Novel Tissue Sample Collection Methods for Standard and Automated Rodent Genotyping. National AALAS Meeting, virtual platform.

Garzel LM, et al. 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 FC, et al. 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 FC, et al. 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, NRC. 2010. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C.

Irwin MH, et al. 1996. Identification of transgenic mice by PCR analysis of saliva. Nature Biotechnology **14:**1146-8.

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

Office of Animal Care and Use, NIH. 2022. Guidelines for Tissue Collection for Genotyping of Mice and Rats. { https://oacu.oir.nih.gov/system/files/media/file/2022-01/b3-rodent_genotyping.pdf}

Okada M, et al. 2017. An Efficient, Simple, and Noninvasive Procedure for Genotyping Aquatic and Nonaquatic Laboratory Animals. J Am Assoc Lab Anim Sci. **56**(3): 570–573.

Paluch L, et al. 2014. Developmental and Behavioral Effects of Toe Clipping on Neonatal and Preweanling Mice with and without Vapocoolant Anesthesia. J Am Assoc Lab Anim Sci. **53**: 132-140.

Petkov PM, et al. 2004. Development of a SNP genotyping panel for genetic monitoring of the laboratory mouse. Genomics **83:**902-11.

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

Ren S, et al. 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 EM, et al. 1999. DNA detection in hair of transgenic mice--a simple technique minimizing the distress on the animals. Laboratory Animals **33:**385-9.