Animal Research Protection Program
Policy

Tissue Collection for Genotyping Genetically Altered Rodents

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Background

It is often necessary to obtain tissues samples from potentially genetically altered rodents (rats and mice) to determine or confirm their genotype. A number of methods exist for tissue sampling and the chosen technique should balance the humane treatment of the animal with research needs. The most commonly used method is the removal of tail tissue ("tailing") for DNA analysis by Polymerase Chain Reaction (PCR) or Southern Blot. Less invasive methods are available to obtain DNA including ear punches, hair samples, saliva/oral swabs, blood, or fecal analysis. Furthermore, if tailing, PCR is encouraged since it requires smaller tissue samples when compared to Southern Blot. Other factors for consideration to ensure that pain and distress are minimized include the age of the rodent sampled, use of local or general anesthesia and analgesia, and documentation of both procedures and training of personnel performing the procedure.

Scope

This affects all rodents at OHSU that will be genotyped.

Policy

A complete description of all tissue collection procedures should be included in the eIACUC protocol. If using invasive collection procedures (e.g. tail biopsy), investigators should provide justification as to why less invasive methods are unsuitable for their genotyping. If a tail biopsy is to be used, the following procedures will be followed:

I. A single tail sample 2mm in length is the maximum allowed. If more than a single sample of tail is needed, then justification should be provided. Larger sections of tail will consist mainly of bone thus increasing the chance for pain and may not increase the DNA yield.
II. Ideally, tail tissue should be obtained at approximately 2-3 weeks of age. At this age, the caudal vertebrae are not fully ossified, less trauma is induced, and the maximum sample (see table) can be obtained. Obtaining tail samples in young rodents less than 2 weeks of age is discouraged, unless justification is provided.
III. All tail sampling in rodents older than 3 weeks of age must be done under general anesthesia (e.g. isoflurane,). Additional analgesia may be needed under these circumstances. Consult the DCM veterinarians to discuss pain management prior to submitting the animal care protocol.
IV. A sterile scalpel, blade, or sterile surgical scissors can be used. New or disinfected instruments should be used in-between animals to decrease cross-contamination of samples.
V. Hemostasis must be provided by using pressure, silver nitrate, or tissue glue (Vetbond). It is required that animals have stopped bleeding before being placed back into their cages
VI. To prevent repeat sampling, investigators are encouraged to freeze a portion of tail as a backup sample.
VII. If additional genomic DNA is needed, the use of less invasive techniques should be

considered. Repeat tail samples should be justified in the animal protocol and preemptive analgesia is required (contact DCM veterinarians for agents and dosages).

Procedure	Less than 2 weeks of age	2-4 weeks of age	Greater than 4 weeks of age
Saliva or fecal samples	Acceptable	Acceptable	Acceptable
Buccal or rectal epithelium	Not recommended[1] Acceptable w/justification	Acceptable	Acceptable
Tail biopsy ("tailing")	Not recommended; Acceptable with justification	Acceptable[2]	Acceptable w/justification[2]
Ear notching	Not recommended[1]	Acceptable	Acceptable
Blood	Not recommended[1]	Acceptable	Acceptable
Hair bulb	Acceptable[3]	Acceptable	Acceptable
Toe amputation	Not recommended Acceptable with justification[4]	Not acceptable	Not acceptable

1Size makes sample collection difficult and potentially harmful to pup.
2General anesthesia is required for rodents older than 3 weeks of age.
3Early neonates do not have hair; therefore this method will not be applicable until at least 10 days of age.
4Toe amputation as a method for genotyping or identification is not recommended, except in exceptional circumstances where no other method is feasible. These should be scientifically justified in the animal use protocol and should only be performed in neonatal animals that are less than 12 days of age (eyes should not be open).

References and Additional Resources:

1. Refinement and reduction in production of genetically modified mice. Lab Anim 2003;37 Suppl 1:S1-S49.

2. Cinelli P, Rettich A, Seifert B, et al. Comparative analysis and physiological impact of different tissue biopsy methodologies used for the genotyping of laboratory mice. Lab Anim 2007;41(2):174-184.

3. Hankenson FC, Garzel LM, Fischer DD, et al. Evaluation of tail biopsy collection in laboratory mice (Mus musculus): vertebral ossification, DNA quantity, and acute behavioral responses. J Am Assoc Lab Anim Sci 2008;47(6):10-18.

4. Irwin MH, Moffatt RJ, Pinkert CA. Identification of transgenic mice by PCR analysis of saliva. Nat Biotechnol 1996;14(9):1146-1148.

5. Lahm H, Hoeflich A, Rieger N, et al. Identification of transgenic mice by direct PCR analysis of lysates of epithelial cells obtained from the inner surface of the rectum. Transgenic Res 1998;7(2):131-134.

6. Meldgaard M, Bollen PJ, Finsen B. Non-invasive method for sampling and extraction of mouse DNA for PCR. Lab Anim 2004;38(4):413-417.

7. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. 2011;.

8. Schmitteckert EM, Prokop CM, Hedrich HJ. DNA detection in hair of transgenic mice--a simple technique minimizing the distress on the animals. Lab Anim 1999;33(4):385-389.

9. Suematsu N, Isohashi F. Rapid and simple screening of transgenic mice: novel extraction-free, filter-based PCR genotyping from blood samples. Acta Biochim Pol 2006;53(3):613-616.

Authority
Guide for the Care and Use of Laboratory Animals, Eighth Edition, 2011

U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training

PHS Policy