Guidelines for Tissue Collection for Genotyping of Mice and Rats

Purpose: The correct genetic identification of genetically modified rodents is critical to the efficiency and reproducibility of research and for reducing the number of animals involved in a research project. The genotype is most often determined by analysis of DNA extracted from tissues of young rodents. Historically, tissue biopsies (e.g., pinna, tail, and distal phalanx) have been the most common methods used, but biopsies must be carefully performed because they have the potential to result in some level of pain and/or distress (1-3). Other less invasive but more technically challenging testing methods using hair follicles, blood, feces, ocular tear samples, or oral swabs have been described (1, 4-15).

Researchers should use the least invasive method that is practical for their research and should collect the smallest sample necessary for reliable results. Prompt collection and analysis of tissue allows the desired mice/rats to be identified prior to weaning and will facilitate more efficient use of cage space. The Principal Investigator must ensure sufficient training for individuals performing these technical procedures.

When performing sample collection for genotyping, the following guideline should be considered to minimize the risk of cross contamination and ensure high quality DNA samples are used to produce accurate results:

- Ensure the work surface is cleaned with 70% ethanol before getting started.
- Start with clean, sterilized instruments and disinfect with 70% ethanol between each animal after ensuring that all sample material has been transferred to the appropriate labeled sample collection tube.
- Transfer animals from their home cage to a new clean cage as they are sampled/identified to ensure each animal is sampled only once.
- Verify the animal's ID and double check that the animal ID and sample tube label match.
- Confirm the sample material is fully placed within the sample tube and that the tube is closed.
- Store samples at -20°C until genotyping.
- Keep in mind that DNA yield and quality for genotyping is often better from younger animals (12, 16).
- Try to collect a uniform sample from each animal – too little or too much tissue can interfere with the efficiency of DNA extraction and PCR reactions.

All tissue collection procedures must be described in either an approved Animal Study Proposal (ASP) or referred to in an ACUC approved Standard Operating Procedure (SOP). Basic recommendations for each biopsy method are provided below.

Pinna Biopsy:

Pinna biopsy or ear punch offers the advantage of having tissue collection and permanent identification completed in one procedure. In rodents, the ear is sufficiently developed around

14 days of age to allow suitable tissue collection, although earlier timepoints have been suggested with use of specialized technique and training (9). Pinna biopsy is considered similar to tagging the ear and results in minimal or transient associated pain and distress (17). A two (2) millimeter ear punch or marginal notch is recommended. If repeated biopsies are required, the use of the contralateral pinna or an alternate method should be considered. Pinna biopsies performed as described do not require the use of anesthetics or analgesics.

Tail Biopsy:

Tail biopsy is an effective and humane method of tissue collection analysis when performed correctly and has minimal effects on long-term pain responses (18). Pain perception of mid-tail clamping in rats is reported to develop between 12 to 14 days of age (19), so performing tail biopsy as early as possible in rodents should minimize potential pain (4, 20, 21). The recommendations provided below for tail biopsy are based on the referenced journal articles and are intended to minimize or alleviate any transient pain that may occur.

Tail biopsy length should be limited to the smallest amount possible. In general, a biopsy of approximately 2 mm is sufficient to generate DNA for multiple PCR reactions. Initial biopsies of 2mm or less in young animals (<21 days) likely prevents the cutting of ossified bone, a potentially painful procedure (16). If larger sample sizes are required at any age, a justification should be included in the ASP.

For preweaning animals (<21 days of age), the use of anesthesia is recommended. For mice 21 days of age or older and for rats 21-35 days of age, the use of anesthesia is required unless justified in the ASP or otherwise approved by the ACUC. For rats >35 days of age general anesthesia is required.

Anesthetics and analgesics should be chosen in consultation with the Attending Veterinarian. Potential procedural anesthetics and analgesics for tail biopsy may include but are not limited to the following:

- Local anesthesia by immersion of the tail tip in ice cold ethanol for 10 seconds prior to biopsy may provide sufficient anesthesia for the biopsy procedure (22).
- General anesthesia with isoflurane is used safely in many programs for chemical restraint and procedural analgesia.
- Although used in some programs, the use of vapocoolants (e.g., ethyl chloride) for local procedural anesthesia/analgesia has been reported to result in undesirable aftereffects (23-25).

Post-procedural analgesia should be considered. Topical analgesics and non-steroidal anti-inflammatory agents are used in many programs. The need to provide an effective analgesic (e.g., an opioid such as buprenorphine) post-biopsy will increase with the age of the rodent post weaning, length of the biopsy, and/or with repeated biopsies.

The investigator must monitor the animals to assure hemostasis after the rodents are returned to the cage. To achieve hemostasis, digital pressure, styptic powder (i.e., KwikStop), heat cautery (briefly), silver nitrate, or other effective methods can be used. If silver nitrate is used, the tissue must be washed free of the chemical with saline following hemostasis to neutralize the chemical reaction. Heat cautery should only be applied to animals under local or general anesthesia.

Distal Phalanx Biopsy:

Distal phalanx biopsy (DPB) is the removal of a portion of a digit corresponding to the third phalanx, P3 (3, 26). DPB is used as a method of identifying small rodents by using a predetermined numbering code and the technique may simultaneously be used as a method to obtain biopsy tissue for genotyping by PCR. DPB should only be used in altricial pre-weaning rodents (e.g., mice and rats; NOT guinea pigs) after the toes are no longer webbed (usually between postnatal days 4 and 5) and up to seven days of age.

Studies in mice indicate that DPB produces no more acute pain or distress than other commonly used rodent identification procedures when performed from five to seven days of age (3, 9, 16, 18, 27). These studies also reported no long-term effects of this procedure in test batteries evaluating physiological, developmental, and behavioral assessments (3, 5, 26, 28). It may be the preferred method for neonatal mice up to seven days of age, especially if toe clipping and genotyping can be combined (6).

Every reasonable effort should be made to minimize pain or distress, including limiting the number of digits clipped to one digit per rodent. It is preferable to remove a digit from a hind paw rather than a forepaw, especially if the animal will be used in studies that include grip strength testing (3, 10). 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. To ensure pain and distress is minimized, small sharp scissors should be used and personnel performing the procedure should be trained and proficient in the technique.

References:

1. Balafas E, Katsila T, Melissa P, Doulou A, Moltsanidou E, Agapaki A, et al. A Noninvasive Ocular (Tear) Sampling Method for Genetic Ascertainment of Transgenic Mice and Research Ethics Innovation. Omics. 2019;23(6):312-7.
2. Hankenson FC, Braden-Weiss GC, Blendy JA. Behavioral and activity assessment of laboratory mice (Mus musculus) after tail biopsy under isoflurane anesthesia. J Am Assoc Lab Anim Sci. 2011;50(5):686-94.
3. Paluch LR, Lieggi CC, Dumont M, Monette S, Riedel ER, Lipman NS. Developmental and behavioral effects of toe clipping on neonatal and preweanling mice with and without vapocoolant anesthesia. J Am Assoc Lab Anim Sci. 2014;53(2):132-40.
4. Bonaparte D, Cinelli P, Douni E, Hérault Y, Maas M, Pakarinen P, et al. 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. 2013;47(3):134-45.
5. Castelhano-Carlos MJ, Sousa N, Ohl F, Baumans V. Identification methods in newborn C57BL/6 mice: a developmental and behavioural evaluation. Lab Anim. 2010;44(2):88-103.
6. Chen Z, Mantha RR, Chen JS, Slivano OJ, Takahashi H. Non-invasive genotyping of transgenic animals using fecal DNA. Lab Anim (NY). 2012;41(4):102-7.
7. Hamann M, Lange N, Kuschka J, Richter A. Non-invasive genotyping of transgenic mice: comparison of different commercial kits and required amounts. Altex. 2010;27(3):185-90.
8. Meldgaard M, Bollen PJ, Finsen B. Non-invasive method for sampling and extraction of mouse DNA for PCR. Lab Anim. 2004;38(4):413-7.
9. Morales ME, Gereau RW. The effects of tail biopsy for genotyping on behavioral responses to nociceptive stimuli. PLoS One. 2009;4(7):e6457.
10. Murgatroyd C, Bilko D, Spengler D. Isolation of high-quality DNA for genotyping from feces of rodents. Anal Biochem. 2006;348(1):160-2.
11. Otaño-Rivera V, Boakye A, Grobe N, Almutairi M, Kursan S, Mattis L, et al. A highly efficient strategy to determine genotypes of genetically-engineered mice using genomic DNA purified from hair roots. Laboratory Animals. 2016;51.
12. Pinkert CA. Transgenic animal technology: alternatives in genotyping and phenotyping. Comp Med. 2003;53(2):126-39.
13. 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):613-6.
14. Symonds EL, Fenech M. A method for non-invasive genotyping of APCmin/+ mice using fecal samples. Biol Proced Online. 2012;14(1):1.
15. Zhang YH, Huang BL, Eastman K, McCabe LL, MacLennan NK, McCabe ER. Mouth cell collection device for newborn mice. Mol Genet Metab. 2006;89(1-2):164-7.
16. Hankenson FC, Garzel LM, Fischer DD, Nolan B, Hankenson KD. 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):10-8.
17. Kalippke K, Werwitzke S, von Hornung M, Mischke R, Ganser A, Tiede A. DNA analysis from stool samples: a fast and reliable method avoiding invasive sampling methods in mouse models of bleeding disorders. Lab Anim. 2009;43(4):390-3.
18. Chen DD, Molk DM, Palley LS, Jarrell DM. Pinna Edge Biopsy of 7 and 21 Day Old C57BL/6 Mice as a Method for Identification and Genotyping. J Am Assoc Lab Anim Sci. 2023;62(5):438-48.
19. Diesch TJ, Mellor DJ, Johnson CB, Lentle RG. Electroencephalographic responses to tail clamping in anaesthetized rat pups. Lab Anim. 2009;43(3):224-31.
20. Jacquot S, Chartoire N, Piguet F, Hérault Y, Pavlovic G. Optimizing PCR for Mouse Genotyping: Recommendations for Reliable, Rapid, Cost Effective, Robust and Adaptable to High-Throughput Genotyping Protocol for Any Type of Mutation. Curr Protoc Mouse Biol. 2019;9(4):e65.

21. Silverman J, Hendricks G. Sensory neuron development in mouse coccygeal vertebrae and its relationship to tail biopsies for genotyping. PLoS One. 2014;9(2):e88158.
22. Dudley ES, Johnson RA, French DC, Boivin GP. Effects of Topical Anesthetics on Behavior, Plasma Corticosterone, and Blood Glucose Levels after Tail Biopsy of C57BL/6NHSD Mice (Mus musculus). J Am Assoc Lab Anim Sci. 2016;55(4):443-50.
23. Braden GC, Brice AK, Hankenson FC. Adverse effects of vapocoolant and topical anesthesia for tail biopsy of preweanling mice. J Am Assoc Lab Anim Sci. 2015;54(3):291-8.
24. Jones CP, Carver S, Kendall LV. Evaluation of common anesthetic and analgesic techniques for tail biopsy in mice. J Am Assoc Lab Anim Sci. 2012;51(6):808-14.
25. Matthias N, Robinson MA, Crook R, Lockworth CR, Goodwin BS, Jr. Local cryoanalgesia is effective for tail-tip biopsy in mice. J Am Assoc Lab Anim Sci. 2013;52(2):171-5.
26. Schaefer DC, Asner IN, Seifert B, Bürki K, Cinelli P. Analysis of physiological and behavioural parameters in mice after toe clipping as newborns. Lab Anim. 2010;44(1):7-13.
27. National Research Council. Guide for the Care and Use of Laboratory Animals: Eighth Edition. Washington, DC: The National Academies Press; 2011. 246 p.
28. Dahlborn K, Bugnon P, Nevalainen T, Raspa M, Verbost P, Spangenberg E. Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification. Lab Anim. 2013;47(1):2-11.

Approved - 06/12/2002
Revised - 01/12/2005, 09/12/2007, 05/12/2010, 09/11/2013, 02/28/2018, 01/26/2022, 12/11/2024