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) are 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; 14; 28]. Recently, noninvasive testing methods using hair follicles, blood, feces, ocular tear samples, or oral swabs have been described and used successfully in many laboratories [1; 2-5; 13; 18; 22-25; 27; 29; 31-33].

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 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 once and 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. 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[17].

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. 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. Pinna biopsy is considered similar to tagging the ear and results in minimal or transient associated pain and distress[19]. A two (2) millimeter ear punch or marginal notch is recommended. If repeated biopsies are required, the use of the

alternate 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. Pain perception of mid-tail clamping in rats is reported to develop between 12 to 14 days of age[9], so performing tail biopsy as early as possible in rodents should minimize potential pain[2; 10; 15; 30]. 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[15]. If larger sample sizes are required at any age, the justification should be described in the ASP.

For preweaning animals (<21 days of age), the use of anesthesia is recommended. For mice 21 days of age or older, the use of anesthesia is required unless justified in the ASP or otherwise approved by the ACUC. For rats 21-35 days of age, the use of local or general 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. Among the methods tested, 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 [10]. 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 [3;18; 21; 6].

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 or with repeated biopsies.

The investigator must monitor the animals to assure hemostasis after the rodents are returned to the cage. If needed, apply digital pressure, heat cautery (briefly), silver nitrate, or some other means of hemostasis. 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

Removal of a portion of a digit[26; 28], distal phalanx biopsy (DPB), is used as a method of identifying small rodents by using a predetermined numbering code and may simultaneously be used as a method to obtain biopsy tissue for genotyping by polymerase chain reaction (PCR). DPB should only be used in altricial pre-weaning rodents (i.e., mice and rats, NOT guinea pigs) after the digits are no longer webbed and before they reach eight (8) days of age. Every reasonable effort should be made to minimize pain or distress, including limiting the number of digits clipped to one digit per rodent. If possible, 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[16; 26; 28]. 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.

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[4; 8; 16; 26; 28]. These studies also reported no long-term effects of this procedure in test batteries evaluating physiological, developmental, and behavioral assessments[4; 8; 26]. It may be the preferred method for neonatal mice up to seven days of age[7].

Approved - 06/12/02
Revised - 01/12/05, 09/12/07, 05/12/10, 09/11/13, 02/28/18, 01/26/22

References:

1. Balafas E, Katsila T, Melissa P, Doulou A, Moltsanidou E, Agapaki A, Patrinos GP, Kostomitsopoulos N. 2019. A Noninvasive Ocular (Tear) Sampling Method for Genetic Ascertainment of Transgenic Mice and Research Ethics Innovation. OMICS J Integ Biol 23(6):312-317.
2. Bonaparte D, Cinelli P, Douni E, Herault 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 47:134-145.
3. Braden GC, Brice AK, Hankenson FC. 2015. Adverse effects of vapocoolant and topical anesthesia for tail biopsy of preweanling mice. Journal of the American Association for Laboratory Animal Science: JAALAS 54:291-298.
4. Castelhano-Carlos MJ, Sousa N, Ohl F, Baumans V. 2010. Identification methods in newborn C57BL/6 mice: a developmental and behavioral evaluation. Lab Anim 44:88-103.
5. Chen Z, Mantha RR, Chen JS, Slivano OJ, Takahashi H. 2012. Non-invasive genotyping of transgenic animals using fecal DNA. Lab animal 41:102-107.
6. Cinelli P, Rettich A, Seifert B, Bürki K, Arras M. 2007. Comparative analysis and physiological impact of different tissue biopsy methodologies used for the genotyping of laboratory mice. Laboratory Animals 41:174-184.
7. Council NR. 2011. Guide for the Care and Use of Laboratory Animals: Eighth Edition. Washington, DC: The National Academies Press.

8. Dahlborn K, Bugnon P, Nevalainen T, Raspa M, Verbost P, Spangenberg E. 2013. Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification. Lab Anim 47:2-11.
9. Diesch TJ, Mellor DJ, Johnson CB, Lentle RG. 2009. Electroencephalographic responses to tail clamping in anaesthetized rat pups. Lab Anim 43:224-231.
10. Dudley ES, Johnson RA, French DC, Boivin GP. 2016. Effects of Topical Anesthetics on Behavior, Plasma Corticosterone, and Blood Glucose Levels after Tail Biopsy of C57BL/6NHSD Mice (Mus musculus). Journal of the American Association for Laboratory Animal Science: JAALAS 55:443-450.
11. Fink D, Yau TY, Kolbe T, Rulicke T. 2015. Non-invasive instant genotyping of fluorescently labelled transgenic mice. Altex 32:222-227.
12. Garrels W, Cleve N, Niemann H, Kues WA. 2012. Rapid non-invasive genotyping of reporter transgenic mammals. BioTechniques 52.
13. Hamann M, Lange N, Kuschka J, Richter A. 2010. Non-invasive genotyping of transgenic mice: comparison of different commercial kits and required amounts. Altex 27:185-190.
14. Hankenson FC, Braden-Weiss GC, Blendy JA. 2011. Behavioral and activity assessment of laboratory mice (Mus musculus) after tail biopsy under isoflurane anesthesia. Journal of the American Association for Laboratory Animal Science: JAALAS 50:686-694.
15. Hankenson FC, Garzel LM, Fischer DD, Nolan B, Hankenson KD. 2008. Evaluation of Tail Biopsy Collection in Laboratory Mice (Mus musculus): Vertebral Ossification, DNA Quantity, and Acute Behavioral Responses. Journal of the American Association for Laboratory Animal Science: JAALAS 47:10-18.
16. Iwaki S, Matsuo A, Kast A. 1989. Identification of newborn rats by tattooing. Lab Anim 23:361-364.
17. Jacquot S, Chartoire N, Piguet F, Herault Y, Pavlovic G. (2019) 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 Prot Mouse Biol 9 (e65):1-28.
18. Jones CP, Carver S, Kendall LV. 2012. Evaluation of Common Anesthetic and Analgesic Techniques for Tail Biopsy in Mice. Journal of the American Association for Laboratory Animal Science: JAALAS 51:808-814.
19. Kalippke K, Werwitzke S, von Hornung M, Mischke R, Ganser A, Tiede A. 2009. DNA analysis from stool samples: a fast and reliable method avoiding invasive sampling methods in mouse models of bleeding disorders. Lab Anim 43:390-393.
20. Mach DB, Rogers SD, Sabino MC, Luger NM, Schwei MJ, Pomonis JD, Keyser CP, Clohisy DR, Adams DJ, O'Leary P, Mantyh PW. 2002. Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. Neuroscience 113:155-166.
21. Mathias N, Robinson MA, Crook R, Lockworth CR, Goodwin BS. 2013. Local Cryoanalgesia Is Effective for Tail-Tip Biopsy in Mice. JAALAS 52(2), 171-175.
22. Meldgaard M, Bollen PJA, Finsen B. 2004. Non-invasive method for sampling and extraction of mouse DNA for PCR. Laboratory Animals 38:413-417.
23. Mitrecic D, Mavric S, Branica BV, Gajovic S. 2008. Mice genotyping using buccal swab samples: an improved method. Biochemical genetics 46:105-112.
24. Murgatroyd C, Bilko D, Spengler D. 2006. Isolation of high-quality DNA for genotyping from feces of rodents. Analytical Biochemistry 348:160-162.
25. Otaño-Rivera V, Boakye A, Grobe N, Almutairi MM, Kursan S, Mattis LK, Castrop H, Gurley SB, Elased KM, Boivin GP, Di Fulvio M. 2016. A highly efficient strategy to determine genotypes of genetically-engineered mice using genomic DNA purified from hair roots. Laboratory Animals 51:138-146.

26. Paluch LR, Lieggi CC, Dumont M, Monette S, Riedel ER, Lipman NS. 2014. Developmental and behavioral effects of toe clipping on neonatal and preweanling mice with and without vapocoolant anesthesia. Journal of the American Association for Laboratory Animal Science: JAALAS 53:132-140.
27. Pinkert CA. 2003. Transgenic animal technology: alternatives in genotyping and phenotyping. Comp Med 53:126-139.
28. Schaefer DC, Asner IN, Seifert B, Burki K, Cinelli P. 2010. Analysis of physiological and behavioural parameters in mice after toe clipping as newborns. Lab Anim 44:7-13.
29. Schmitteckert EM, Prokop C-M, Hedrich HJ. 1999. DNA detection in hair of transgenic mice-a simple technique minimizing the distress on the animals. Laboratory Animals 33:385-389.
30. Silverman J, Hendricks G. 2014. Sensory Neuron Development in Mouse Coccygeal Vertebrae and Its Relationship to Tail Biopsies for Genotyping. PLoS ONE 9: e88158.
31. Suematsu N, Isohashi F. 2006. Rapid and simple screening of transgenic mice: novel extraction-free, filter-based PCR genotyping from blood samples. Acta biochimica Polonica 53:613-616.
32. Symonds EL, Fenech M. 2012. A method for non-invasive genotyping of APC(min/+) mice using fecal samples. Biological Procedures Online 14:1-1.
33. Zhang YH, Huang BL, Eastman K, McCabe LL, MacLennan NK, McCabe ERB. 2006. Mouth cell collection device for newborn mice. Molecular Genetics and Metabolism 89:164-167.