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	Title: Tissue Sampling of Rodents and Other Species	
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Standard Operating Procedures for Tissue Sampling of Rodents and Other Species

1.0 Scope:

1.1 This SOP applies to all WSU researchers and technicians who collect tissue samples for determination of genotype or similar purposes unless an alternative method is approved by the IACUC in the Animal Subjects Approval Form.

2.0 Introduction:

Tissue biopsies (tail, ear, toe, or fin) are frequently used to obtain tissue for genetic monitoring in rodents and fish. Some of these techniques may also be used in larger species for the purpose of determining genotype or, in some instances, as a method to screen for disease. Polymerase chain reaction (PCR) is widely used as the primary method for genotypic analysis and may require less tissue than other assays. Where possible, principal investigators are strongly encouraged to utilize less invasive procedures for obtaining tissue. These alternative techniques include collection of feces, saliva, or hair bulbs. Specific procedures for collecting these tissues are outlined according to species below.

3.0 Guidelines for Tissue Sampling from Rodents:

3.1 Tail biopsy

3.1.1 Overview: The potential for pain associated with tail biopsy increases in animals ≥ 21 days of age, if multiple tail biopsies are required, or if more than 5 mm of tail is taken. In these cases, anesthesia is required. These procedures must be described in the ASAF and approved by the IACUC.

3.1.1.1 Before weaning (less than 21 days of age): Anesthesia is not required in rodents less than 21 days of age if less than 5 mm of length is taken. Optimally, tail biopsies should be performed when rodents are between 10-17 days of age, since ossification of the tail is not completed at this age. The skin should be pushed toward the tip of the tail so that the vertebrae are avoided. (Category C)

3.1.1.2 After weaning (21 days of age or older): Anesthesia is required in any animal 21 days of age or greater, regardless of the biopsy length. (Category D)

3.1.1.3 Tail tip samples > 5 mm: Anesthesia is required in rodents of any age if > 5 mm of tail is biopsied. Samples of this length will probably damage the coccygeal vertebrae and therefore be associated with more pain. (Category D) These procedures must be described in the ASAF for approval by the IACUC.

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3.1.2 Possible Anesthetic Choices:

- Local: topical lidocaine or benzocaine
- Injectable: ketamine/xylazine, ketamine/dexmedetomidine
- Inhalant: isoflurane

3.1.3 Standard Technique:

- Gloves should be worn when handling laboratory animals.
- Anesthetize rodent (if required based on age or sample length).
- Gently, but securely, restrain rodent.
- Swab tail with alcohol (povidone-iodine or chlorhexidine solutions may interfere with DNA detection).
- Push skin toward tip of tail.
- Snip skin sample with sterile instrument such as scalpel or scissors.
- Apply gentle pressure to site until bleeding stops.
 - If needed, apply surgical glue or silver nitrate powder (Kwik-stop) to tail tip.
- Release rodent.
- Observe rodent for bleeding or abnormal behavior. Check for healing over the next 1-2 days.

3.2 Ear Tissue Biopsy

3.2.1 As an alternative to tail biopsy, tissue can be collected from the ears of rodents. These biopsies may be collected with a standard rodent ear punch instrument. The instrument punctures a small hole in the pinna of the ear and provides the researcher with a small amount of tissue for analysis. This procedure does not require the use of anesthetics or analgesics when performed by a trained individual. It is recommended to ear punch rodents when their pinnae are large enough to take a clean sample, usually after 14 days of age. An advantage to utilizing this procedure is that the hole that remains in the ear of the rodent may be used for individual animal identification. Unlike ear tags, ear punch identification systems have not been associated with ear infection or wound-induced carcinomas. At a minimum, the ear punch instrument should be appropriately disinfected between cages of animals (alcohol or hot bead sterilizer).

3.2.2 Standard Technique

- Gloves should be worn when handling laboratory animals
- Gently, but securely, restrain rodent.
- Position ear punch instrument and take sample (<2mm)
- A small amount of bleeding may occur; if so, apply gentle pressure to site until bleeding stops.
- Release rodent and observe mouse for bleeding or abnormal

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behavior.

3.3 Fecal Sample

3.3.1 Stool samples from rodents can easily be obtained either by collecting fresh samples from the cage bottom or by direct collection from the animal (rodents routinely defecate when gently handled). Procedures for genotyping rodents using stool samples have been described:

- 3.3.1.1** Broome, Rosemary L., et al. "Non-invasive transgenic mouse genotyping using stool analysis." *FEBS letters* 462.1-2 (1999): 159-160.
- 3.3.1.2** Hamann, Melanie, et al. "Non-invasive genotyping of transgenic mice: comparison of different commercial kits and required amounts." *Altex* 27.3 (2010): 185-190.
- 3.3.1.3** Kalippke, Katy, et al. "DNA analysis from stool samples: a fast and reliable method avoiding invasive sampling methods in mouse models of bleeding disorders." *Laboratory animals* 43.4 (2009): 390-393.
- 3.3.1.4** Symonds, Erin L., and Michael Fenech. "A method for non-invasive genotyping of APC min/+ mice using fecal samples." *Biological procedures online* 14.1 (2012): 1.

3.4 Hair Bulb Sample

3.4.1 Hairs must contain the bulb (root) for DNA extraction. Hair bulbs collected from rodents can also be used for genetic analysis. Hair bulbs can be treated with an alkaline lysis step and subsequently used in PCR assays. This sampling method is efficient and non-invasive. Procedures for genotyping rodents using hair bulb samples have been described:

- 3.4.1.1** Schmitteckert, Eva Maria, Christa-Maria Prokop, and Hans J. Hedrich. "DNA detection in hair of transgenic mice-a simple technique minimizing the distress on the animals." *Laboratory Animals* 33.4 (1999): 385-389.
- 3.4.1.2** Otaño-Rivera, Víctor, et al. "A highly efficient strategy to determine genotypes of genetically-engineered mice using genomic DNA purified from hair roots." *Laboratory animals* 51.2 (2017): 138-146.

3.5 Saliva Sampling

3.5.1 Studies have shown that a small amount of saliva contains enough oral epithelial cells and lymphocytes to yield sufficient DNA for PCR analysis. Using a plastic pipette tip, an oral wash can be performed on rodents at weaning age or older to collect saliva and cells. This non-invasive technique has been described in the following articles:

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- 3.5.1.1 Irwin, Michael H., R. Jeffrey Moffatt, and Carl A. Pinkert. "Identification of transgenic mice by PCR analysis of saliva." *Nature biotechnology* 14.9 (1996): 1146.
- 3.5.1.2 Meldgaard, Michael, P. J. A. Bollen, and Bente Finsen. "Non-invasive method for sampling and extraction of mouse DNA for PCR." *Laboratory Animals* 38.4 (2004): 413-417.
- 3.5.1.3 Mitrečić, Dinko, et al. "Mice genotyping using buccal swab samples: an improved method." *Biochemical genetics* 46.3-4 (2008): 105-112.
- 3.5.1.4 Zhang, Y-H., et al. "Mouth cell collection device for newborn mice." *Molecular genetics and metabolism* 89.1 (2006): 164-167.

3.6 Distal Phalanx Biopsy

- 3.6.1 Distal phalanx biopsy is another method of obtaining tissue for genotyping. While this method is often considered equally as invasive as amputation of the distal tail, it carries the added benefit of being able to be used for identification purposes. When performed appropriately in neonatal rodents up to 7 days of age, there appear to be few adverse effects. Toe clipping should only be used "when no other individual identification method is feasible" in mice or rats up to 7 days of age (Guide pg 75). Only the distal phalanx may be removed, with no more than one toe per paw being clipped. Aseptic practices, including sterilized instruments and disinfection of the skin, must be used. This method of identification and genotyping must be approved by the IACUC. Anesthetics and analgesics may be used in accordance with animal age and species.

4.0 Guidelines for Tissue Sampling from Aquatic Species:

4.1 Fin Clip (fish)

- 4.1.1 An adequate tissue sample may be collected by removing a small portion of one fin.
- 4.1.2 Location of the fin clip will vary depending on the species. For example, the caudal fin is most commonly used for zebrafish, while the adipose fin is usually clipped in salmonids.
- 4.1.3 Anesthesia is required for this procedure.
- 4.1.4 Wear gloves when handling fish. Pre-surgical skin preparation is not necessary.
- 4.1.5 Use a sterile razor blade, scalpel or sharp surgical scissors to remove a small portion of the fin lobe, approximately 2-3 mm.
- 4.1.6 Recover fish in fresh system water. Individual identification or housing may be required until genotyping results are obtained.

4.2 Skin swab (fish or amphibians)

- 4.2.1 Collecting skin mucus with a sterile swab has been used as a non-invasive method to obtain DNA samples from some species of fish and amphibians

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and has recently been validated for zebrafish and three-spined sticklebacks.

- 4.2.1.1 Breacker, C, et al. “A low-cost method of skin swabbing for the collection of DNA samples from small laboratory fish.” Zebrafish 14.1 (2017): 35-41

4.3 Buccal Swab (amphibians)

- 4.3.1 This non-invasive method collects epithelial cells from the buccal mucosa.
- 4.3.2 Wear clean exam gloves.
- 4.3.3 Place a sterile swab in the oral cavity and gently rotate the swab against the inner surface of the cheek for about 20 seconds.
- Avoid touching the swab or buccal mucosa with your hands

4.4 Tail or Toe Clip (amphibians)

- 4.4.1 An adequate tissue sample may be collected by removing a small portion of the tail (in tadpoles) or a single toe. Amphibians generally regenerate this tissue within 2-3 weeks of collection.
- 4.4.2 Anesthesia may be used for this procedure.
- 4.4.3 Wear gloves. Pre-surgical skin preparation is not necessary.
- 4.4.4 Use a sterile razor blade, scalpel or sharp surgical scissors to remove a small portion of the tail or toe, approximately 2-3 mm.
- 4.4.5 Release amphibian and monitor for any signs of bleeding or abnormal behavior. Individual identification or housing may be required until genotyping results are obtained.

5.0 Guidelines for Tissue Sampling from Large Animals:

5.1 Ear tissue biopsy

- 5.1.1 Aseptic technique is used to obtain a small sample of skin from the pinna.
- 5.1.2 Clean and disinfect the skin with povidone-iodine or chlorhexidine solution and rinse with 70% isopropyl alcohol.
- 5.1.3 Inject lidocaine 2% subcutaneously around the biopsy site.
- 5.1.4 Use a fresh, 3-4 mm biopsy punch to create the circular skin incision.
- 5.1.5 Gently grasp the edge of the biopsy with forceps and cut away the underlying attachment with sterile iris scissors.
- 5.1.6 Place a single cruciate suture or staple to close the defect.

5.2 Ear notch

- 5.2.1 Ear notches are commonly used to screen for bovine viral diarrhea virus (BVDV) infection in cattle, but notching may also serve the dual purpose of providing individual identification
- 5.2.2 An ear notching tool should be used to collect the sample. Contact OCV for recommendations
- 5.2.3 The ear notch tool should be disinfected between animals. Be sure to rinse

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the disinfectant off thoroughly before use as it may affect downstream analysis

- 5.2.4 Take an appropriately sized ear notch (~ 1 cm in cattle) from a clean portion of the ear. The site should be appropriately disinfected before tissue collection. Bleeding can be controlled by applying pressure to the site.
- 5.2.5 Monitor animal for bleeding or abnormal behavior

5.3 Fecal sample

- 5.3.1 Intestinal epithelial cells contained within and on the surface of feces are a less invasive source of DNA for genotyping.
- 5.3.2 Fecal samples are obtained from the pen or stall floor or directly from the animal.

5.4 Hair bulb sample

- 5.4.1 Hairs must contain the bulb (root) for DNA extraction.
- 5.4.2 With the animal restrained, remove dirt and debris from the area to be sampled.
- 5.4.3 Use a clean pair of pliers or forceps to grasp the hairs near the skin and pull up and away from the skin, not at an angle.
- 5.4.4 Inspect the hair sample to ensure bulbs are intact and that the hair is dry. Avoid touching the roots. As many as 40-60 hairs may be needed for a sufficient sample.
- 5.4.5 Cleanse hands and instruments between animals to avoid cross contamination.

5.5 Buccal swab

- 5.5.1 This non-invasive method collects epithelial cells from the buccal mucosa.
- 5.5.2 Wear clean exam gloves.
- 5.5.3 With the animal restrained, rinse the mouth using clean water to remove food particles and other potential contaminants.
- 5.5.4 Place a sterile swab between the cheek and gums, and gently rotate the swab against the inner surface of the cheek for about 20 seconds.
- 5.5.5 Avoid touching the swab or buccal mucosa with your hands.

5.6 Blood sample

- 5.6.1 Nucleated blood cells contain DNA and may be used for genotyping.
- 5.6.2 Refer to Guideline #9: Blood Collection for guidance on collection site and maximum volume.

6.0 References:

- 6.1 Baron BW, Langan G, Huo D, Baron JM, Montag A. Squamous cell carcinomas of the

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skin at ear

tag sites in aged FVB/N mice. *Comp Med.* 2005 Jun;55(3):231-5. PMID:16089170

- 6.2 Bonaparte, Dolores, 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." *Laboratory animals* 47.3 (2013): 134-145.
- 6.3 Castelhana-Carlos MJ, Sousa N, Ohl F, Baumans V. Identification methods in newborn C57BL/6 mice: a developmental and behavioural evaluation. *Lab Anim.* 2010 Apr;44(2):88-103. PMID:19854756.
- 6.4 Cover CE, Keenan CM, Bettinger GE. Ear tag induced *Staphylococcus* infection in mice. *Lab Anim.* 1989 Jul;23(3):229-33. PMID:2761227
- 6.5 Hankenson, F. Claire, Gillian C. Braden-Weiss, and Julie A. Blendy. "Behavioral and activity assessment of laboratory mice (*Mus musculus*) after tail biopsy under isoflurane anesthesia." *Journal of the American Association for Laboratory Animal Science* 50.5 (2011): 686-694.
- 6.6 Hankenson, F. Claire, et al. "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* 47.6 (2008): 10-18.
- 6.7 Jones, Carissa P., Scott Carver, and Lon V. Kendall. "Evaluation of common anesthetic and analgesic techniques for tail biopsy in mice." *Journal of the American Association for Laboratory Animal Science* 51.6 (2012): 808-814.
- 6.8 National Research Council. *Guide for the care and use of laboratory animals*. National Academies Press, 2011.
- 6.9 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: 7–13.
- 6.10 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.
- 6.11 Vachon, P. Anatomical and histological observations of fore- and hind limb toes in adult mice after amputations performed at the age of two weeks. *Can J Vet Res* 1998; 62: 311–313.