Policies and Procedures

SECTION: IACUC	NUMBER: 7.04			
CHAPTER: Miscellaneous Experimental Animal Use Policies	ISSUED: 10/28/07	REV. A: 4/7/09	REV. B: 11/2012	REV. C: 3-30-15
POLICY: Identification and Genotyping of Mice and Rats	REV. D: 08/2016	REV. E: 07/2019	PAGE 1 OF 5	

Purpose

The proper identification of genetically engineered animals in a litter is critical to the efficient pursuit of research and in reducing the number of animals involved in a research project. Most often, the genotype is determined by analysis of DNA extracted from tissues of young rodents. Analysis by the Polymerase Chain Reaction (PCR) requires the least amount of DNA. DNA for PCR analysis can be obtained from ear punches, hair or fecal samples, oral or rectal swabs. Depending on the requirements of the study, investigators are urged to consider these noninvasive alternatives. Larger amounts of DNA are required for Southern Blot determination of the genotype and require a larger tissue sample. Whenever possible the method of identifying an animal should be simultaneously used as a method to obtain biopsy tissue. Different methods of identification and genotyping are detailed below.

Repeat tissue sampling for the purpose of genotyping must be performed only on an as needed basis and must be described in an approved IACUC protocol. If repeat sampling is needed the IACUC strongly encourages non-invasive sampling or ear biopsy if it does not compromise previous ear marks that are used as a method of identification. [1]

Temporary Markings

Use of indelible ink (permanent marker or fur stain) on the fur, skin or tail of animals used to identify the animals and does not require anesthesia and does not need to be included on an IACUC protocol.

Ear Tags

Tags must be appropriately size for species and age. Tags should be properly placed to avoid irritation or trauma. Ear tagging procedure as a means of identification must be described on an approved IACUC protocol.

Tattooing

Tattooing is a means of permanent identification. FDA approved tattoo pigment must be used. Tattoos may be applied to the tail, toe or ear. Tattooing does not require the use of anesthesia regardless off the age of the animal, however anesthesia is recommended if more than one colored dot will be applied. Needles should be sterile and sharp. Needles should be changed between each group of animals or when the needle is becomes blunted. Tattooing procedure must be described on an approved IACUC protocol.

Implantable Transponders

Transponders and implantation cannulas must be sterile. Anesthesia is not required but is recommended. The lateral aspect of the neck/shoulder is the recommended site of insertion. This method of identification must be described in an approved IACUC protocol.

Tail Biopsy

Obtaining tissue from a mouse or rat for DNA analysis via tail biopsy is a safe, effective and humane procedure that causes minimal or transient pain and distress when performed properly. DNA prepared from tail biopsies is suitable for analysis by either Southern Blot or PCR. Care must be taken that only a small tissue sample is obtained, since the tail is the major thermoregulatory organ in mice and rats. In general, the tail tissue is harvested before ossification of the tail has occurred, since this procedure is painful with a greater risk of severe bleeding and prolonged healing in animals where ossification has occurred. This procedure is approved by the Creighton University Institutional Animal Care and Use Committee (IACUC). All investigators will follow this policy unless scientific justification is provided and approved by the IACUC.

Application

1. Procedures for tail biopsy for DNA analysis and/or genotyping must be described in an approved IACUC Protocol.

2. Ideally, mice and rats should be ~**7-21** days old. At this age, the tail tissue is soft (vertebra are not yet calcified) and the yield of DNA is highest. In addition, prompt analysis of tail tissue allows the desired mice and rats to be identified prior to weaning which can facilitate more efficient use of cage space.

    a. **For mice and rats 7-21 days of age**: Because pain sensory development may be complete, and to minimize further any transient pain or distress, investigators are strongly encouraged to apply local anesthesia to the tail. Local anesthesia may be achieved by immersion of the tail in ice-cold ethanol for 10 seconds. The use of vapocoolants is discouraged. [2] Because the discomfort of the tail biopsy is minimal and the young animal is extremely vulnerable to general anesthesia-induced mortality, only local anesthetic is used.

    b. **For mice and rats greater than 21 days of age**: The use of general anesthetic is required prior to collection of tissue. An appropriate agent should be recommended by the attending veterinarian. The use of analgesia should also be considered

3. Manually restrain the mouse or rat between thumb and forefinger. This is a convenient time to identify the animals using the appropriate method (i.e. ear punch, ear tag, transponder etc).

4. With a sterile scalpel or razor blade or very sharp sterile scissors cleanly excise <5 mm of the distal tail. If the proper procedures are followed, the yield of DNA from < 5 mm of tail should exceed 50 micrograms, enough for multiple analyses. The yield of DNA does not proportionally increase as tail fragments larger than 5 mm are used. If small amounts of DNA are required, investigators should consider taking ~ 2-3 mm of tail. If the analysis of the DNA is to be performed by PCR, great care should be taken to remove all tissue from the razor blade or scalpel after each animal. Disinfect the scalpel or razor blade between animals and the work surface on which the tail is placed between animals.

5. The investigator must monitor the animals to assure hemostasis after the animals are returned to the cage. Apply digital pressure or other means of hemostasis. If necessary, treat the tail with Kwik Stop™ powder (Styptic powder, Gimborn) to stop the bleeding, and return the animal to the cage. Monitor mice and rats for 5 minutes for bleeding or subtle signs of unusual movement or grooming behavior. If bleeding recurs, treat the wound with Kwik Stop powder. If the re-treatment with Kwik Stop does not work, then cauterize the wound to minimize loss of blood and observe the site for any abnormal bleeding. Silver Nitrate cautery sticks may be used, but remnant of silver nitrate must be wiped off after hemostasis is achieved to ensure the reaction has stopped. Check the animals again after 1 hour, and then on a daily basis until wounds are healed, for infections of the wound or behavior indicating despair or distress. The mice are expected to appear normal with no bleeding following either the Kwik Stop treatment or cauterization. The discomfort of the animal is minimal and equivalent to a quick injection with a very fine needle. With clean technique, infections are not expected and so no antibiotics are used. Consult the attending veterinarian if an animal appears to be suffering chronic pain (as noticed through behavioral assessment).

Repeat tail biopsies require general anesthesia and must be justified. The use of post procedural analgesia should be considered.

Ear Punch

Ear punch is preferred method of choice after 14 days of age when both permanent identification and collection of DNA for genotyping is needed. [1] A 2 mm punch is recommended as this will yield enough DNA for PCR analysis. Ear punch does not require the use of anesthesia. In general hemostasis is not required, but if needed Kwik Stop™ or silver nitrate cautery may be used. Excess silver nitrate must be wipe off after hemostasis is achieved. Ear punch must be described on an IACUC protocol.

Toe Clipping or Distal Phalanx Biopsy (DPB

DPB consists of removal of the distal phalanx of one or more toes of a newborn animal. This tissue can then be used as a source of DNA. This method is also used as a means of identification. Recent studies have shown that refined distal phalanx biopsy of neonates, if properly performed, does not seem to affect mice more than tail or ear biopsy.[1,3,4] **The Guide for the Care and Use of Laboratory Animals states "***As a method of identification of small rodents, toe clipping should be used only when no other individual identification is feasible. It may be the preferred method for neonatal mice up to 7 days of age as it appears to have few adverse effects on behavior and well being at this age, especially if toe clipping and genotyping can be combined. Under all circumstances aseptic practices should be followed. Use of anesthesia or analgesia should be commensurate with the age of the animals."*

Application

1. The IACUC allows DPB on pups 7-8 days of age. Performing DPB on pups >8 days of age and performing DPB solely as a mean of identification must be justified in an approved IACUC protocol. The IACUC may require anesthesia and analgesia for DPB procedures performed on pups >8 days of age.
2. Sharp sterile scissors are to be used and are to be disinfected between animals.
3. Hemostasis must be performed if needed with gentle pressure or application of KwikStop™ or Silver Nitrate Cautery (with excess removed once hemostasis is achieved.) Animals are to be monitored for 5 minutes once hemostasis has been achieved to ensure there is no recurrence of bleeding and no signs of pain or distress.
4. The most distal phalanx of only one toe per paw should be removed. It is preferable for the hindpaws to be used. If a forepaw is to be used, it is preferable not to cut the hallux ("dewclaw") as this may decrease the rodent's grasping ability. Every reasonable effort should be made to limit the number of digits clipped.
5. If additional tissue must be harvested for genotyping using a different method, this must be justified in an approved IACUC protocol.
6. The use of vapocoolant anesthesia is not recommended.[2]
7. This procedure must be described in an approved IACUC protocol.

References

1. Bonaparte (Covenor) D, Cinellis P, Douni E, Heralt Y, Maas, A, Pakarinen P, Poutanen M, Lafuente MS, Scavizzi F. FELASA guidelines for the refinement of methods for genotyping genetically-modified rodents. 2013. *Lab Animal*;(47)3 : p134-145.
2. Paluch L, Lieggi C, Dumont M, Monette S, Riedel E, Lipman N. Developmental and Behavioral Effects of Toe Clipping on Neonatal and Preweanling Mice with and without Vapocoolant Anesthesia. 2014. *J Am Assoc Lab Anim Sci*.; 53(2): p 132-140.
3. Dahlborn K, Bugnon P, Nevalainen T, Raspa M, Verbost P and Spangeberg E. Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification. *Lab Anim* 2013; 47: 2-11.
4. Scaefer DC, Asner IN, Seifert B, Burki K, and Cinelli P. Analysis of physiological and behavioral parameters in mice after toe clipping as newborns. *Lab Anim* 2010;44:7-13.
5. *The Guide for the Care and Use of Laboratory Animals*. National Research Council, National Academy Press, 2011.
6. National Institutes of Health, ARAC, Guidelines for Toe Clipping of Rodents, Revised 5/12/04.