Title: Guidelines for Genotyping of Mice and Rats, IACUC Administrative Policy P-0015-00
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I. Purpose

The specific genetic 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. The genotype is most often 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 tail biopsies, ear punches, hair, and blood, fecal or oral samples (1-9). Larger amounts of DNA are required for Southern Blot determination of the genotype.

Obtaining tissue from a mouse or rat for DNA analysis via tail biopsy is a safe, effective and humane procedure. Pain perception in rats does not start to develop until 12 to 14 days of age (10), so performing tail biopsy earlier in rodents may cause less pain. When performed properly in adult mice it causes only minimal or transient pain and distress, and induces no more "physiological impact" (change in heart rate, body temperature, or activity level) than just restraining the animal for the procedure11. DNA prepared from tail biopsies is suitable for analysis by either Southern Blot or PCR. Depending on the requirements of the study, investigators are urged to consider noninvasive alternatives such as hair, fecal or oral samples.

II. Procedures

Guidelines for Tail Biopsy

1. Procedures for tail biopsy for DNA analysis and/or genotyping must be described in an approved Animal Study Proposal (ASP).

2. Ideally, mice and rats should be **10-21** days old. At this age, the yield of DNA is highest (4,9). 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 10-21 days of age**: Based on the physiological impact and rodent pain ontogeny studies, investigators are strongly encouraged to apply local anesthesia to the tail and to conduct tail biopsy as early as possible within this age range. Local anesthesia may be achieved by immersion of the tail in ice

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cold ethanol for 10 seconds, by an application of ethyl chloride spray or by the use of another anesthetic as recommended by the attending veterinarian.

b. **For mice and rats greater than 21 days of age**: The use of a local or general anesthetic is required prior to collection of tissue. If a general anesthetic is to be used, an appropriate agent should be recommended by the attending veterinarian.

c. **For rats greater than 35 days of age**: The use of a general anesthetic is required.

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 sterile scalpel, razor blade, or scissors cleanly excise the distal 2 mm (maximum 5 mm) of the tail. If the proper procedures are followed, the yield of DNA from 5 mm of tail should be enough for multiple analyses. The yield of DNA does not proportionally increase as tail fragments larger than 5mm are used. If small amounts of DNA are required, investigators should consider taking only 2 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 scissors or scalpel after each animal. Disinfect the scalpel or scissors between animals. If a scalpel is used, also disinfect 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. If needed, apply digital pressure, silver nitrate, or some other means of hemostasis.

6. If additional DNA is needed for retesting, alternatives to a second tail biopsy should be considered (11). Repeat tail biopsies require anesthesia and must be justified in the ASP. The use of post-procedural analgesia should be considered.

Procedure for Ear Punch/Notch

Procedures for ear punch for DNA analysis and/or genotyping must be described in an approved Animal Study Proposal (ASP) if different from this guideline. This procedure does not require the use of anesthetics or analgesics as long as the procedure is performed by a trained individual.

1. Disinfect the cutting end of the ear punch or scissors with 70% ethanol, chlorhexidine or betadine before using and between each animal. A 1-2 mm ear punch is used to produce a small hole in the ear pinnae or along the pinnae margin.

2. Manually restrain the mouse by hand.

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3. Punch a hole 1-2 mm from the ear pinnae margin. If using iris scissors, notch the pinnae margin.

Blood Collection

Blood collection for DNA analysis and/or genotyping must be described in an approved Animal Use Protocol. An advantage of using blood as a source of DNA is that multiple samples can be collected from the same mouse with very little trauma to the animal.

Please refer to the IACUC Guidelines for Rodent Blood Collection for a description of the various collection techniques and permissible volumes.

Generally, a sample of 50 to 100 µl is needed for adequate DNA yield.

Procedure for Toe Clip

Use of toe clipping for genotyping is a strongly discouraged method and you must obtain IACUC approval to use this method solely for genotyping purposes. Genotyping of the offspring will be determined by analysis of DNA utilizing a procedure that necessitates acquisition of a small sample of tissue from the pups between 7-14 days of age. Analysis by the Polymerase Chain Reaction (PCR) requires the least amount of DNA. DNA for PCR analysis will be obtained from toe snips which is safe, effective, and humane. Most importantly, the toe clips serve, at the same time, for identification of individual pups, making it unnecessary to perform tail biopsies for tissue sampling.

The following procedures will be adhered to:

- No more than 1 toe per paw is clipped.
- The 1st digit/toe (i.e. thumb) on either fore paw is never clipped.
- Only the 3rd phalanx (i.e. last bone of a digit) is being removed (amputation at the joint between the 2nd and 3rd bones/phalanges).
- The digit is aseptically prepared before clipping (i.e. wiped with 70% alcohol or chlorhexidine).
- Very sharp scissors are being used (fine pointed tips work best).
- Scissors are cleaned with alcohol between animals.
- While in animals this age there rarely occurs any bleeding, if it does, monitor animals continuously until bleeding has stopped (bleeding can easily be stopped using a piece of gauze with gentle pressure between finger tips).
- Veterinary staff will be contacted promptly if toe does not heal properly or if the animal cannot ambulate normally following the procedure.
- Toe clipping in animals over 14 days of age is discouraged.
- Analgesia/Anesthesia must be used in animals over 14 days of age and this must be described in the animal use protocol.
- Animals should be placed into a clean cage post toe-clip
- PI should monitor periodically for signs of infection

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