Arizona State University
Institutional Animal Care and Use Committee
STANDARD INSTITUTIONAL GUIDELINE

TISSUE COLLECTION FOR GENOTYPING MICE & RATS

Tissue collection for genotyping mice and rats, whether performed by DACT personnel or investigators, will follow the protocol described here unless alternate methods are described in an IACUC-approved protocol. Much of this guideline was developed from the NIH's Animal Research Advisory Committee (ARAC) "Guidelines for Tissue Collection for Genotyping Mice and Rats" (revised 2/28/18).

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 Polymerase Chain Reaction (PCR) requires the least amount of DNA. DNA for PCR analysis can be obtained from tail biopsies, ear punches, hair, 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 procedure[11]. 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.

Protocols that include tissue collection for genotyping must include a statement that confirms that tissue collection will strictly follow these guidelines or it may include a detailed description of an alternate approach with scientific justification for deviating from the approach described here.

Pinna Biopsy

1. Ear punching can be a superior source of tissue for animals over 21 days of age because:
    a. It does not require anesthesia.
    b. The ear punch can also serve to identify the individual.
    c. The sample size is consistent for every animal.

2. A two (2) millimeter ear punch or marginal notch is recommended. If repeated biopsies are required, the use of the alternate pinna or an alternative method should be considered. Pinna biopsies performed as described do not require the use of anesthetics or analgesics.

3. In rodents, the ear is sufficiently developed around 14 days of age to allow suitable tissue collection.

4. Pinna biopsy is considered similar to tagging the ear and results in minimal or transient associated pain and distress[12].

5. The animal is restrained by firmly holding its scruff in one hand, and then, using a 2-3 mm commercial rodent ear punch in the other hand, collecting a small tissue sample from the pinna. If DNA analysis is performed, great care should be taken to remove all tissue from the instruments after each animal. **Disinfect the instruments between animals** (e.g., using detergent followed by 70% ethanol or a bead sterilizer).

Tail Biopsy

1. Ideally, mice and rats should be **10-21** days old at the time of tissue collection. 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 cold ethanol for 10 seconds, by an application of ethyl chloride spray, by application of 2% lidocaine jelly, or by the use of another suitable anesthetic as recommended by the ASU veterinary team.

    b. **For mice greater than 21 days and rats 22-35 days of age**: The use of a local or general anesthetic is required prior to collection of tissue. Topically applied lidocaine with epinephrine is the preferred local anesthetic, since the epinephrine will constrict the blood vessels locally and will enhance the longevity of the lidocaine-induced local anesthesia. After applying the lidocaine, wait 1-2 minutes before cutting the tail. Isoflurane is the preferred general anesthetic, because it has a high degree of safety and provides rapid induction and recovery.

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

3. Rodents can be restrained by hand between thumb and forefinger or in a rodent restraint device. Once restrained, it is a convenient time to identify the animals using the appropriate method described in the protocol (e.g., ear punch, ear tag, transponder, etc.).

4. With a sterile scalpel, scissors, or razor blade 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 5 mm are used. If small amounts of DNA are required, investigators should consider taking only 2 mm of tail. If DNA analysis is performed, great care should be taken to remove all tissue from the instruments after each animal. Disinfect the instruments between animals (e.g., using detergent followed by 70% ethanol or a bead sterilizer). 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 **ensure hemostasis** after the animals are returned to the cage. If needed, apply digital pressure, silver nitrate, or Kwik Stop with benzocaine. Alternatively, the scissors or blade used to cut the tail can be heated in a hot bead sterilizer just prior to use. Pre-heating the instrument does not change the response of the animal to the procedure but effectively cauterizes the tissue being cut and prevents post-procedure bleeding.

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 protocol. The use of post-procedural analgesia should be considered.

Distal Phalanx Biopsy (i.e., toe clipping)

The following is predominantly based on the ARAC "Guidelines for Toe Clipping of Rodents" (revised 2/27/19):

Removal of a portion of a digit[13,14], 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 (e.g., 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[13-15]. 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. If DNA analysis is performed, great care should be taken to remove all tissue from the instruments after each animal. Disinfect the instruments between animals (e.g., using detergent followed by 70% ethanol or a bead sterilizer).

Studies in mice indicate that toe-clipping produces no more acute pain or distress than other commonly used rodent identification procedures when performed from five to seven days of age[13-17]. These studies also reported no long-term effects of this procedure in test batteries evaluating physiological, developmental, and behavioral assessments[12, 18-19]. It may be the preferred method for neonatal mice up to seven days of age[20].

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