Guidelines for Tissue Collection for Genotyping 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 or oral swabs have been described and used successfully in many laboratories [1; 4; 12; 17; 20-23; 25; 27; 29-31].

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.

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[17]. A two (2) millimeter ear punch or marginal notch is recommended. If repeated biopsies are required, the use of the alternate pinna or an alternated 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[8], so performing tail biopsy as early as possible in rodents should minimize potential pain[1; 9; 14; 28]. 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[14]. If larger sample sizes are required at any age, the justification should be described in the ASP.

For preweanling animals (<21 days of age), the use of anesthesia is suggested. 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[9]. 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 [2; 16; 19; 24].

Post-procedural analgesia should be considered. 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.

Recommended Tail Biopsy Procedure:

1. Manually restrain the rodent between thumb and forefinger.

2. Starting with a sterile scalpel, razor blade, or scissors, cleanly excise the defined length of distal 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. Sanitize the scalpel or scissors between animals using an appropriate method (e.g. using detergent followed by 70% ethanol, bead sterilizer, etc.). If a scalpel is used, also sanitize the work surface on which the tail is placed between animals.

3. 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.

<u>Distal Phalanx Biopsy</u>

The following are the ARAC Guidelines for Distal Phalanx Biopsy of Rodents (revised 10/26/16):

Removal of a portion of a digit[24; 26], 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[15; 24; 26]. 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[3; 7; 15; 24; 26]. These studies also reported no long-term effects of this procedure in test batteries evaluating physiological, developmental, and behavioral assessments[3; 7; 24]. It may be the preferred method for neonatal mice up to seven days of age[6].

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