PURDUE UNIVERSITY | Office of Research

IACUC Policy – approved 6/19/2024

Genotyping Policy for Rodent and Fish Studies

1. PURPOSE

The correct genetic identification of genetically modified animals 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. Tissue biopsies (e.g., pinna, tail and distal phalanx for rodents; fin for fish) must be carefully performed because they have the potential to result in some level of pain and/or distress. Recently, noninvasive testing methods using hair follicles, blood, feces, ocular tear samples, or oral swabs have been described and used successfully in many laboratories.

Researchers should use the least invasive method needed 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 decreases risk of adverse events. The PI must ensure sufficient training for individuals performing these technical procedures.

Basic recommendations for each biopsy method are provided below. Any deviations from these recommendations must be justified in the protocol.

2. RODENT PROCEDURES

2.1 Tail biopsy

2.1.1 Perform tail biopsy as early as possible to minimize potential pain and unwanted side effects.

2.1.2 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

2.1.3 Initial biopsies of 2mm or less in young animals (<21 Days), likely prevents the cutting of ossified bone, a potentially painful procedure which may put the animal at risk for infection.

(a) If larger sample sizes are required at any age, the justification must be described in the IACUC protocol.

(b) If animals older than 21d are used, justification must be described in the IACUC protocol due to risk of adverse events

2.1.4 Anesthesia

(a) For preweaning animals (<21 days of age), the use of anesthesia is recommended.

(b) For mice 21 days of age or older, the use of anesthesia is required unless justified in the protocol and approved by the IACUC.

(c) For rats 21-35 days of age, the use of local or general anesthesia is required unless justified in the protocol and approved by the ACUC.

(d) For rats >35 days of age general anesthesia is required.

(e) 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.

(f) General anesthesia with isoflurane is used safely in many programs for chemical restraint and procedural analgesia.

2.1.5 Analgesia

(a) Post-procedural analgesia should be considered.

(b) Topical analgesics and non-steroidal anti-inflammatory given once likely provide adequate post-procedural analgesia.

(c) The need to provide an effective analgesic post-biopsy will increase with the age of the rodent post weaning, length of the biopsy, or with repeated biopsies.

2.1.6 Hemostasis must be assured after the animals are returned to the cage

2.2 Pinna Biopsy

2.2.1 Pinna biopsy or ear punch offers the advantage of having tissue collection and permanent identification completed in one procedure.

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

2.2.3 Pinna biopsy is considered like tagging the ear and results in minimal or transient associated pain and distress and therefore does not require analgesia.

2.2.4 A two (2) millimeter ear punch or marginal notch is recommended.

2.2.5 If repeated biopsies are required, the use of the alternate pinna or an alternate method should be considered.

2.2.6 Pinna biopsies as described do not require the use of anesthetics or analgesics.

2.3 Distal Phalanx Biopsy

2.3.1 Removal of a portion of a digit, distal phalanx biopsy (DPB), may simultaneously be used for identification and as a method to obtain tissue for genotyping by PCR.

2.3.2 DPB should only be used in altricial pre-weaning rodents when the digits are no longer webbed and before they reach eight (8) days of age.

2.3.3 Every reasonable effort should be made to minimize pain or distress, including limiting the number of digits clipped to one digit per rodent

2.3.4 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.

2.3.5 To ensure pain and distress is minimized, small sharp scissors should be used and personnel performing the procedure should be trained.

2.3.6 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.

2.3.7 These studies also reported no long-term effects of this procedure in test batteries evaluating physiological, developmental, and behavioral assessments.

2.4 General guidelines

2.4.1 Ensure a clean surface and clean, sterilized instruments are used to avoid contamination or infection.

2.4.2 Store samples at -20°C until genotyping.

2.4.3 Keep in mind that DNA yield and quality for genotyping is often better from younger animals.

2.4.4 Try to collect a uniform sample from each animal – too little or too much tissue can interfere with the efficiency of DNA extraction and PCR reactions

3. **FISH PROCEDURES**

3.1 Fin biopsy

3.1.1 Fish are anesthetized in buffered MS-222 (Tricane) until gill movement is slowed

3.1.2 The anesthetized fish is transferred immediately onto a petri dish or clean surface

3.1.3 The fin is clipped with a sterile blade or scissors at a point not greater than halfway between the tip of the fin and the point where scales end

(a) The total length of fin clipped should be 2-3 mm

(b) No more than 50% of the caudal fin area should be removed

(c) This should not result in bleeding

3.1.4 The fish should be put into a labeled tank with fresh water for recovery

3.1.5 Analgesia

(a) Analgesia should be considered for fin clipping

(b) Lidocaine at 2-5mg/L provides analgesia and has been demonstrated to improve activity levels and opercular beat rate in fin-clipped zebrafish

3.2 Skin swab for Genotyping

3.2.1 There is evidence that skin swabbing has a lesser impact on fish welfare when compared to fin clipping, which provides an opportunity to refine DNA sampling procedures.

3.2.2 Remove a single fish from the home tank using a small hand net

3.2.3 Gently restrain the fish on top of a wetted surface

3.2.4 Expose the uppermost surface of the fish to air

3.2.5 Stroke a sterile swab along the flank of the fish, from head to tail, five to ten times.

3.2.6 Place the swab in a clean labelled Eppendorf tube

3.2.7 Place the fish in a clean labeled holding tank until DNA extraction is complete

3.3 General guidelines

3.3.1 Ensure a clean surface and clean, sterilized instruments are used to avoid contamination or infection.

3.3.2 Store samples at -20°C until genotyping.

3.3.3 Keep in mind that DNA yield and quality for genotyping is often better from younger animals.

3.3.4 Try to collect a uniform sample from each animal – too little or too much tissue can interfere with the efficiency of DNA extraction and PCR reactions

4. **APPLICABLE REGULATIONS AND GUIDELINES**

4.1 NIH genotyping policy: https://oacu.oir.nih.gov/system/files/media/file/2022-01/b3-rodent_genotyping.pdf

4.2 NIH toe clipping policy: https://oacu.oir.nih.gov/system/files/media/file/2022-03/b9_toe_clipping.pdf

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