SOP: Collecting Tissues for Genotyping Rodents

These SOPs were developed by the Office of the University Veterinarian and reviewed by Virginia Tech IACUC to provide a reference and guidance to investigators during protocol preparation and IACUC reviewers during protocol review. They can be used as referenced descriptions for procedures on IACUC protocols. However, it is the sole responsibility of the Principal Investigator to ensure that the referenced SOPs adequately cover and accurately represent procedures to be undertaken in any research project. Any modification to procedure as described in the SOP must be outlined in each IACUC protocol application (e.g. if the Principal Investigator plans to use a needle size that is not referenced in the SOP, simply state that alteration in the IACUC protocol itself).

Table of Contents

I. Procedure Summary and Goal .................................................................................................................. 1
II. Personal Protective Equipment and Hygiene .............................................................. 1
III. Supply List...................................................................................................................................... 1
IV. Detailed Procedure: Tail Biopsy (Tail Snip) ........................................................................... 2
V. References........................................................................................................................................ 3
I. Procedure Summary and Goal
Describes procedures for collecting tissue samples to use for genetic analysis of mice and rats. This SOP is intended for use by research staff approved to perform these techniques on an IACUC approved protocol. Besides those techniques described below, DNA for PCR analysis can also be obtained from toe amputation, ear punch or biopsy, blood, hair and fecal samples, and oral or rectal swabs. Tail biopsy is the most commonly used method for collecting tissue for DNA analysis at Virginia Tech and is described below.

Considerations
a. The method of genotyping must be described in the IACUC protocol.

b. Mice and rats at or less than weaning age (21-25 days of age).
   i. No anesthesia or analgesia required for tail biopsies (1-5mm) at this age.
   ii. Higher DNA yield has been reported from tail biopsies in rodents 18 days or younger.

c. Mice and rats beyond day of wean or older than 25 days old.
   i. Performing tail biopsies on these animals is discouraged.
   ii. Procedures for performing tail biopsies in mice older than day of wean must be described and justified in the approved IACUC protocol.
   iii. General and local anesthesia must be used to alleviate pain and distress.

d. Anesthesia must also be used if more than 5mm tail tissue is removed, or if the animal is undergoing a second tail biopsy.

e. In young mice (<10 days) the tissue near the tip of the tail is soft and the bones have not completely mineralized. Therefore, removing of the tail tip of a young mouse most likely amounts to no more than momentary pain for the animal. As the animal ages, however, mineralization of the bone and increased vascularity progresses in this region, requiring anesthesia to perform the same procedure in the same location. Anesthetic and analgesic regimens tested by Jones et.al demonstrated that isoflurane anesthesia alone resulted in sufficient anesthesia at the time of biopsy but had little lasting effect and may have led to post-anesthetic distress or anxiety. Therefore, the Virginia Tech OUV and IACUC has required the use of both general and local anesthesia for use in mice greater than 25 days of age undergoing tail biopsies.

II. Personal Protective Equipment and Hygiene
a. Ensure appropriate PPE is used to protect technician from accidental exposure to blood and other body fluids, such as:
   i. Gloves
   ii. Eye protection
   iii. Mask
   iv. Other PPE as required by protocol/facility

b. Promptly dispose of used sharps in the provided leak-proof, puncture resistant sharps container.

III. Supply List
a. Scissors or disposable scalpel blades, ear punch, forceps
b. Gauze

c. Tissue sample tubes

d. Restraint device if necessary

e. Chemical cautery agent (silver nitrate, Kwik Stop)
f. Agent or device for decontamination of scissors between animals (chlorine dioxide, hot bead sterilizer, etc.).
g. 70% Isopropyl Alcohol

IV. Detailed Procedures

Tail Biopsy (Tail Snip): Initial Sampling in Rodents ≤ 25 Days of Age

a. Securely restrain the animal either manually or with a restraint device in animals less than 25 days of age.
b. Wipe the tail tip gently with an alcohol soaked gauze pad; let the intended biopsy site dry.
c. Quickly and precisely cut the distal-most aspect of the tail with sanitized sharp scissors or a disposable blade.
   i. Do NOT remove more than 5mm tail tissue.
   ii. 2-4mm is typically adequate for genotyping
d. Place tail tip into a collection tube separate from other samples.
e. Place the animal back in the home cage and observe the biopsy site for bleeding immediately post biopsy and again 5 minutes after the procedure.
   i. Bleeding should be controlled by gentle pressure to the biopsy site with gauze until bleeding has stopped.
   ii. If continuous pressure (up to 2 minutes) does not stop the bleeding, utilize a chemical cautery agent (Kwik Stop or silver nitrate).
   iii. Sterilize scissors, blade, or change scalpel blades between each animal to prevent DNA cross-contamination.

Tail Biopsy (Tail Snip): Second Sampling in Rodents ≤ 25 Days of Age and All Rodents > 25 Days of Age

a. Anesthesia: General and local anesthetic should be used in rodents 25 days of age or older, if a second tail biopsy is being performed, or if more than 5mm tissue is to be removed.
   i. 3-5% isoflurane in 100% oxygen or injectable general anesthesia (see recommended dosages in the guidelines section of the OUV website)
   ii. Soak tail in 0.75% bupivacaine solution for 30 seconds after the biopsy.
b. Adverse Events, Mitigation, and Treatment
   i. Administer analgesic if signs of pain are evident (i.e. the animal is hunched, ruffled, does not appear to be grooming normally, or has experienced weight loss).
   ii. Follow instructions above for bleeding and hemostasis.
   iii. Contact the Office of the University Veterinarian for treatment recommendations if any distress or pain is noted (see link above for contact information).

Ear Punch or Notch
a. See Rodent Identification SOP for detailed procedure.

Toe Clipping
b. See Rodent Identification SOP for detailed procedure and restrictions.
SOP: COLLECTING TISSUES FOR GENOTYPING RODENTS

Blood Collection
c. See Blood Collection SOPs for mice and rats.

II. References