Purpose

The proper 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. Most often, the genotype is 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 ear punches, hair or fecal samples, oral or rectal swabs. Depending on the requirements of the study, investigators are urged to consider these noninvasive alternatives. 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 that causes minimal or transient pain and distress when performed properly. DNA prepared from tail biopsies is suitable for analysis by either Southern Blot or PCR. Care must be taken that only a small tissue sample is obtained, since the tail is the major thermoregulatory organ in mice and rats. In general, the tail tissue is harvested before ossification of the tail has occurred, since this procedure is painful with a greater risk of severe bleeding and prolonged healing in animals where ossification has occurred. This procedure is approved by the Creighton University Institutional Animal Care and Use Committee (IACUC). All investigators will follow this policy unless scientific justification is provided and approved by the IACUC.

Application

1. Procedures for tail biopsy for DNA analysis and/or genotyping must be described in an approved IACUC Protocol.

2. Ideally, mice and rats should be ~**7-21** days old. At this age, the tail tissue is soft (vertebra are not yet calcified) and the yield of DNA is highest. 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 7-21 days of age**: Because pain sensory development may be complete, and to minimize further any transient pain or distress, investigators are strongly encouraged to apply local anesthesia to the tail. Local anesthesia may be achieved by immersion of the tail in ice-cold ethanol for 10 seconds. Alternatively, the tail can be disinfected with 70% ethanol and allowed to dry, followed by an application of ethyl chloride spray or other

suitable anesthetic as recommended by the attending veterinarian. Because the discomfort of the tail biopsy is minimal and the young animal is extremely vulnerable to general anesthesia-induced mortality, only local anesthetic is used.

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

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 a sterile scalpel or razor blade cleanly excise <5 mm of the distal tail. If the proper procedures are followed, the yield of DNA from < 5 mm of tail should exceed 50 micrograms, 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 ~ 2-3 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 razor blade or scalpel after each animal. Disinfect the scalpel or razor blade between animals and 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. Apply digital pressure or other means of hemostasis. If necessary, treat the tail with Kwik StopTM powder (Styptic powder, Gimborn) to stop the bleeding, and return the animal to the cage. Monitor mice and rats for 5 minutes for bleeding or subtle signs of unusual movement or grooming behavior. If bleeding recurs, treat the wound with Kwik Stop powder. If the re-treatment with Kwik Stop does not work, then cauterize the wound to minimize loss of blood and observe the site for any abnormal bleeding. Check the animals again after 1 hour, and then on a daily basis until wounds are healed, for infections of the wound or behavior indicating despair or distress. The mice are expected to appear normal with no bleeding following either the Kwik Stop treatment or cauterization. The discomfort of the animal is minimal and equivalent to a quick injection with a very fine needle. With clean technique, infections are not expected and so no antibiotics are used. Consult the attending veterinarian if an animal appears to be suffering chronic pain (as noticed through behavioral assessment).

Repeat tail biopsies require general anesthesia and must be justified. The use of post procedural analgesia should be considered.