Guidelines for Genotyping Mice and Rats

Background

Identification of individual genotype for animals in a litter is critical for research and in reducing the number of animals used in a protocol. Genotype can be determined by analysis of DNA extracted from tissues of young rodents. Analysis by the Polymerase Chain Reaction (PCR) requires the least amount of DNA which is easily obtained from ear punches or tail biopsy (1-7) although alternative methods are available (4,6,7). Obtaining tissue from a mouse or rat for DNA analysis by ear punch or tail biopsy is safe, effective and humane. When preformed properly techniques 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. This document is based on the NIH Intramural Animal Care and Use Guidelines for Genotyping (8).

Guidelines for Tail Biopsy

1. The procedures for tail biopsy for DNA analysis or genotyping must be described in an approved IACUC Protocol.

2a. Ideally, mice and rats should be **less than 21** days old for genotyping. At this age, the tail tissue is soft (vertebra are not yet calcified) and the yield of DNA is high. Prompt analysis of tail tissue allows the desired mice and rats to be identified prior to weaning which can facilitate efficient use of cage space.

2b. **For mice and rats 21-35 days of age**: Because sensory development is complete, the use of a local or general anesthetic is required prior to collection of tissue. An appropriate agent should be recommended by the attending veterinarian.

2c. **For rats older than 35 days of age**: The use of a general anesthetic is required. 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 sterile scalpel, razor blade, or scissors cleanly excise the distal 2mm (maximum 5 mm) of the 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 5mm are used. If small amounts of DNA are required, investigators should take only 2 mm of tail. If analysis of DNA is to be by PCR, particular care should be taken to remove all tissue from the scissors or scalpel after each animal. Disinfect the scalpel or scissors between animals. If a scalpel

or razor blade is used, also clean and disinfect 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. If needed, as indicated by bleeding greater than 10-20 microliters or multiple streaks of blood in the cage, apply digital pressure, silver nitrate, or other means of hemostasis. Monitoring is easier if animals are place in a clean cage.

6. If additional DNA is needed for retesting alternatives to a second tail biopsy should be considered (1). Repeat tail biopsies require anesthesia and must be justified in the IACUC protocol. Post-procedural analgesia should be used.

References

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