FSU ACUC Guidelines and Policies

DNA Genotyping of Mice

With the ever expanding interest in genetically engineered mice (transgenics and knock-outs), there is an accompanying need to reliably identify the genetic identity of individual animals. To accomplish this, tissue from individual animals must be obtained for in vitro analysis. For genotyping, the two most commonly used methods are Southern Blotting and Polymerase Chain Reaction (PCR). The technique utilized may dictate the amount of tissue needed for analysis and hence, the method of biopsy acquisition. As a rule of thumb, Southern Blotting will require more extracted DNA than PCR techniques.

There are several methods that can be used to obtain tissue for DNA extraction. These include toe clipping and ear punching (which can double as a means of identification), tail biopsy, blood, hair, feces, rectal scrapings and saliva. Alternative methodology that is minimally invasive or less painful (e.g. hair, saliva, feces) must be considered and if one cannot be used, justified in writing to the ACUC. Each of the aforementioned methods has drawbacks and the literature should be consulted before choosing a technique. The selection of method will depend on the amount of tissue needed for analysis (e.g. ear punch biopsies generate enough tissue for PCR but not Southern Blots) as well as the age of the animal when tissue is to be collected. An additional consideration when choosing a method is how to minimize any associated pain or distress that might occur as well as any possible refinement of technique to reduce pain or distress. A combination of techniques may be used to obtain adequate amounts of tissue but must be scientifically justified. Listed below are some basic premises of the most commonly used methods for rodent genotyping:

Toe Clipping: A simple procedure performed in pre-weanling rodents that simultaneously permits permanent identification. This method is usually performed in 8-12 day old animals without anesthetic and can be used in younger animals. The method is not recommended for use in older weanling or adult animals. Should it be used in older weanling or post-weanling animals, appropriate anesthesia and analgesia must be used. The *Guide* states: *"Toe-clipping, as a method of identification of small rodents, should be used only when no other individual identification method is feasible and should be performed only on altricial neonates."*

Ear Punching: Another method which does not generally require anesthesia and provides permanent identification. Usually cannot be performed in young pre-weanling animals if being used also for identification due to small ear size. Animals should be at weaning age or older in order to have adequate ear size for permanent identification. Usually generates enough tissue for PCR but not for Southern Blot. May be less distressing or painful than either toe clipping or tail biopsy, especially in older animals.

Tail Biopsy: Provides significantly larger quantities of DNA than either of the other methods. Is not used for identification. Can be done one or more times, but only a limited overall quantity of tail may be taken. May be done without anesthesia in young pre-weanling animals but anesthesia must be used in older animals. In the mouse, the distal tail is completely ossified and innervated between 2 to 4 weeks of age. Thus, tail sampling

is recommended in mice less than 3 weeks of age to avoid undue stress and discomfort to the animals.

FSU ACUC policies relating to the above three methods:
Unless otherwise scientifically justified, the following procedures must be performed under anesthesia with the exceptions of mice less than 21 days old and rats less than 8 days old. Consult with an FSU veterinarian for assistance with these procedures or selection of an appropriate anesthetic or analgesic. Toe clipping is permissible only if no alternative methods are available and must be justified.

Toe Clipping:

- Use a sterile scalpel blade or sharp surgical scissors. Disinfect with an appropriate method between animals.
- No anesthetic is necessary for mice younger than 13 days and rats younger than 8 days. Older animals should be appropriately anesthetized.
- No more than two digits should be removed from each limb. A chart is available as a reference for numerical identification. In animals 8-12 days old or older, usually no more than one half of a digit is necessary for both biopsy material and identification.
- Minimal bleeding should be expected in pre-weanling animals and can usually be controlled with gentle pressure. Bleeding is expected in older animals. Adequate hemostasis can be achieved through the use of manual pressure, cautery, tissue adhesives or coagulation powder/sticks. Analgesia must be considered for older animals.

Ear Punching:

- Use a sterile ear punch. Disinfect with an appropriate method between animals.
- Anesthesia should be used (see below for recommendations) for older animals. Scientific justification must be provided should the investigator not wish to use anesthesia in older animals and approved by the ACUC as part of the protocol.
- Several samples may be obtained from the same animal. A chart is available for numerical identification of individual animals.
- Minimal bleeding should be expected and can usually be controlled with gentle pressure. Animals must be rechecked several hours after the procedure (as well as daily for several days) to determine whether analgesia is needed.

Tail Biopsy:

- Use a sterile scalpel blade or sharp surgical scissors. Disinfect with an appropriate method between animals.
- For mice 10-21 days of age: Because pain sensory development may be complete, and to further minimize 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. Mice older than 21 days must be appropriately anesthetized.
- It is recommended that only 0.5 cm of tail should be obtained per biopsy and a single biopsy length may not exceed 1 cm. No more than 3 biopsies may be performed per animal and the total length of tail collected may not exceed 2 cm. Repeat biopsies require anesthesia.
- Minimal bleeding should be expected in pre-weanling animals and can usually be

controlled with gentle pressure. Bleeding is expected in older animals. Adequate hemostasis can be achieved through the use of cautery, tissue adhesives or coagulation powder/sticks.

- Animals under anesthesia should be recovered in a warm environment until they regain consciousness. Recheck the animal several hours later to determine whether analgesia is needed. Check daily to ensure that healing has occurred.
- Re-biopsy should not be performed within 72 hours of the first tail biopsy procedure.

Anesthesia

Local: ethyl chloride or alternative hypothermic method
Injectable: Avertin, Ketamine/Xylazine, Pentobarbital
Inhalant: Isoflurane

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