OHSU IACUC POLICY ON TISSUE COLLECTION FOR GENOTYPING GENETICALLY ALTERED MICE

Background: It is often necessary to obtain tissues samples from potentially genetically altered mice to determine or confirm their genotype. A number of methods exist for tissue sampling and the chosen technique should balance the research needs with the humane treatment of the animal. Most common is the removal of tail tissue ("tailing") for DNA analysis by Polymerase Chain Reaction (PCR) or Southern Blot. However, less invasive methods are available to obtain DNA including ear punches, hair samples, saliva/oral swabs, and blood or fecal analysis. Further, PCR is encouraged since it may require smaller tissue samples when compared to Southern Blot. Other considerations to ensure that pain and distress are minimized include the age of the mouse when sampled, use of local or general anesthesia and analgesia, and proper training of personnel performing the procedure.

Method	Less than 2 weeks of age	3-4 weeks of age	Greater than 4 weeks of age
Saliva or fecal samples	Acceptable	Acceptable	Acceptable
Tail biopsy	Acceptable w/ justification	Acceptable[1]	Acceptable w/justification
Ear notching	Not recommended	Acceptable	Acceptable
Blood	Not recommended	Acceptable	Acceptable
Toe amputation	Not recommended, but acceptable with justification[2]	Not acceptable	Not acceptable

[1]General anesthesia is required for mice older than 3 weeks of age.
[2]Toe amputation as a method for genotyping or identification is not recommended, except in exceptional circumstances where no other method is feasible. These should be scientifically justified in the animal use protocol and should only be performed in neonatal animals that are less than 12 days of age (eyes should not be open).

Policy: A complete description of the tissue collection procedure should be included in an approved animal usage protocol. Investigators should provide scientific justification that the less invasive methods are unsuitable for their research needs. If "tailing" is to be used, the following procedures will be followed:

1. The maximum tail sample size should be 2mm. If more than 2mm of tail is needed, than scientific justification should be provided. Larger sections of tail will consist mainly of bone thus increasing the chance for pain and may not increase the DNA yield.
2. Ideally, tail tissue should be obtained at approximately 2-3 weeks of age. At this age, the caudal vertebrae are not fully ossified, less trauma is induced, and the maximum sample can be obtained. Obtaining tail samples in young mice less than 2 weeks of age is discouraged, unless scientific justification is provided and a minimum amount of tail is taken.
3. All tail sampling in mice older than 3 weeks of age must be done under general anesthesia (isoflurane, ketamine/xylazine, etc.).
4. A sterile scalpel, blade, or surgical scissors can be used. New or disinfected instruments should be used in-between animals to decrease cross-contamination of samples.
5. Hemostasis must be provided by using pressure, silver nitrate, or tissue glue (Vetbond).
6. To prevent repeat sampling, investigators are encouraged to freeze unused tail tissue for further DNA extraction if PCR contamination occurs.
7. If another tissue sample is needed, the use of less invasive techniques should be considered. Repeat tail samples should be scientifically justified in the animal usage protocol and preemptive analgesia is **required** (contact DCM veterinarians for agents and dosages).

References:

Institute of Laboratory Animal Resources, National Research Council. Guide for the Care and Use of Laboratory Animals. 1996 National Academy Press, Washington, DC.
Refinement and reduction in production of genetically modified mice. Members of the Joint Working Group on Refinement. Lab Anim. 2003 Jul;37 Suppl 1:S1-S49.