

NIH GUIDELINES FOR THE GENOTYPING OF RODENTS

Purpose

The proper identification of transgenic 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 mice. Analysis by the Polymerase Chain Reaction (PCR) requires the least amount of DNA. DNA for PCR analysis can be obtained from ear punches, hair samples, or oral swabs (see references 1-6). Depending on the requirements of the study, investigators are urged to consider these alternatives. Larger amounts of DNA are required for Southern Blot determination of the genotype. The ARAC has determined that obtaining tissue from a mouse 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.

Guidelines for Tail Biopsy

1. Procedures for tail biopsy for DNA analysis and/or genotyping must be described in an approved Animal Study Proposal (ASP).
2. Ideally, mice should be **10-21** 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 to be identified prior to weaning which can facilitate more efficient use of cage space.
 - a. **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.
 - n.b. An ARAC subcommittee is currently evaluating pain sensitivity specifically during tail biopsy in 10-21 day old mice. Investigators should note that this guideline is likely to become more definitive when the results of these studies are complete.
 - b. **For mice greater than 21 days of age:** The use of a local or general anesthetic is required prior to collection of tissue. If a general anesthetic is to be used, an appropriate agent should be recommended by the attending veterinarian.
3. Manually restrain the mouse 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 5 mm of 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 consider taking only 2 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 scissors or scalpel after each animal. Disinfect the scalpel or scissors between animals. If a scalpel is used, also 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. Apply digital pressure, silver nitrate, or other means of hemostasis.
6. Repeat tail biopsies require general anesthesia and must be justified in the ASP.

References

1. Hofstetter JR, Zhang A, Mayeda AR, Guscar, T, Numberger JI and Lahiri DK. Genomic DNA from Mice: A Comparison of Recovery Methods and Tissue Sources. *Biochem Mol Med* 1997 Dec; 62(2):197-202.
2. Dennis, MB. IACUC Review of Genetic Engineering. *Lab Animal* 2000 Mar; 29(3):34-37.
3. Irwin MH, Moffatt RJ and Pinkert CA. Identification of Transgenic Mice by PCR Analysis of Saliva. *Nat Biotechnol* 1996 Sep;14(9): 1146-8.
4. Schmitteckert EM, Prokop CM and Hedrich HJ. DNA Detection in Hair of Transgenic Mice - A Simple Technique Minimizing the Distress on the Animals. *Laboratory Animals* 1999; 33/4: 385-389.
5. Couse JF, Davis VL, Tally WC and Korach KS. An Improved Method of Genomic DNA Extraction for Screening Transgenic Mice. National Institute of Environmental Health Sciences, National Institutes of Health. *BioTechniques* 1994; 17:1030-1032.
6. Malumbres M, Manges R, Ferrer N, Lu S and Pellicer A. Isolation of High Molecular Weight DNA for Reliable Genotyping of Transgenic Mice. *BioTechniques* 1997; 22/6:1114-1119.

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