

# Guidelines on Genotyping of Mice

## Purpose

Obtaining tissue from a mouse for DNA analysis is a safe, effective and humane procedure when performed properly. DNA can be obtained from multiple sites, including ear punches and tail biopsies.

DNA prepared from tail biopsies is suitable for analysis by either Southern Blot or PCR.

## Procedure for Ear Punch

Procedures for ear punch for DNA analysis and/or genotyping must be described in an approved Animal Study Proposal (ASP) if different from this guideline. This procedure does not require the use of anesthetics or analgesics as long as the procedure is performed by a trained individual.

1. Disinfect the cutting end of the ear punch or scissors with 70% ethanol or betadine before using and between each animal. A 1-2 mm ear punch is used to produce a small hole in the ear pinnae or along the pinnae margin.
2. Manually restrain the mouse by hand.
3. Punch a hole 1-2 mm from the ear pinnae margin. If using iris scissors, notch the pinnae margin.

## Procedures for Tail Biopsy

Procedures for tail biopsy for DNA analysis and/or genotyping must be described in an approved Animal Study Proposal (ASP) if different from this guideline. Ideally, mice should be 10-14 days old as DNA yield is the highest at this time. 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. Some strains have been shown to undergo vertebral ossification as early as day 14. For mice greater than 21 days of age, the use of anesthesia is required prior to collection of tissue.

1. 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.)
2. With sterile scalpel or scissors cleanly excise the distal 2-5 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. Samples larger than 5 mm

need to be justified in the ASP. If small amounts of DNA are required, investigators should consider taking only 2 mm of tail. For the analysis of RNA and DNA great care should be taken to avoid cross contamination of samples; decontaminate the scalpel or scissors with ethanol between animals or use fresh instruments on animals in different groups. Scissors may be sterilized using a dry bead sterilizer. Scissors must remain in the sterilizer for at least 15 seconds and cooled for 30-60 seconds prior to the start of tail excision. Return the scissors to the sterilizer for at least 15 seconds after each tail sample is collected. If blood/tissue is observed on the scissor blades between collections, please wipe prior to returning the scissors into the dry bead sterilizer. Using multiple scissors will ensure that proper temperature is maintained between samples.

3. The investigator must monitor the animals to assure hemostasis after the animals are returned to the cage. Apply digital pressure, silver nitrate, electric cautery, surgical glue or other means of hemostasis.
4. Repeat tail biopsies require anesthesia (five percent of the total animal number must be placed in Pain/Distress Category 2 to account for repeat tail biopsies).

### **Procedures for Blood Collection**

Procedures for blood collection for DNA analysis and/or genotyping must be described in an approved Animal Study Proposal (ASP) if different from this guideline. An advantage of using blood as a source of DNA is that multiple samples can be collected from the same mouse with very little trauma to the animal.

1. Please refer to the ACUC Guidelines for Rodent Blood Collection for a description of the various collection techniques and permissible volumes.
2. Generally, a sample of 50 to 100 ul is needed for adequate DNA yield.

### **References**

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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. M Fitzgerald and S Gibson. The postnatal physiological and neurochemical development of peripheral sensory C fibres. *Neuroscience* 1984 13(3):933-944.
5. M Fitzgerald. Post-natal development of cutaneous afferent fibre input and receptive field organization in the rat dorsal horn. *J Physiol* 1985 364: 1-18.
6. F Hankenson, L Garzel, D Fischer, B Nolan, and K Hankenson. Evaluation of Tail Biopsy Collection in Laboratory Mice (*Mus musculus*): Vertebral Ossification, DNA Quantity, and Acute Behavioral Responses. *JAALAS* 2008 47(6): 10-18.