

# UT Health Science Campus IACUC Guidelines for Rodent Genotyping Methods

## **I. Purpose:**

To describe some common techniques used to genotype rodents. Genotyping is the process by which animals are genetically identified. This is important when working with genetically manipulated animals (e.g. transgenics). Most often the genotype is determined by analysis of DNA extracted from tissues of young rodents. Analysis by the Polymerase Chain Reaction (PCR) requires the least amount of DNA. These samples can be obtained from ear tissue, blood, buccal/saliva swabs and hair bulbs. Larger DNA samples are sometimes required (some cases of Southern Blot analysis). Tail Clipping for sample collection can be justified in some cases. The IACUC strongly encourages researchers to use the least invasive procedures for obtaining samples for genotyping.

In essentially all cases, animals to be genotyped must be individually identified so that the sample can be traced back. Ear Punch sample collection is a minimally-invasive technique that provides a convenient, one-step ID'ing system.

## **II. Basic Information:**

- A. Genotyping is the process through which the genetic makeup of an animal is determined using a tissue sample.
- B. Identification is the method in which individual animals are marked to be able to distinguish one animal from another.
- C. Both genotyping and identification methods must be described in the IACUC Protocol.
- D. The existing information relating to welfare of tail-clipping applies to mice.

## **III. Ear Tissue:**

- A. Information: This method is less invasive than tail clipping. Ear tissue can be gathered either by ear punching or ear snipping. Special ear punching tools or sharp scissors can be utilized. The procedure is quick, easy, and should not cause bleeding if done properly. If bleeding does occur take proper measures to ensure the bleeding has stopped before returning the animal to its cage.
- B. Procedures:
  - 1. The procedure is best performed at 10-21 days of age but is humane and acceptable at all rodent ages without anesthesia.
  - 2. *Ear Punch*: Ear punching is also a method of identification used on rodents. After performing the identification procedure, the tissue that is "punched" out can be used for genetic analysis. Tissue from a single punch is sufficient for PCR but multiple samples are routine for ID'ing.
  - 3. *Ear Snip*: A small portion (2-3 mm) of the ear pinna is cut off with sharp scissors to obtain tissue.
  - 4. Ear punch device and scissors should be disinfected between animals. This can be done by wiping with 70% ethyl alcohol.

## **IV. Alternative Genotyping Methods:**

- A. Buccal Swabs/Saliva: This is a non-invasive procedure that can be performed on rodents of any age and does not require anesthesia. Cotton swabs are used to retrieve cheek cells from the mouths of mice. Samples are processed and subjected to genetic analysis via PCR.
- B. Blood: Blood samples can be obtained using any standard blood collection method. The sample can be used for PCR analysis.
- C. Hair Bulbs: This method is a non-invasive procedure in which hair is plucked from the animal and for use in genetic analysis.
- D. Fecal Pellets: Collecting stool is a non-invasive procedure. Stool can be collected directly from the animal or from the cage. Rodents routinely defecate when picked up.

**V. Tail Clipping:**

- A. Information: The tail of a mouse contains a variety of tissues, including bone, cartilage, blood vessels and nervous tissues. In a young mouse (<18 days) the tissue near the tip of the tail is soft and the bones have not completely mineralized. Therefore, removing of the tail tip of a young mouse probably amounts to momentary pain for the animal. As the animal ages, tissue maturation includes mineralization of the bone and increased vascularity. Tail tip sampling performed on an older animal (>21 days) is likely to involve more than momentary pain and distress as well as the potential for significant hemorrhage.
- B. Procedures: The IACUC believes that tail tip removal should be performed at a young age. In most, if not all, cases the procedure can be performed prior to weaning and there is nothing to be gained by genotyping at an older age. Therefore the IACUC has adopted the following guidelines for tail tip removal.
  - 1. Ideally, mice should be **10-17** 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 10-17 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 numbed by an application of ethyl chloride spray or other suitable anesthetic as recommended by the attending veterinarian.
    - b. **For mice greater than 17 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.

2. 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.).
3. 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 5 mm are used. Investigators routinely recover sufficient DNA for multiple PCR runs from samples of 1-2 mm. 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.
4. The investigator must monitor the animals to assure hemostasis and recovery from any anesthesia provided. If less than 2 mm is taken then hemostasis can usually be achieved by direct pressure on the end of the tail. If greater than 2 mm is taken or if direct pressure does not work, the use of chemical cauterizing agents are required and these should always be on hand as a precautionary measure. Styptic powder and silver nitrate are two very effective cauterizing agents commonly used for these procedures.
5. Repeat tail biopsies require general anesthesia and must be justified in the Protocol. If additional tail clips are required, the rationale must be justified to the IACUC in the animal use protocol and the use of anesthesia is mandatory regardless of age or amount of sample taken. If you anticipate the possibility of needing an additional sample from a mouse at a later date, cut the original sample in half and preserve the extra piece at -20°C or -80°C.

## **VI. Tissue Processing Method:**

- A. Information: The following tissue processing technique has been found to be easy and efficient and is offered as a suggestion.

*PBND Buffer* (For 500 ml final volume):

50 mM KCl	(1.87 g KCl)
10 mM Tris (pH 8.3)	(5 ml 1M Tris-HCl Stock)
2.5 mM MgCl <sub>2</sub>	(1.25 ml 1M stock MgCl <sub>2</sub> )
0.1 mg/ml gelatin	(50 mg)
0.45% v/v NP40	(2.25 ml)
0.45% v/v Tween 20	(2.25 ml Tween 20)
bring to 500 ml with ddH <sub>2</sub> O and autoclave	

- B. Procedure:

1. 1-2mm of tail or ear punch tissue (the younger the mouse, the better); place into a 1.5 ml microfuge tube. (Tissue can be stored frozen until use.) Add 200ul 1X PBDN buffer.
2. Add 1 ul 10 mg/ml Proteinase K to each sample and place on a rocker at 55°C. Incubate (3 hours to overnight).
3. Use 1-2 ul processed lysed tail/ear punch DNA in PCR reaction. It can help to either pellet hair and junk before using or at least to let it settle out.

## VII. References:

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