

## **Guidelines for Tail and Toe Clipping Rodents**

The University of Texas at Austin Institutional Animal Care and Use Committee

*These guidelines have been written to assist faculty, staff, and students in performing vertebrate animal procedures in a humane manner and complying with pertinent regulatory requirements. Under some circumstances deviations from these procedures may be indicated but such variances must be approved in advance by the IACUC.*

This document provides information to be used when planning and performing tail and toe-clipping procedures in rodents used for research, teaching, or other purposes at The University of Texas at Austin. It is organized into six sections:

Section A – Background

Section B – Humane Considerations

Section C – Tail Clipping Methods

Section D – Toe Clipping Methods

Section E – Alternatives

Section F – Acknowledgements and References

### **Section A – Background**

DNA analysis by tail clipping some experimental animals is necessary to monitor production of the desired genotype. Similarly, toe clipping, when done humanely, is a method that allows simultaneous animal marking and genotyping.

### **Section B – Humane Considerations**

Tail sampling for genotyping routinely involves the removal of the tail tip of a rodent that is of weaning age (21-28 days) or younger. This procedure involves minimal potential for prolonged pain or distress, and under these conditions can be performed without anesthetic. As the animal ages, tissue maturation results in mineralization of bone and increased vascularity. Tail tip sampling performed on an older animal (>28 days) is likely to cause more than momentary pain and distress as well as in increased potential for significant bleeding. Therefore, animals over four weeks of age (>28 days) should be first anesthetized with a short acting anesthetic (e.g., isoflurane).

Toe clipping is both a method used for animal marking (in order to individually identify neonatal animals within a litter) and obtaining DNA for genotyping. The ideal age for this procedure is 5-7 days old because younger pup's toes are still partially grown together, creating more of a challenge for the experimenter to accurately clip them. Literature has shown that toe clipping generally does not have negative effects on growth and physical development of pups and clipped pups do not suffer from rejection by their mother<sup>1</sup>. Regardless, the UT Austin IACUC subscribes to the recommendations of The Guide (pg. 75), which stipulates that, "toe-clipping should be used only when no other identification method is feasible." This method of identifying an animal must be justified in the protocol and must be performed in the most painless and humane way consistent with current veterinary practice and standards.

## Section C – Tail Clipping Methods

- Tail tip removal should be performed at as young an age as possible.
- In animals less than twenty-eight ( $\leq 28$ ) days of age clipping of the tail can be performed without general anesthesia.
- Sampling should be performed using sharp, sterile scalpel blades or scissors. If tail biopsies are performed on multiple mice, instruments must be disinfected appropriately between animals. Additionally, instruments must be replaced (scalpel blades) or sharpened (scissors) regularly to minimize tissue trauma caused by blunted instruments.
- The smallest possible section should be removed (3 mm is recommended), but no more than one cm may be taken at ANY age without the use of anesthesia. Some DNA kits may recommend larger samples be taken but experience has shown this is often not required.
- It is understood that sampling may occasionally have to be repeated for a variety of reasons. Under these guidelines, tail clips can be performed twice without a specific justification in the approved protocol for repetitive sampling. If possible, samples should be frozen to reduce the number of tail clips required.
- If three (3) or more tail clips are required for any reason, the rationale must be justified within the relevant protocol, submitted to and approved by the IACUC prior to performing the procedure.
- More than one tail clip requires the use of anesthesia.
- Regardless of the age of the mouse or the size of the sample, bleeding must be controlled by applying direct pressure to the wound or through the application of heat (cautery), silver nitrate, or tissue adhesive. Care should be taken when silver nitrate is used to minimize the amount of time that the silver nitrate stick is applied, and water should be used to rinse off and neutralize the silver nitrate after application.
- The animal should be closely monitored until it is fully recovered from the procedure and/or the anesthetic and shows no evidence of active bleeding at the sampling site.

## Section D – Toe Clipping Methods

- The use of toe clipping methods must be described, justified, and approved in the IACUC protocol.
- The study must require both identification and genotyping at an early age. It is recommended to be performed on mice that are 5-7 days old.
  - There is evidence that it is humane to perform toe clipping on mice up to 17 days of age<sup>2</sup>, but approval should be reserved for persons with demonstrated proficiency in the technique because the older animals will be more difficult to restrain.

- Sampling should be performed using fine sharp scissors. If toe clipping is to be performed on multiple animals, scissors must be disinfected appropriately between animals (e.g. with alcohol). Additionally, scissors must be sharpened regularly to minimize tissue trauma caused by blunted instruments.
- The coding system used must require no more than one toe per foot to be removed (four toes total per neonate as a maximum).
- The cut must remove the entire distal phalanx (toe segment farthest from the body) by cutting through the second phalanx near the joint with the distal phalanx. This will result in a toe that is not cut extremely short but heals without a nail, allowing the clipped toe to readily be identified throughout the lifespan.
- Bleeding must be controlled by applying direct pressure to the wound or through the application of hemostatic agents.
- The animal should be closely monitored until it is fully recovered from the procedure. ARC veterinarians must be contacted promptly if the toe does not heal properly or if the animal cannot ambulate normally following the procedure.

## **Section E – Alternatives**

Alternatives to tail clipping that may be considered:

- Small quantities of blood from distal veins (e.g., saphenous vein) may be used.<sup>3</sup>
- Tissue can be obtained by ear punching, which can also serve as identification.<sup>4</sup>

Alternatives to toe clipping that may be considered:

- Neonatal mice may be identified through the use of toe or tail tattoos or subcutaneous implantation of a small transponder.<sup>5</sup>
- Upon weaning, tail tattoos combined with a cage number can generate a unique combination to identify neonatal mice.<sup>6</sup>
- Genotype may be determined from hair follicles by using a DNA extraction kit (e.g. Chelex®).<sup>7</sup>

## **Section F – Acknowledgements and References**

This document contains content that was adapted from materials obtained from Stanford University and Tufts University.

<sup>3</sup>Campbell DB, Hess EJ. 1997. Rapid genotyping of mutant mice using dried blood spots for polymerase chain reaction (PCR) analysis. *Brain Research Protocols* 1: 117-123.

<sup>5</sup>Castelhano-Carlos, M. J., Sousa, N., Ohl, F., & Baumans, V. (2010). Identification methods in newborn C57BL/6 mice: a developmental and behavioural evaluation. *Laboratory Animals*, 44(2), 88–103. <https://doi.org/10.1258/la.2009.009044>

<sup>6</sup>Chen, Miao et al. “Tattooing Various Combinations of Ears, Tail, and Toes to Identify Mice Reliably and Permanently” *Journal of the American Association for Laboratory Animal Science* : JAALAS vol. 55,2 (2016): 189-98.

<sup>7</sup>Otaño-Rivera, Víctor, et al. “A Highly Efficient Strategy to Determine Genotypes of Genetically-Engineered Mice Using Genomic DNA Purified from Hair Roots.” *Laboratory Animals*, vol. 51, no. 2, Apr. 2017, pp. 138–146, doi:10.1177/0023677216646088.

<sup>2</sup>Paluch, Lee-Ronn et al. “Developmental and behavioral effects of toe clipping on neonatal and preweanling mice with and without vapocoolant anesthesia” *Journal of the American Association for Laboratory Animal Science* : JAALAS vol. 53,2 (2014): 132-40.

<sup>4</sup>Ren S, et al. 2001. A Simplified Method to Prepare PCR Template DNA for Screening of Transgenic and Knockout Mice. *Contemporary Topics in Laboratory Animal Medicine* 40 (2): 27-30.

<sup>1</sup>Schaefer, D. C., Asner, I. N., Seifert, B., BÜRki, K., & Cinelli, P. (2010). Analysis of physiological and behavioural parameters in mice after toe clipping as newborns. *Laboratory Animals*, 44(1), 7–13. <https://doi.org/10.1258/la.2009.009020>

Sørensen DB, Stub C, Jensen HE, Ritskes-Hoitinga M, Hjorth P, Ottesen JL, Hansen AK. 2007. The impact of tail tip amputation and ink tattoo on C57BL/6JBTac mice. *Lab Animal*: 41(1): 19-29.