Policy 309

Identification and Genotyping Methods in Rodents

Responsible Official:	Research Administration
Administering Division/Department:	IACUC / Research Compliance and Regulatory Affairs
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309.1 Policy:
Emory University permits several biopsy methods in rodents to provide a source of genomic DNA for molecular genotyping as well as identification options. Many procedures allow for identification of animals during the time of biopsy collection. All procedures must have prior IACUC approval and personnel must be trained in the methods to be used.

Specific techniques and recommendations are listed below. Please note that training is available from both the Division of Animal Resources and the Emory Primate Center.

309.2 Definitions of Key Terms Specific to this Policy:

309.2.1 Genotyping- the process of determining the genetic make-up of an animal using a tissue sample.

309.2.2 Identification- the method used to distinguish one animal from another.

309.3 Applicability:
This policy applies to rodents under the Emory IACUC's jurisdiction.

309.3.1 Biopsy/genotyping procedures: Please note that only one of these procedures may be used on a given animal at a time. If it is necessary to take additional genotyping material from an animal, a second technique can be utilized. This must be indicated and approved in your IACUC protocol. For any of the procedures, bleeding must be controlled, and animals must be observed until they have recovered from anesthesia (if anesthesia is required). Sampling must be performed using sharp, clean instruments. Bleeding after sampling should be treated by use of chemical cauterizing agents (styptic powder and silver nitrate are recommended), compression of the sample site by direct pressure, or if necessary, by electrocautery. Instruments **must be disinfected between animals** and sharpened regularly to ensure minimization of tissue injury. None of the methods below are considered surgical procedures.

309.4 Genotyping and identification methods:

309.4.1 Toe-clipping: Toe-clipping of neonatal mice is a procedure that provides permanent identification as well as genotyping material. The technique can be applied to animals up to seven (7) days of age without anesthesia. **Note that under this policy, toe-clipping is not allowed after day 7**. As per the 8th edition of *The Guide for the Care and use of Laboratory Animals***,** use of toe-clipping as an identification method should only be used when no other individual identification method is feasible[1]. However, it is also indicated in the *Guide* that *"It may be the preferred method for neonatal mice up to 7 days of age as it appears to have few adverse effects on behavior and well-being at this age* [2-3]*"* Therefore, toe-clipping must be scientifically justified, and used as both an identification method and for genotyping material in order to obtain IACUC approval. **It is also restricted to a maximum of 3 paws and 2 digits**

per paw. It is recommended to avoid using the front paws, if possible. Note that it is unnecessary to remove the entire toe. Removal of the distal third of each toe in question is sufficient to permit identification.

309.4.2 Ear-punching: Ear punching is a method that removes small pieces of ear tissue using an ear punch device. To be able to apply the combination of punches required for an effective numbering system, the ear must be almost fully developed. Therefore, the full-circle punches or more than one partial punch per ear should be performed on weanling rodents. A partial ear notch can be done on rodents as young as 10 days old, with a maximum of two per ear for animals of the appropriate size. Ear punching does not require anesthesia. However, application of multiple punches requires skill as torn punches can make identification difficult or impossible. Animals greater than 12 days of age have a more developed vascular system, so hemostasis must be ensured before returning the animal to its housing location.

309.4.3 Ear Snipping: This practice of tissue collection requires the removal of 2-3 mm of tissue from the edge of the ear pinna with sharp scissors. Ear biopsies should be taken from animals between the ages of 8 and 12 days of age because there is a lack of nervous system development, and bleeding is minimal. Animals greater than 12 days of age have a more developed vascular system, so hemostasis must be ensured before returning the animal to its housing location. Ear snips do not require anesthesia, however, repeat ear snips from the same ear and ear snips greater than 3 mm require prior IACUC approval.

309.5 Genotyping methods only

309.5.1 Tail snipping: This method refers to the amputation of the distal 5mm or less of tail tissue in order to obtain DNA for genotyping. Tail snips are not useful for identification of mice. Tail biopsies should preferably be taken from animals between the ages of 8 and 12 days of age because there is a lack of nervous system development, bleeding is minimal, and anesthesia is not required. After day 12, anesthesia is required because of the developed vascular and nervous system. Therefore, general anesthesia (i.e. isoflurane, ketamine/xylazine) (preferred), or local anesthesia must be applied to the tail (i.e., immersion of tail in cold ethanol) when snips are performed after day 12. The use of ethyl chloride or topical analgesic cream for tail snips requires individual IACUC approval and should be justified in the protocol. **Note: no more than 5 mm of tail can be collected over the life of the animal.**

309.5.2 Buccal swabs/saliva: The method is non-invasive and can be performed without anesthesia on any age animal. Cotton swabs are used to retrieve cheek cells from the mouths to be used for genotyping.

309.5.3 Blood: Samples may be obtained using any of the standard blood collection methods as approved by IACUC blood collection policies. However, the IACUC protocol must specifically state the blood collection method utilized and the amount to be collected. The veterinary staff will decide if anesthesia is necessary for the blood collection method.

309.5.4 Hair bulbs: This method involves plucking a small number of hairs from the animal to use for genetic analysis. The method is non-invasive and does not require anesthesia at any age.

309.5.5 Fecal pellet: Stool can be collected for use in genetic sampling. Collecting stool is non-invasive and can be collected directly from the animal or the cage.

309.6 Identification methods only

309.6.1 Ear tagging identification: This method includes attaching a metal tag to the ear of the rodent that corresponds to a unique identification number. Anesthesia is not required for the procedure, but rodents must be at weaning age or older. Very seldom are there complications with tagging such as feet getting caught in the ear tag, or the development of severe irritation around the ear tag site. The ear tag must be disinfected or sterilized before being attached to the animal.

309.6.2 Tattooing: A permanent mark is made on the tail, toes, ears, or possibly foot pads by using a needle or appropriate micro-tattooing device. Anesthesia is not required for this method.

309.6.3 Micro-chipping: This method is used for identification by injecting a small microchip transponder subcutaneously between the scapulae of the rodent. The microchip is detected by use of a reader. Anesthesia is not required for the procedure, but rodents must be at weaning age or older.

309.6.4 Permanent Marker: For a temporary identification procedure, a permanent marker can be used to uniquely color the skin/tail for the purpose of short-term identification. This method is non-invasive and does not require anesthesia.

309.8 References

1. Guide for the Care and Use of Laboratory Animals 8th edition. 2011. National Research Council. The National Academies Press Washington DC.
2. Castelhano-Carlos MJ et.al., 2010. Identification methods in newborn C57BL/6 mice: A developmental and behavioral evaluation. Lab Anim 4:88-103
3. Schaefer DC et.al. 2010. Analysis of physiological and behavioral parameters in mice after toe clipping as newborns. Lab Anim. 44:7-13.

309.9 Genotyping/ Identification Summary Chart

Method	Purpose	Age	Anesthesia/Analgesic Requirements	Welfare Concerns/additional comments
Toe Clipping	Genotyping and Identification	Up to 7 days of age	None required	Permanent method. Must be scientifically justified. Risk of permanent injury if more than the distal 1/3 of the toe is removed.
Ear Punching	Genotyping and Identification	Weaning: when ear is developed	None required	Skill and technique are important to conduct punches.
Tail Snipping- 5 mm or less	Genotyping	Before weaning age is preferred	No anesthesia if conducted before 12 days of age. General anesthesia or local anesthesia is required for animals older than 12 days of age.	Permanent method. Risk of bone injury or tissue necrosing. No more than 5mm can be collected over the life of the animal.
Ear Snipping 2-3 mm	Genotyping and identification	Between 8-12 days of age	Ear snipping **does not** require anesthesia	Permanent method. There is a risk of injury. The operator should be aware of increased bleeding risk after 12 days Hemostasis should always be achieved before returning the animal to their cage (regardless of age). Repeated ear snips from the same ear OR >3mm tissue collected requires individual IACUC approval and should be stated in the protocol.
Buccal Saliva Swabs	Genotyping	Any age	None required	There is a risk of being bitten.

Blood Sample	Genotyping	Dependent on collection method	Dependent on collection method	Skill is important for proper blood collection.
Hair Bulb Samples	Genotyping	Any age if hair is present	None required	Skill is important for proper hair removal.
Fecal Pellet Samples	Genotyping	Any age	None required	Skill is important if manually collecting stool. Risk of injury in neonate animal
Ear Tagging	Identification	At weaning or older	None required	Skill and technique are important. Risk of infection or tag falling out.
Tattooing	Identification	Any age	None required	Skill and technique are important. Permanent method of identification.
Micro-chip	Identification	At weaning or older	None required	There is a risk of infection from implantation of foreign object. Must have reader to detect the micro-chip.
Permanent Marker	Identification	Any age	None required	Temporary mark for short procedure

Contact Information

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Clarification of Policy	Office of Research Compliance and Regulatory Affairs - IACUC	404-712-0734	iacuc@emory.edu

Revision History

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