



Division of Research

SUBJECT: Genotyping, Identification and Use of Proper Nomenclature of Rodents	Effective Date: 9/27/2019	Policy Number: 10.4.8
	Supersedes: 8/30/2016	Page of 1 7
	Responsible Authorities: Vice President, Research Institutional Animal Care and Use Committee Director, Research Integrity Director, Comparative Medicine	

- I. Background
 The Public Health Service (PHS) policy and the “Guide for the Care and Use of Laboratory Animals” (Guide) require the Institutional Animal Care and Use Committee (IACUC) and the Attending Veterinarian to provide oversight of all experimental procedures and guarantee the welfare of the animals used in biomedical research. As stated in the NIH Guidelines for the Genotyping of Mice and Rats, specific genetic identification of pups in a litter is critical to the efficient pursuit of research and reducing the number of animals involved in a research project. Likewise, this applies to the individual identification of rodents depending on the particular study design. The Guide emphasizes that accurate recording, with standardized nomenclature, of both the strain and substrain or of the genetic background of research animals is essential.

- II. Purpose
 The purpose of this document is to provide guidelines to researchers and animal care staff regarding acceptable methods of tissue collection for the purpose of rodent genotyping, individual identification of mice and rats as well as the proper nomenclature of genetically modified animals (GMA).

- III. General Statement
 Properly executed procedures will protect the welfare of animals used, avoid exposing the animals to undue stress and pain and provide physiologically stable biologic models for research. The immense variety of mouse and rat strains including genetically modified animals requires adequate nomenclature to ensure the name reflects the most relevant information of the strain/substrain, which can carry spontaneous or induced mutations, transgenes or knock-out/knock-in alleles existing in numerous different genetic backgrounds.

- IV. Policy
 - A. Genomic DNA is required to genotype any genetically modified animal. The amount of DNA needed is dependent upon the analysis method employed. Polymerase Chain

Reaction (PCR) and Southern Blot are two commonly used testing methodologies. Flow cytometry can also be used as a method of genotyping for certain specific applications.

- B. For PCR analysis the least invasive methods of DNA collection include the use of small tissue samples from ear punching/notching, buccal/rectal swabs, hair samples, and blood. Based on the requirements of the study, researchers are urged to consider these noninvasive tissue collection sites.
- C. Tail biopsies, suitable for Southern Blot and PCR and often used in mice and rats, involve removing a piece of tissue from the terminal end of the tail and should only be used after alternatives have been considered. The tail contains a variety of tissues, including bone, cartilage, blood vessels and nervous tissue.
- D. The ideal time to collect tail tissue is in between post-natal days 10 and 15. At this age, the tail tip is soft, bones have not completely mineralized and the yield of DNA is highest. In addition, prompt analysis of tissue allows the desired mice and rats to be identified prior to weaning.
- E. Biopsy of the tail tip can be performed **without anesthesia on animals less than or 21 days** of age but requires **anesthesia** if animals are **older than 21 days**. Brief general anesthesia with isoflurane (either via vaporizer or open drop method in a fume hood) is recommended.
- F. The total amount of tissue removed should be the minimum necessary. For tail tissue this would be ideally 2mm of the tip (usually sufficient for PCR) but **not more than 5mm** to avoid developing coccygeal bones.
- G. **Repeated tail biopsies** on a single animal are discouraged, requires general anesthesia and **must be justified in the IACUC protocol**. The use of post-procedural analgesia should be considered.
- H. Cage cards are used for the identification of all animals following the recommendation of the Guide. However, for many studies it is advantageous to identify experimental animals individually. Temporary marking systems can be satisfactory for short time periods while permanent identification systems will not wear off rapidly and are more reliable.
- I. **Temporary marking systems** acceptable for rodents include fur clipping and shaving in distinct configurations on the animal's body, non-toxic waterproof dyes and non-toxic colored markers applied in a variety of patterns on light-colored fur or hairless areas on the tail.
- J. **Permanent identification systems** considered acceptable for mice and rats include ear punch/notch, ear tags, microchip transponder and tattoos. Neonates can be identified via digit marking (tattoo) using non-toxic ink injections and toe clipping.
- K. **Toe clipping** may be approved by the IACUC after all other alternatives have been considered and a sound justification must be provided, especially if toe clipping serves a dual purpose of identification and tissue collection for genotyping. Toe clipping must only be used in altricial pre-weanling rodents **up to 7 days of age in rats and up to 17 days in mice**. Only the most distal bone of the toe (3rd phalanx) and only one toe per paw can be removed.
- L. Research faculty and staff performing tissue collection procedures for genotyping and permanent identification methods must be trained and proficient in the procedures.
- M. The Mouse Genome Informatics Database and the Rat Genome Database are the authoritative source of official names for genes, and alleles as well as strains of mice and rats, respectively. The International Committee on Standardized Genetic Nomenclature for Mice established the rules and guidelines for nomenclature of mouse and rat strains.

Policy Number:	
10.4.8	
Page	of
3	7

N. Investigators who use rodent models are responsible for accurate reporting of the identification of research rodents with regard to strain/substrain and genetic background. To that end, **researchers are strongly encouraged to follow nomenclature guidelines** published by the above mentioned sources, which can be found on the respective websites (MGI-Mouse Nomenclature www.informatics.jax.org/mgihome/nomen/).

V. Definitions

1. **Genetically modified animal (GMA)** is an animal whose genetic material has been altered by adding, changing or removing certain DNA sequences in a way that does not occur under natural conditions.
2. A mouse or rat **strain** is a group of animals that is genetically uniform, which can be inbred, mutated or genetically engineered/modified. Inbred strains can genetically diverge into **substrains** under a number of circumstances, e.g. a breeding colony of an inbred strain maintained at different locations.
3. **Genotyping** is the process of determining differences in the genetic make-up (genotype) of an individual by examining the individual's DNA sequence using biological assays and comparing it to another individual's sequence or a reference sequence.
4. **Individual identification** means the marking of animals in a unique way to identify individuals in contrast to a group of animals housed in the same enclosure (e.g. via cage cards).
5. **Nomenclature** is a system of terms used in a particular science. In regards to this policy it is an international system of standardized genetic terms and symbols applied to rodent strains/substrains.

VI. Accountability

The Principal Investigator (PI) will be responsible for:

- Describing all procedures performed on an animal including the tissue collection for genotyping and individual identification method in sufficient detail in any IACUC protocol.
- Ensuring appropriate training of personnel performing the genotyping or identification procedures and contact the Comparative Medicine staff for support or any concern/problem resolution.
- Securing the usage of proper nomenclature of mouse and rat strains/substrains during the conduct of the research study and in publications.

The IACUC will be responsible for:

- Reviewing and approving, requiring modifications in (to secure approval) or withholding approval of IACUC protocols and/or amendments, especially assessing the appropriateness of the procedures described for genotyping and identification of animals.
- Reviewing records on a regular basis during semi-annual site inspections, post-approval monitoring reviews, and whenever concerns regarding the welfare of a particular animal(s) arise.

The Research Integrity office will be responsible for:

- Administrative support of the IACUC members to facilitate their regulatory function.
- Maintaining policy and assure regular review and update as necessary by the IACUC.
- Organizing, supporting and recording outcomes of regular inspections of research labs in regards to procedures performed and nomenclature used.

The Attending Veterinarian/Office of Comparative Medicine (CM) will be responsible for:

- Ensuring adequate oversight of experimental procedures including consultation during the design of the study, evaluation of recordkeeping, health surveillance of the animal colonies, and the assessment of the well-being of animals on a one-by-one basis.
- Provide training to research and animal care personnel performing collection of tissues for genotyping and identification procedures.

VII. Procedures

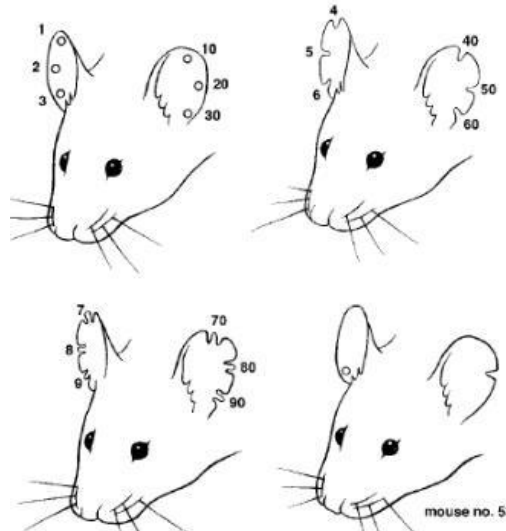
A. Tail Biopsy

1. Collection of distal tail tissue ($\leq 5\text{mm}$) can be performed without anesthesia in young animals but general anesthesia is required for animals older than 21 days or if a repeated sampling in a single animal is necessary. Topical cetacaine or ethyl chloride sprays have been shown **not** to provide appropriate anesthesia/analgesia in mice and should not be used. Anesthetized animals have to be continuously monitored until fully awake.
2. The tail has to be swabbed at least once with a disinfectant such as iodine or chlorhexidine. Instruments used to cut the tail tip have to be sterile at the start of the procedure and should be sterilized between animals. This can be achieved by but is not limited to using a hot bead sterilizer, which not only will sterilize the scalpel blade or scissors but also can accomplish hemostasis of the tail wound.
3. Instruments used for cutting need to be sharp (autoclaving will dull cutting parts of instruments and regular sharpening is required). Note that disposable scalpel blades are not designed to be used on multiple animals.
4. Bleeding has to be stopped before returning the animal to the cage. Hemostasis can be achieved either by cauterization with hot instrument tips (see above comment), styptic powder, silver nitrate or simple digital pressure.
5. The working surface where the tail is cut should be wiped with alcohol in between animals to reduce DNA contamination between samples.

B. Ear Punch/Notch

1. Ear tissue can be collected either by ear punching of a circle of tissue or ear notching by removing an edge of the pinna, and can be used for genotyping and identification of the animal at the same time.
2. These methods do not require anesthesia and should be performed on animals close to weaning age or older to ensure the pinnae are large enough. If very young animals are ear punched, holes might close again and identification may be lost.
3. If done correctly ear tissue collection should not cause bleeding. If bleeding occurs ensure proper hemostasis.
4. The instruments used, either commercial punch devises or scissors, must be sterilized before usage (e.g. autoclaving or cold sterilants) and disinfected between animals. Confirm that the instruments are sharp before starting the procedure.

- When using the punch holes and/or notches in the ears as identification follow an identification chart. See below example.



From AALAS Learning Library (<http://aalaslearninglibrary.org>)

C. Toe Clipping

- Although toe clipping can be used for identification and genotyping purposes it can only be performed on mice up to 17 days and rats up to 7 days of age. Only the most distal phalanx bone and only one toe per paw can be removed. Following these guidelines no anesthesia is required.
- Instruments used for cutting must be sharp, sterilized before usage and disinfected between animals.
- Ensure hemostasis before releasing animals into regular housing (see VII.A.4).

D. Other Genotyping Methods

- None of the methods described in this section are invasive and therefore they do not require anesthesia.
- Buccal swabs/saliva collection can already be performed on animals a 2-3 days old. Use individual sterile mini-cotton swabs rubbing against the inner cheeks to collect epithelial cells from the mouth. Care should be taken to ensure gentle swabbing.
- Hair samples can be collected at the neck line between the shoulder blades. Two (2) tufts of hair are plucked using forceps or hemostats. It is important to avoid contamination with hair/skin cells from cage mates.
- Fecal pellets can be collected directly from the animal during defecation while being picked up or from the cage floor of single housed animals. Epithelial cells shed in the feces are the target tissue for genotyping.
- A blood sample (50-100ul) can be utilized for flow cytometric assessment using clonotypic monoclonal antibodies. This method is used when either the sequence for making primers is not available or the expression of transgene product on specific cells is of interest.

E. Ear Tagging

- The attachment of a metal or plastic tag with a unique identification number to the base of the ear is a common identification system. Improper placement can lead to

Policy Number:	
10.4.8	
Page	of
6	7

pressure necrosis, inflammation and infection, which can result in removing the tag by the animal.

2. Instruments used must be sterilized before usage and disinfected between animals.
3. The site of placement has to be disinfected prior to attachment of the tag to avoid infections and should be monitored regularly after the procedure.
4. Note that ear tags may not be compatible with advanced imaging procedures.

F. Microtattooing

1. This is a method using needle and ink to apply a permanent distinctive mark (e.g. number) at the dermis of the tail, toes, ears or foot pad of a rat or mouse. It can be performed on animals as young as 2 days old depending on the tattooing system used. Manual or electric equipment can be used.
2. Anesthesia is not required when it is performed by trained personnel. Adult animals might need to be anesthetized for immobilization.
3. The tattooing site has to be disinfected to minimize risk of infection.
4. Commercial electrical equipment is available from various vendors requiring differing proficiencies of personnel.
5. Injecting tattoo ink subcutaneously into the tail, hocks or toes producing a pattern of dots is used for identification of neonatal rats and mice.

G. Microchipping

1. Microchip transponders implanted usually subcutaneously but also intraperitoneally are often used to track large numbers of animals and data (some microchips can also measure physiologic parameters) when interfaced with compatible computer software.
2. Microchips have to be sterile and implantation has to follow aseptic technique.
3. Subcutaneous microchip placement is performed like an injection with a large bore needle while intraperitoneal transponder implantation is considered a survival surgery and has to be described accordingly in the IACUC protocol.
4. Microchip transponders can lead to tissue inflammation response or neoplasia with prolonged placement. It might not be compatible with advanced imaging procedures.

VIII. Policy Renewal Date 9/27/2022

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Policy Number:	
10.4.8	
Page	of
7	7

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POLICY APPROVAL

Initiating Authority

Signature: _____

Date: _____

Name: Daniel C. Flynn, Ph.D., Vice President for Research

Executed signature pages are available in the Initiating Authority Office(s)