GENOTYPING AND IDENTIFICATION
(Including Tail and Toe Clipping)

PERFORMANCE STANDARD: Methods to identify individual mice and collect a DNA sample for genotyping will focus on assuring appropriate animal welfare and minimizing pain and distress. All methods used must be approved in your Duke IACUC protocol. All Duke animals will be: 1) correctly identified; and 2) directly associated with a protocol, SOP, and/or medical record.

BACKGROUND: The Animal Welfare Act, PHS Policy, and the Guide for the Care and Use of Laboratory Animals (Guide) require that animals engaged in studies be properly identified to assure adequate provision of care and limitation of distress and discomfort. The Guide also includes an encouragement to consider identification in field studies, and places identification on the same level of significance as sedation, anesthesia, recovery, holding, and transportation. According to The Guide, there are several forms of identification which may be considered for effective and consistent identification of animals. Identification of individual animals, which may be combined or separate to obtaining sufficient DNA to perform genotyping, is necessary and frequently employed practices in biomedical research.

APPLICABILITY: This protocol applies to all persons who work with animals for research, testing, or teaching at Duke, as a member of the Duke University community, or in a Duke IACUC approved study.

ROLES: All participants shall treat the animals with respect and compassion. The regards to tail or toe clipping shall be performed with expertise and skill, in a fashion that will prevent infection.
   1. PI: Shall provide or ensure proper training for individuals who will be performing the activity.
   2. User: Shall practice clean, careful and appropriate technique, ensure appropriate handling and restraint, and monitor the recovery of the animals post-procedure.
   3. Veterinary Staff: Shall provide required training, ensure proper monitoring, and assist with animal care practices as needed to minimize pain or distress.

PROTECTION POSTURES REQUIRED: Proper gowing and gloving is required as dictated by the facility management plan. Adequate procedure site preparation is necessary to ensure that the procedure is done rapidly and appropriately, allow for immediate hemostasis, and prevent undesirable environmental contamination.
POLICY ON IDENTIFICATION: There are several options for identification of animals engaged in a Duke IACUC-approved activity. All Duke animals will be correctly identified and associated with a protocol, SOP, and/or medical record.

1. **Means of Animal Identification:** Means of animal identification include room, rack, pen, stall, and cage cards with written, bar-coded, or radio frequency identification (RFID) information. Animals may wear collars, bands, plates, or tabs or be marked by colored stains, ear notches/punches and tags, tattoos, or subcutaneous transponders.

2. **Group Identification/Cage Card Identification:** The goal of group/cage card identification is establish a relationship of responsibility, confirm the identity of the animals within, and provide a minimum of information about the animal which could assist the veterinary staff with initial assessment of the animal if it is not looking ‘normal.’ Animals may be identified as a group when the animals are all held and managed in the same manner (e.g., four mice in a cage being used in the same study) or when they are being minimally managed for later use (e.g., three guinea pigs housed in the same cage for growth and maturation, a herd of sheep held on pasture to provide time for disease development). A group record must identify the number of animals in the group at the time each entry is made, size, sex, color, or other distinguishing characteristics which will allow a clear confirmation of this record for these animals, the provisions of care to all animals in the group, and diagnostic, therapeutic, or experimental plans for the group of animals.

   A. Identification cards commonly include the following information:
      1. Source of the animal,
      2. Strain or stock (rodents) or breed (larger animals)
      3. Names and contact information for the responsible investigator(s) or laboratory designated person
      4. Pertinent dates (e.g., arrival date, birth date, etc.)
      5. Protocol number (when the animal is associated with a protocol)
      6. Genotype information, when applicable, should also be included.
         i. Consistent, unambiguous abbreviations (e.g. B6, ICR, SW) should be used when the full genotype nomenclature is too lengthy.
         ii. Printing the statement ‘See Protocol’ is an acceptable alternative to specific genotype information. This is appropriate because the veterinary staff have electronic access to all protocols and can review the phenotypic specifics from the protocol file.
3. **Individual Identification:** Animals should be identified by individual records when they are held as individuals (e.g. a rabbit in a cage, a primate in a cage, a pig in a pen), when they are receiving diagnostic or therapeutic care which others would not receive at the same point in time, or when keeping individual records would enhance clarity as to the needs, provisions, and oversight of an animals.

4. **Special Identification Techniques:**
   
   A. Freeze brands are preferred over hot brands of livestock as a means of permanent identification.
   
   B. Toe-clipping may be used as a method of identification of small rodents, but should be used only after Duke IACUC approval and when no other individual identification method is feasible. Toe clipping (see below) is allowed only if the tissue is also used for genotyping.
   
   C. Ear punching or notching (see below).
   
   D. Ear tagging with a unique numbered tag.
   
   E. SQ radio tag / microchip according to manufacturer’s recommendations (products may have slightly different requirements).
   
   F. Indelible markers / temporary dye / ink marking (must be non-toxic).
   
   G. Tattoos according to the DLAR SOP on ‘Animal Identification.’
   
   H. Other: As described and approved in your IACUC protocol.

5. **Association with protocol, SOP, or medical record:** Acceptable methods of animal document association may range from information on identification cards to detailed computerized records for individual animals. The style or type of document required is based upon the type of animal, the use of those animals, and the clinical needs of an individual animal.

**POLICY ON GENOTYPING:** For more information, please reference the FELASA Guidelines. There are several tissue type that may provide the required tissues for genotyping. This include:

1. Blood
2. Hair follicles
3. Colonic and rectal cells (derived from feces)
4. Cells from oral mucosa
5. Ear punching, clipping, or notching

When using ear punching, clipping, or notching for genotyping (this can be used for both identification and genotyping):

1. Mice should be gently and appropriately restrained by hand. A restraining device is not recommended.
2. A very sharp instrument should be used, with a clean, precise cutting gesture. A rodent ear punch device is recommended. If scissors are used, they should be sharp.

3. In case of bleeding, pressure should be applied to the digit to achieve hemostasis. Contact a DLAR veterinarian immediately if excessive bleeding occurs.

4. This procedure is considered to cause only slight or momentary pain and distress. Therefore, anesthesia and analgesia are not required.

5. Ear punching or notching can also be used to provide identification, based on the pattern and location of ear punches/notches.

6. Examples of identification via ear punching include:

![Illustration of a mouse with ear punches]

Other ear punch "codes" may also be appropriate (see Appendix 1). This should be described in your IACUC protocol.

**POLICY ON TOE CLIPPING:** Toe clipping is the practice of removing a toe from the most distal joint (phalanx) to the tip of the toe. This is generally performed in a fashion that allows identification as well as providing tissue for genotyping (mice).

1. Toe clipping is not allowed unless scientifically justified and is NOT allowed solely for identification.

2. If toe clipping is to be performed, it MUST occur on or before 12 days of age.

3. It is preferable to remove toes from the hind paw rather than the forepaw.

4. It is preferred to remove only one toe per foot.

5. A very sharp instrument should be used, with a clean, precise cutting gesture. If scissors are used, they should be sharp.

6. In case of bleeding, pressure should be applied to the digit to achieve hemostasis. Contact a DLAR veterinarian immediately if bleeding continues beyond a few seconds.

7. Mice should be gently hand-restrained. Use of a restraining device is not recommended.

8. Toe clipping is not considered a surgical procedure, therefore the following modifications to aseptic technique are acceptable:
   a. **Skin preparation:** Assure the skin is free of debris; if not, then alcohol may be used to wipe down the foot.
   b. **Instrument preparation:** All instruments must be clean and free of visible debris. Instruments that have been exposed to autoclave, glass bead sterilizer, or chemical disinfectants are preferred; however sterile instruments are not required (this is not a surgical procedure).
POLICY ON TAIL CLIPPING: Tail clipping is the practice of removing the tip of the tail to provide tissue for genotyping. It is not considered a surgical procedure (mice).

1. If tail clipping is done before a mouse is weaned at 21 days, anesthesia is not necessary.

2. If tail clipping must be performed on mice after 21 days of age, then:
   a. Alternate methodologies should be considered (consult DLAR veterinarians); or
   b. Anesthesia and/or analgesia must be used. Investigators and research staff should be aware that tail ossification rates may be altered in genetically modified mice and they should be vigilant to the appearance of pain and distress following the tail snip. If pain or distress is suspected after the procedure, the DLAR veterinary staff should be consulted regarding anesthetic and/or analgesic recommendations and the IACUC protocol amended accordingly.

3. No more than 5 mm of tail tissue should be taken.

4. A very sharp instrument should be used, with a clean, precise cutting gesture. If scissors are used, they should be sharp.

5. In case of bleeding, digital pressure should be applied to achieve hemostasis. Contact a DLAR veterinarian immediately if bleeding continues beyond a few seconds.

6. Mice should be gently hand-restrained. Use of a restraining device is not recommended.

7. Tail clipping is not considered a surgical procedure, therefore the following modifications to aseptic technique are acceptable:
   a. Skin preparation: Alcohol may be used to wipe down the tail prior to cutting.
   b. All instruments must be clean and free of visible debris. Instruments that have been disinfected via autoclave, glass bead sterilizer, or chemical disinfectants are preferred for this procedure; however sterile instruments are not required.
REFERENCES


APPENDIX 1
Numbering systems

From the Jackson Laboratories website, 3/9/2015
http://jaxmice.jax.org/support/husbandry/identification.html

**Universal Mouse Numbering System (within entire colony)**
Ear punch used to make 1) holes, 2) single notch, or 3) double notch. Scissors used to make 'V'.


Dorsal view