1) Proper identification of research animals is an essential component of a research design. It allows an easy method for tracking an animal throughout a research project and assists animal care staff in providing appropriate care to the appropriate animal. This document identifies various methods of identifying individual or groups of mice and rats i.e. rodents for this policy, and provides guidance concerning the biopsy of tissue for genotyping.

   a) **Cage Cards**
      i) Cage card information includes: species, strain or stock, source of animal, names and contact numbers of responsible investigators, date of birth/arrival and protocol number
      ii) Cage card can be used as only method of identification for individually housed rodents or a breeding pair or groups of rodents on protocols where individual identification is not necessary

   b) Temporary Markings
      i) Use an indelible marker to write numbers, bars, or other distinguishable markings, on the tail or the ears.
      ii) Temporary marking can be used for short-term individual identification; this marking can last up to 3-4 days depending on the age of the animal. Dams will clean the markings off of nursing pups within a few hours, so this may not be the best way to identify young animals.

   c) Tattooing
      i) Use an electric tattoo machine to write numbers on the tail
      ii) Use only sterile and sharp tattoo needles.
      iii) This procedure is easier to perform under general anesthesia
      iv) If not using general anesthesia, apply a local anesthetic on the tail before tattooing (EMLA cream or a local anesthetic spray.

   d) **Micro-tattooing**
      i) Use a micro-tattooer to inject tattoo ink in the toe pads and/or the ears
      ii) Whenever possible, use a simple identification code to limit the number of toes tattooed
      iii) Have the identification code chart readily available in the animal room to allow prompt identification of individuals

   e) **Ear Tags**
      i) Rodents should be ear tagged at weaning age or older
      ii) Use tags that are about 5 mm long for rodents
      iii) Rinse tag in 70% alcohol before use to help prevent ear infection
      iv) Position the tag in the applicator so that the end with the hole is positioned over the notched area of the applier; the pointed end should be opposite the hole
      v) Scruff the animal so that the ears are easily accessible
      vi) Place the ear between the point and the hole of the tag; the numbers should be in an upward configuration so that they can be more easily read

vii) The tag should be positioned at the lateral base of the ear, approximately 3 mm from the edge of the ear pinna
viii) Once the tag is positioned correctly, squeeze the applicator firmly to apply
ix) Monitor the tag implantation intermittently for signs of local infection

f) **Microchips**
   i) **Do not** implant microchips in rodents less than 3 weeks old
   ii) Use appropriate anesthesia and analgesia to implant the microchip,
   iii) Apply disinfectant on the skin (e.g. chlorexidine, betadine)

g) Microchips (con't)
   i) Implant microchips subcutaneously in the dorsal neck area;
   ii) The standard size for a mouse microchip is about 2 x 13 mm
   iii) Have a compatible reader available to allow identification of the mice
   iv) Reuse microchips only after proper cleaning and sterilization (follow manufacturer's recommendation)

2) **Identification and genotyping methods-**Tail snip, ear punch, and toe clip are methods used for identifying individual animals and obtaining tissue for genotyping in small rodents.
3) Procedures for tissue biopsy for DNA analysis and/or genotyping must be described in an approved protocol.
4) **The PI must include a justification for IACUC approval prior to using the toe-clip method**. This explanation must clearly indicate why alternate methods of identification are not possible and cannot be based solely on the number of animals requiring identification.
5) The proper identification of genetically engineered animals in a litter is critical to the efficient pursuit of research and in reducing the number of animals involved in a research project. 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.
6) DNA for PCR analysis can be obtained from ear punches, hair or fecal samples, oral or rectal swabs. Depending on the requirements of the study, investigators are urged to consider these noninvasive alternatives. Larger amounts of DNA are required for Southern Blot determination of the genotype. (See references)

7) **Tail snip/ biopsy**
   a) Obtaining tissue from a rodent for DNA analysis via tail biopsy is a safe, effective and humane procedure. Tail clipping will not distinguish between animals but will serve as a source of DNA for genotyping.
   b) When performed properly it causes only minimal or transient pain and distress, and induces no more "physiological impact" (change in heart rate, body temperature, or activity level) than just restraining the animal for the procedure.
   c) Ideally, rodents should be **10-21** 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 rodents to be identified prior to weaning which can facilitate more efficient use of cage space.

d) 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.
e) Local anesthesia may be achieved by immersion of the tail in ice cold ethanol for 10 seconds, by an application of ethyl chloride spray or by the use of another suitable anesthetic as recommended by the attending veterinarian.
f) **For rodents greater than 21 days of age**: The use of a local or general anesthetic (e.g. isoflurane) is required prior to collection of tissue: Recent studies have proven that tail snips can cause hypersensitivity even six months after the tail has been snipped.
g) The last 5mm of a rodent tail has tendons, nerves and coccygeal vertebrae that partly ossify by the time a mouse is two weeks of 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.

h) Tail snip/ biopsy procedure

i) Manually restrain the rodent between thumb and forefinger and cradle the body with the palm of the hand. This is a convenient time to identify the animals using the appropriate method (i.e. ear punch, ear tag, transponder etc.).

i) **Tail snip procedure (con't**)

i) With sterile scalpel, razor blade, or scissors cleanly excise the distal 2mm (maximum 5 mm) of the 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 5mm are used. It is good practice to divide the tissue into several pieces after collection, label and freeze the extra samples at -20C in case you have to verify your samples.
ii) If small amounts of DNA are required, investigators should take only 2 mm of tail.
iii) 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 and disinfect after each animal.
iv) If a scalpel is used, also disinfect the work surface on which the tail is placed.
v) The investigator must monitor the animals to assure hemostasis after the animals are returned to the cage. Apply digital pressure, silver nitrate, as needed.
vi) The maximum number of tail snips that can be performed for genotyping purposes is one; therefore any further requirement for tissue should be obtained by ear punch

8) Ear Notching/Punching

a) This method involves collecting a sample of tissue from the ear using a punch designed for this task. The method is often used for identification purposes and so is ideal to fulfill both needs at the same time. 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. The procedure is best performed at 10-21 days of age but is humane and acceptable at all rodent ages without anesthesia or analgesia.

b) **Do not** use this method in rodents under 2 weeks of age
c) Restrain the animal by the scruff and using the ear punch to create holes and/or notches in the ears, following an identification chart
d) Whenever possible, use a simple code to limit the number of notches/punches
e) Have the identification chart readily available in the animal room to allow prompt identification of individuals
f) If possible, use the excised tissue as a sample for genotyping, replacing the need for a tail biopsy.
g) Ear punch devices and scissors should be disinfected between animals. This can be done by wiping with 70% ethyl alcohol.
h) Instruments used for ear notching can become dull after use and should be replaced often, as dull instruments can cause trauma to the notch site.

9) Toe clipping

a) **Note:** According to *The Guide for the Care and Use of Laboratory Animals*, as a method of identification for small rodents, should be used only when no other individual identification method is feasible and should be performed only on altricial neonates."

b) Toe clipping can only be performed in 1-12 day old mice and 1-7 day old rats without anesthesia. The ideal time is between postnatal day five and seven when the toes are large enough to work with yet the bones are not calcified. Older animals will require anesthesia.

c) Toe Clipping procedure

i) Digit removal is limited to two toes per foot, two feet per animal.
ii) Whenever it is feasible, amputating half a digit is sufficient.
iii) Instruments (surgical scissors, scalpel blades, etc.) must be sterilized before use and cleaned and disinfected between animals.
iv) Confirm bleeding has stopped prior to returning animals to their cage.
v) Apply a local anesthetic (e.g., lidocaine, bupivacaine, local anesthetic spray) on the site of amputation
vi) The removed tissue should be used for genotyping.
vii) Currently, it is generally accepted that anesthesia is not required for this procedure in mice up to 12 days old and rats up to 7 days old. However, evidence exists suggesting that surgical procedures performed without anesthetics in altricial rodents can induce long lasting or permanent changes in the nervous system (NRC, 2003 p 106). Investigators are advised to consult with DLAR on the use of anesthesia on older animals.

References

1. Definitions (see Glossary)
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