TITLE: Genotyping of Mice and Rats

Purpose: To establish consistent standards for genotyping of mice and rats in the FIT animal program.

PROCEDURE: The proper genetic 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. DNA for PCR analysis can be obtained from ear punches, hair, fecal samples, oral or rectal swabs [1-9]. 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. Obtaining tissue from a mouse or rat for DNA analysis via tail biopsy is a safe, effective, and humane procedure that causes minimal or transient pain and distress when performed properly. An alternative method is removal of 1 of the distal phalanges of a foot ("toe clipping"), which has the added advantage of permanently identifying the animal. DNA prepared from tail biopsies or toe clips is suitable for analysis by either Southern Blot or PCR. PIs are encouraged to use the least invasive alternative possible. If animals are genotyped, they must be accounted for in the animal numbers.

Tail biopsy:

1. Procedures for tail biopsy for DNA analysis and/or genotyping must be described in an approved Animal Care and Use Protocol. If animals are genotyped, they must be accounted for in the animal numbers.
2. Mice and rats must be 28 days old or less at the time of tail biopsy if no anesthesia is used. The optimal time is age 12-14 days. At this age the yield of DNA is highest [8,10,11]. In addition, the prompt analysis of tail tissue allows the genetically desirable animals to be identified prior to weaning which will facilitate more efficient use of cage space.
3. For animals more than 28 days old, local or general anesthesia is required prior to collection of tissue as well as perioperative analgesia as recommended by a laboratory animal veterinarian. This must be described in the approved Animal Care and Use Protocol.
4. Samples between 2 and 5 mm may be taken. The yield of DNA does not proportionally increase as tail fragments larger than 5 mm are used due to the presence of cartilage and bone that are not as rich in DNA as the more metabolically active distal end [14]. Requests to sample greater amounts must be requested in the context of an animal use protocol and approved by the IACUC.
5. All animals must be monitored to assure effective hemostasis. Hemostasis can be achieved by methods such as digital pressure, cautery, or the use of silver nitrate.
6. Repeat tail biopsies on animals older than 24 days require anesthesia and analgesia and must be justified in the Animal Care and Use Protocol and approved by the IACUC.

Toe clipping:

1. Procedures for toe clipping for DNA analysis and/or genotyping must be described in an approved Animal Care and Use Protocol. Toe clipping may only be used when no other method of individual identification is feasible [12].
2. Mice and rats must be less than 14 days old at the time of toe clipping. Performing the procedure at day 7-10 is technically easier than earlier when toes have not completely separated [13]. In addition, the prompt analysis of toe tissue allows the genetically desirable animals to be identified prior to weaning which will facilitate more efficient use of cage space.
3. Toe clipping is not permitted in animals >14 days of age.
4. No more than 1 toe per foot may be sampled; the front feet should not be sampled if future experimental use could include tests of grip strength.
5. All animals must be monitored to assure effective hemostasis. Hemostasis can be achieved by methods such as digital pressure, cautery, or the use of silver nitrate.
6. If repeat genotyping is necessary, another method must be utilized for DNA collection.

Ear punching:

1. Procedures for ear punching for DNA analysis and/or genotyping must be described in and approved Animal Care and Use Protocol.
2. Mice and rats can be ear punched at any age after the ears have separated from the head during neonatal development. The prompt analysis of ear tissue allows genetically desirable animals to be identified prior to weaning.
3. Ear punching can be used as both a method of identification and of tissue collection for genotyping.
4. It is recommended to use a 2 mm ear punch, since 1 mm punches do not provide enough tissue for DNA analysis.
5. No anesthesia or analgesia is required for ear punching.
6. If additional genotyping is necessary, ear punching can be repeated.

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Approved by the IACUC 14 July 2015
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