Wm. S. Middleton Memorial Veterans Hospital
VA IACUC Policy #28-13

Genotyping of Mice and Rats

Purpose: To establish consistent standards for genotyping of mice and rats on all VA approved protocols.

Background and relevant information: 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. DNA for PCR analysis can be obtained from ear punches, hair, fecal samples, oral or rectal swabs. Depending on the requirements of the study, investigators are urged to consider these noninvasive alternatives. 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 one 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 are suitable for analysis by either Southern Blot or PCR.

Ear punching

1. Procedures for ear punching for DNA analysis and/or genotyping must be described in an approved ACORP.
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.

Tail biopsy

1. 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 (and lower per diem charges.)
2. Procedures for tail biopsy for DNA analysis and/or genotyping must be described in an approved ACORP. If animals are genotyped, they must be accounted for in the animal numbers.
3. Mice and rats should be 10-21 days old for this procedure. (less than 0.5 cm of tissue). Local anesthesia may be used as recommended by attending veterinarian. At or before 21 days of age is considered pain category C.
4. For animals >21days old, local or general anesthesia is required prior to collection of tissue as well as peri-operative analgesia as recommended by a laboratory animal veterinarian. This must be described in the approved ACORP. This is considered a category D procedure since the animals are anesthetized.
5. No more than 5 mm of tail may be sampled. The yield of DNA does not proportionally increase as tail fragments larger than 5 mm are used.
6. All animals must be monitored to assure effective hemostasis. Hemostasis can be achieved by digital pressure or the use of silver nitrate.
7. Repeat tail biopsies on animals older than 21 days require anesthesia and analgesia and must be justified in the ACORP.

Toe clipping

1. Toe clipping may only be used when no other method of individual identification is feasible.
2. Procedures for toe clipping for DNA analysis and/or genotyping must be described in an approved ACORP. If animals are genotyped, they must be accounted for in the animal numbers.
3. It may be the preferred method for neonatal mice up to 7 days of age Toe clipping is not permitted in animals >14 days of age. This is considered pain category C.
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 digital pressure or the use of silver nitrate.
6. If repeat genotyping is necessary, another method must be utilized for DNA collection and must be approved on the VA ACORP.

Principal Investigator Responsibility:
PIs are responsible for following the guidelines above regarding genotyping and incorporating these procedures into their ACORP(s) when applicable.

Role of the Veterinary Medical Consultant (VMC) and Animal Research Facility (ARF) staff:

Educate PIs and animal users of proper method for genotyping.

VA IACUC Oversight: The IACUC shall review proposed protocols for conformity to these guidelines, and enforce compliance.

Questions: Any questions on this policy should be directed to the Administrative Officer, 280-7007.

References: *The Guide for the Care and Use of Laboratory Animals* (2011)

Effective date: Reviewed approved at the 11/07/2011 IACUC meeting.
Subsequent review and approval: **11/07/2012, 11/06/2013**