

RODENT GENOTYPING GUIDELINES

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I. General principles

- A. These guidelines describe methods for obtaining DNA extracted from tissues for PCR analysis on genetically engineered rodents.
- B. The IACUC requires researchers to use the least invasive techniques possible for genotyping. Although tail biopsy is a safe, effective and humane procedure for mice and rats, research investigators must consider less invasive alternatives such as ear punches as part of animal identification and noninvasive alternatives such as blood, hair, fecal or oral saliva samples. Genotyping details and collection techniques must be described and approved in the IACUC protocol.

II. Noninvasive techniques

These methods of DNA sampling produce minimal animal discomfort:

- A. Blood Sampling
1. Follow the accepted methods of blood collection as written in the IACUC blood collection policy.
- B. Hair sampling
1. Hair follicles plucked from the animal can be used as a source of DNA for

genotype identification.

C. Fecal sampling

1. Colonic and rectal cells collected from fecal pellets can be used as a DNA source for genotyping.
2. In general, simple rodent restraint will cause an animal to defecate.

D. Saliva sampling

1. The oral cavity can be swabbed to retrieve cells for DNA sampling.

III. Invasive techniques

A. Ear punching and ear notching

1. Ear punching is intended for rodent identification purposes; use the tissue for DNA analysis.
2. This procedure can be done at any age, no analgesia or anesthesia required.
3. Proper animal restraint for ear punch and ear notch technique must be employed.
4. The Principal Investigator is responsible to ensure all individuals performing the task are appropriately trained.

B. Tail clipping

1. For mice greater than 16 days or rats greater than 14 days of age, local or general anesthesia is required; post procedural analgesia is optimal.
 - Dipping the tail in ice-cold ethanol for at least 10 seconds prior to tail snip and/or application of bupivacaine to the snipped tail as a local anesthetic is recommended.
 - Ethyl chloride and other topical freezing agents are no longer preferred due to associated tissue necrosis.
2. For mice older than 28 days and rats older than 14 days, general anesthesia is required, with 24 hours of post-operative analgesia.
3. The total amount of tail tissue removed should be the minimum necessary, generally 2-3 mm, and no greater than 5 mm. Taking more than five millimeters is not acceptable, regardless of age.
4. Repeated tail clips on a mouse are discouraged. When repeated tail clips are necessary, regardless of age, they require general anesthesia as described in the

protocol and 24 hours of post-procedural analgesia.

5. Clean, sharp, surgical scissors or scalpel blade must be used; apply either gentle pressure or a chemical cautery (Styptic powder or swab) until bleeding has ceased.

C. Toe clipping

Toe clipping is used as a method of identifying mice only by using a predetermined numbering code(1) and may simultaneously be used as a method to obtain biopsy tissue for genotyping. Toe clipping, as a method of identification of mice, should be used only when no other individual identification method is feasible.

1. Toe clipping can only be used in pre-weaning mice less than 28 days.
2. Toe clips are limited to one per foot and one time only.
3. Only the tip of the toe (distal phalanx) can be clipped.
4. Excision of the first digit of either front paw is prohibited. The first digit is the most medial appendage. Avoid clipping digits on the forepaws as much as

possible.

5. The principal investigator must assure all personnel are appropriately trained.
6. Before 7 days of age, no anesthesia is required.
7. Between 8 and 28 days of age, general anesthesia is required.
8. Toe clipping is only acceptable in mice.

IV. References

Please visit <http://www.iacuc.pitt.edu/policies> to view policies on the following:

1. Anesthesia
2. Blood collection
3. Rodent identification
4. Rodent surgical guidelines/aseptic practices
5. Drug formulary

V. Additional Information

Developmental and Behavioral Effects of Toe Clipping on Neonatal and Preweanling Mice with and without Vapocoolant Anesthesia. JAALAS march 2014 Vol 53 No2 Pg 132-140.

Transgenic Animal Technology: Alternatives in Genotyping and Phenotyping. Comparative Medicine. 2003. Vol 53 Pg 126-139.

DNA from tissues of young mice is optimal for genotyping. Electronic Journal of Biotechnology.2015 Pg 83-87.