

Policy for Rodent Genotyping

Policy #A-010

VA Pittsburgh Healthcare System
Pittsburgh, PA 15240

Service Line(s):
Research and Development (R&D)
Department

Signatory Authority:
Dr. Steven Graham, ACOS

Effective Date:
September 15, 2020

Responsible Owner:
Associate Chief of Staff/R&D

Recertification Date:
December 2021

1. PURPOSE AND AUTHORITY

- a. This policy describes methods for obtaining DNA for PCR analysis on genetically engineered rodents.
- b. This Policy sets forth mandatory procedures and processes to ensure compliance with VHA Directive 1200.07, Use of Animals in Research, November 23, 2011.
- c. This policy applies to all VA Pittsburgh Healthcare System (VAPHS) and Veterans Research Foundation employees (including those with without compensation [WOC] appointments) working with rodents within the VAPHS Animal Research Facility (ARF) and VAPHS laboratories as specified in an IACUC-approved Animal Component of Research Protocol (ACORP).

2. PROCEDURES

a. Background.

Transgenic animal colonies require accurate genotyping of litters to achieve research goals and reduce the overall use of animals. The most common methods used in determination of genotype are polymerase chain reaction (PCR) or Southern Blotting.

DNA used for genotyping can be obtained from blood, tissues removed during animal identification (ear punches or toe clips), hair samples, stool, oral swabs or tail snips.

b. Policy.

The IACUC requires VAPHS Principal Investigators (PIs) to use the least invasive techniques possible for genotyping. Although tail biopsy is a safe, effective and humane procedure for mice and rats, PIs 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.

To ensure that less invasive alternatives have been considered for use during genotyping, justification is required when the PI decides to obtain DNA by performing tail snips or toe clips. A justification must be provided in Section 5 of the Breeding and Weaning Supplement that is submitted with the ACORP submission.

c. **Non-Invasive Techniques.**

These methods of DNA sampling produce minimal animal discomfort:

- Blood sampling – Follow the accepted methods of blood collection.
- Hair sampling - Hair follicles plucked from the animals can be used as a source of DNA for genotype identification.
- Fecal sampling – Colonic and rectal cells collected from fecal pellets can be used as a DNA sources for genotyping.
- Saliva sampling – The oral cavity can be swabbed to retrieve cells for DNA sampling.

d. **Invasive Techniques.**

(1) Ear Punching and Ear Notching

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

(2) Tail Clipping

- 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.
- For mice older than 28 days and rats older than 28 days, general anesthesia is required, with 24 hours of post-operative analgesia.
- The total amount of tail tissue removed should be the minimum necessary, generally 2-3 millimeters, and no greater than 5 millimeters. Taking more than 5 millimeters is not acceptable, regardless of age.
- 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.
- Clean sharp surgical scissors or scalpel blades must be used; apply either gentle pressure or a chemical cautery (styptic powder or swab) until bleeding has ceased.

Mice undergoing tail snips are separated into the following categories:

Age at Sampling	Tail sample length (millimeters)	Anesthesia Required?	Surgical description required in ACORP?	Procedure Card Required?	USDA pain category
≤ 16 days	< 5 mm	No	No	No	C
16-28 days	< 5 mm	Yes (local or general)	Yes – Must be described in the ACORP and Appendix 5	No	D
>28 days	< 5 mm	Yes*(general)	Yes – Must be described in the ACORP and Appendix 5	Yes	D
Repeat tail biopsy	< 5 mm	Yes*(general)	Yes – Must be described in the ACORP and Appendix 5	Yes	D

*Additional requirements may be requested by the Veterinarian(s).

(3) Toe Clipping

- Toe clipping is used as a method for identifying mice only by using a predetermined numbering code 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.
- Toe clipping can only be used in pre-weaning mice less than 28 days old.
- Toe clips are limited to one per foot and one time only.
- Only the tip of the toe (distal phalanx) can be clipped.
- 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.
- The PI must assure all personnel are appropriately trained.
- Before 7 days of age, no anesthesia is required.
- Between 8 and 28 days of age, general anesthesia is required.
- Toe clipping is only acceptable in mice.

Mice undergoing toe clips are separated into the following categories:

Age at Sampling	Anesthesia Required?	Surgical description required in ACORP?	Procedure Card Required?	USDA pain category
≤ 7 days	No	No	No	C
8-28 days	Yes	Yes – Must be described in the ACORP and Appendix 5	Yes	D

3. ASSIGNMENT OF RESPONSIBILITIES

Principal Investigator (PI) – PI must use the least invasive techniques possible for genotyping or must consider less invasive alternatives; the PI must also describe genotyping details and collection techniques in the IACUC protocol.

4. DEFINITIONS

None.

5. REFERENCES

- Policy #A-012 VAPHS ARF SOP
- The Guide for the Care and Use of Laboratory Animals, National Research Council, National Academy Press, 8th edition, 2011.
- Developmental and Behavioral Effects of Toe Clipping on Neonatal and Pre-weanling Mice with and Without Vapocoolant Anesthesia. Journal of the American Association for Laboratory Animal Science. March 2014; Vol 53 No. 2, 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.

6. REVIEW

This policy is reviewed at recertification, when there are changes to the governing document (for example, national policy or an accreditation body mandate), and any regulatory requirement for more frequent review.

7. RECERTIFICATION

This Policy is scheduled for recertification fifteen (15) months from the Effective Date. In the event of contradiction with national policy, the national policy supersedes and controls.

8. SIGNATORY AUTHORITY

//signed copy on file //

Gretchen Haas, MD
Chair, Research and Development Committee
Date Approved: September 15, 2020

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Steven Graham, MD, PhD
Associate Chief of Staff Research and Development Department
Date Approved: September 15, 2020

NOTE: *The signature remains valid until rescinded by an appropriate administrative action.*

DISTRIBUTION: SOPs are available at: <https://www.va.gov/pittsburgh-health-care/research/safety-security/animal-research/>