IACUC Guidance:	**TAMU-G-007**	Title:	**IACUC Guidelines on Genetically Modified Animals and Genotyping**

Location	Effective Date	Review By
College Station/Dallas/Galveston/Kingsville	10/20/2022	08/31/2025
Houston	10/01/2022	08/31/2025

1. PURPOSE

1.1. To provide information about animal care and welfare issues that relate to the use of genetically modified (e.g., transgenic) animals for research, teaching, or other purposes at Texas A&M University.

2. SCOPE

2.1. Does not include a thorough discussion regarding analgesics and anesthesia. See TAMU-G-002.

3. RESPONSIBILITY

3.1. Generation or use of genetically modified animals (e.g. transgenic) may require review and approval by the Institutional Biosafety Committee.
3.2. Researchers should use the least invasive method of genotyping that is practical for their research and should collect the smallest sample necessary for reliable results.
3.3. The PI must ensure sufficient training for individuals performing these technical procedures.
3.4. The PI is responsible for describing tissue collection procedures in the approved animal use protocol.
3.5. The PI is responsible for reporting new phenotypes that negatively affect animal health and well-being to the IACUC.

4. DEFINITIONS AND/OR ACRONYMS

4.1. **AUP**: Animal Use Protocol. Document submitted by the PI indicating the housing and procedures involving animals.
4.2. Centrally administered support service for animal research and teaching programs at Texas A&M University:
4.2.1. **ARU**: Animal Resource Unit supports the College of Dentistry vivarium
4.2.2. **CMP**: Comparative Medicine Program supports the Texas A&M College Station campus
4.2.3. **PAR**: Program for Animal Resources supports the Institute of Biosciences and Technology vivarium
4.2.4. **PRF**: Pharmaceutical Research Facility supports the Kingsville Pharmaceutical Science Facility vivarium
4.2.5. **Sea Life:** The Sea Life Facility supports the Galveston campus
4.3. **Cryoanesthesia:** Local anesthesia produced by chilling an area to near-freezing temperature to diminish neural sensitivity to pain during brief/minor surgical procedures, e.g. vapocoolant spray.
4.4. **Epigenetic:** The study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself.
4.5. **ERC:** Early Removal Criteria. Specific, predetermined indicators of pain and distress used to establish early study endpoints without loss of scientific quality.
4.6. **Genotyping:** A molecular technique that will determine the presence or absence of a specific genetic feature, be it an engineered mutation or naturally occurring polymorphism.
4.7. **GMA:** Genetically Modified Animals. Animals that have induced mutations that are human-made alterations in their genetic code. This includes both transgenic and targeted mutations that are created to study the expression, overexpression, or underexpression of a specific gene.
4.8. **Humane Endpoint**: Is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved.
4.9. **IACUC**: Institutional Animal Care and Use Committee. Institutional body responsible for ensuring adherence to federal regulation and institutional policy relating to the care and use of animals in teaching, testing and research. Appointed by the Institutional Official.
4.10. **IBC**: Institutional Biosafety Committee. Institutional body responsible for the review and oversight of research, teaching, and testing activities utilizing biohazardous materials and Dual Use Research of Concern. Appointed by the IO.

4.11. **PCR:** For the purposes of this document, Polymerase Chain Reaction
4.12. **PI:** Principal Investigator. The individual who has ultimate administrative and programmatic responsibility for the design, execution, and management of a project utilizing vertebrate animals.
4.13. **USDA:** United States Department of Agriculture. USDA Animal Care, a unit under the Animal and Plant Health Inspection Service, administers the Animal Welfare Act (AWA) and associated Animal Welfare Act Regulations (AWAR).

5. GUIDELINES OR PROCEDURE

5.1. Genetically modified animals (GMA) represent an increasingly large proportion of animals used in research. Protocols that utilize GMA should:
- 5.1.1. Describe likely health outcomes of any new genetic modification, according to the current understanding of the gene in question.
- 5.1.2. Describe the monitoring program, especially for epigenetic changes that could produce observable alterations in phenotype, function or behavior in later stages of life, or across multiple generations, as applicable.
- 5.1.3. Provide early removal criteria and humane endpoints for negative phenotypes (See TAMU-G-001).
- 5.1.4. Describe method of genotype assessment.

5.2. Tissue Collection for Genotyping Mice and Rats
- 5.2.1. Pinna Biopsy (or ear punch)
  - 5.2.1.1. The ear is sufficiently developed around 14 days of age to allow suitable tissue collection.
  - 5.2.1.2. A two-millimeter ear punch or marginal notch is recommended.
  - 5.2.1.3. If repeated biopsies are required, the use of the alternate pinna or an alternative method should be considered.
  - 5.2.1.4. Pinna biopsies performed as described do not require the use of anesthetics or analgesics.
    - 5.2.1.4.1. EMLA cream may be used as described in the approved protocol.
  - 5.2.1.5. Instrumentation must be sharp, sterilized before use, cleaned, and disinfected between animals (See TAMU-G-013).
- 5.2.2. Tail Biopsy
  - 5.2.2.1. Performed as early as possible to minimize potential pain.
  - 5.2.2.2. Tail biopsy length should be limited to the smallest amount possible.
  - 5.2.2.3. In general, a biopsy of approximately 2 mm is sufficient to generate DNA for multiple PCR reactions.
    - 5.2.2.3.1. If larger individual sample sizes are required, justification should be provided in the AUP.
    - 5.2.2.3.2. No more than 5 mm total tail length is to be removed if additional tail biopsy is performed unless justified in the AUP.
  - 5.2.2.4. For preweanling animals (<21 days of age), the use of anesthesia is suggested; however, the use of vapocoolants (e.g. ethyl chloride) has been reported to result in undesirable effects.
  - 5.2.2.5. For mice 21 days of age or older, the use of anesthesia is required unless otherwise approved by the IACUC.
  - 5.2.2.6. For rats 21-35 days of age, the use of local or general anesthesia is required unless justified in the AUP, or otherwise approved by the IACUC.
  - 5.2.2.7. For rats (>35 days of age) general anesthesia is required.
  - 5.2.2.8. Anesthesia may be general or local, i.e. by immersion of the tail tip in ice cold ethanol for 10 seconds prior to biopsy.
  - 5.2.2.9. Post-procedural analgesia should be considered. The need to provide an effective analgesic (e.g. an opioid such as buprenorphine) post-biopsy will increase with the age of the rodent post weaning, length of the biopsy and/or with repeated biopsies.
  - 5.2.2.10. Consultation with CMP/ARU/PAR/PRF is recommended for direction in the appropriate choice of anesthetics/analgesics, which must be described in the approved AUP.

5.2.2.11. Recommended Tail Biopsy Procedure:

5.2.2.11.1. Manually restrain the rodent between thumb and forefinger.

5.2.2.11.2. Starting with a sterile scalpel, razor blade, or scissors, cleanly excise the defined length of distal tail. 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 after each animal. Sanitize the scalpel or scissors between animals using an appropriate method (e.g., using detergent followed by 70% ethanol, bead sterilizer, etc.). If a scalpel is used, also sanitize the work surface on which the tail is placed between animals.

5.2.2.11.3. The investigator must monitor the animals to assure hemostasis after the rodents are returned to the cage. If needed, apply digital pressure, heat cautery (briefly), silver nitrate, or some other means of hemostasis. If silver nitrate is used, the tissue must be washed free of the chemical with saline following hemostasis to neutralize the chemical reaction.

5.2.3. Distal Phalanx Biopsy (or toe clipping)

5.2.3.1. As a method of identification of small rodents, toe-clipping should be used only when no other individual identification method is feasible. Justification for the selection of the method over other options should be provided in the AUP.

5.2.3.1.1. It may be the preferred method for neonatal mice up to 7 days of age as it appears to have few adverse effects on behavior and well-being at this age, especially if toe clipping and genotyping can be combined.

5.2.3.2. Toe clipping should only be used in altricial pre-weanling rodents (e.g. mice and rats, **NOT** guinea pigs) after the toes are no longer webbed and **before they reach eight days of age**.

5.2.3.3. Every reasonable effort should be made to minimize pain or distress, including limiting the number of digits clipped to **one** digit per rodent.

5.2.3.4. It is preferable to remove toes from a hind paw rather than a forepaw.

5.2.3.5. If the forepaw must be used, it is preferable to not cut the pollux ("dew claw") as this may decrease the rodent's grasping ability.

5.2.3.6. Researchers should consult with CMP/PAR/PRF prior to toe clipping for advice on the need for topical anesthetics or analgesics, which must be described in the approved AUP.

5.2.3.7. Use of a local vapocoolant anesthesia is not recommended.

5.2.3.8. Instruments used for toe clipping must be sharp, sterilized before use and cleaned and disinfected between animals (See TAMU-G-013 and TAMU-G-026).

5.2.3.9. After removing the digit, gentle pressure should be applied until hemostasis occurs.

5.2.4. Noninvasive testing methods

5.2.4.1. Include the use of hair follicles, blood, feces or oral swabs.

5.2.4.2. Can be applied to a variety of species.

5.2.4.3. Are preferred by the IACUC, over invasive methods, when appropriate.

6. EXCEPTIONS

6.1. The PI may request an exception to the above standards by describing the departure in the AUP

6.2. For programmatic exceptions, the facility director or manager may submit a request for the exception using TAMU-F-013

7. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

7.1. References

7.1.1. https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b3-rodent_genotyping.pdf

7.1.2. Guide for the Care and Use of Laboratory Animals, pgs. 75 & 87.

7.1.3. Braden GC, Brice AK, Hankenson FC. 2015. Adverse effects of vapocoolant and topical anesthesia for tail biopsy of preweanling mice. Journal of the American Association for Laboratory Animal Science: JAALAS 54:291-298.
7.1.4. Paluch L, Lieggi C, Dumont M, Monette S, Riedel E, Lipman N. Developmental and Behavioral Effects of Toe Clipping on Neonatal and Preweanling Mice with and without Vapocoolant Anesthesia. 2014. JAALAS. p 132-140.

7.2. Resources
7.2.1. For more information on genotyping, please contact:
7.2.1.1. CMP: at (979) 845-7433
7.2.1.2. PAR: at (713) 677-7471
7.2.1.3. PRF: at (361) 221-0770
7.2.1.4. Sea Life Facility: at (409) 740-4574

7.3. IACUC/AWO Referenced Documents: (requires TAMU NetID authentication)
7.3.1. TAMU-F-013 Request for Programmatic Exception from Animal Welfare Standards
7.3.2. TAMU-G-001 Guidelines on Choosing Appropriate Endpoints
7.3.3. TAMU-G-002 Guidelines on the Use of Anesthesia and Analgesia
7.3.4. TAMU-G-013 Guidelines for Surgical Procedures in Rodents
7.3.5. TAMU-G-026 Guidelines for the Evaluation of Sanitation Practices

7.4. Acknowledgement
7.4.1. This document was partially adapted using materials obtained from Wayne State University.

8. HISTORY

Effective Date	Version #	Description
09/19/2019	000	College Station/Galveston: Updated format and new content; Replaces unnumbered documents "Genetically Altered Animals" and "Guideline for Tail Clipping in Mice".
01/27/2020	001	Houston/Kingsville: Updated format and new content; replaces IBT-206
02/18/2020	002	Dallas: Updated format and new content; replaces CD-206
02/20/2020	003	College Station/Galveston: 5.1.2. 'as applicable' added to denote epigenetic monitoring is activity dependent; new statement discouraging toe clipping in section: 5.2.3.
02/20/2020	003	Typo in 5.2.3.5.: "Pollux" not "Hallux". Processed administratively.
03/24/2022	004	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with College Station/Galveston
09/01/2022	005	College Station/Dallas/Galveston: Renewal; updated definitions, clarification of maximum total length of tail biopsy, require justification for toe clipping method, addition of exceptions section. Reviewed and approved via email.
10/01/2022	006	Houston/Kingsville: Renewal; updated definitions, clarification of maximum total length of tail biopsy, require justification for toe clipping method, addition of exceptions section. Reviewed and approved via email.
10/20/2022	007	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.