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**VTCG**

Institutional Animal Care and Use Committee

**New Protocol Review Form – Breeding – Mice**

*Last updated 2/16/2023*

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| **Section 1: General Information** |

**Protocol Information**

IACUC Protocol Title:

IACUC Protocol Number:

Previous IACUC Protocol Number:

Office Use:

Approval Date:

Expiration Date:

**Principal Investigators Name**

Principal Investigator (PI):

Department:

Phone Number:

**Primary Contact Information**

Same as PI

Contact Person:

Department:

Phone Number:

**Alternate Contact Information (to be used in emergencies when the primary contact is unavailable)**

Same as PI

Alternate Contact Person:

Department:

Phone Number:

**Directions:**

* **Important:** Save this form to your computer’s hard drive before completing it, or your responses may not be captured!
* It is recommended that you read the entire form before completing.
* Upload the completed form to the online IACUC Protocol Management (PM).
* Please respond to all questions in this form. If a particular question does not apply to your breeding protocol, please indicate this. Type responses in the designated shaded boxes or check the designated check boxes.
* For questions, contact the Animal Care and Use Program Office at IACUC@vt.edu or 540/231-0931.

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| **Section 2: Assessment of Unnecessary Duplication** |

1. If this study is an extension of previous work, briefly explain why more work needs to be done. Please check at least one:

Justification for experiments will be provided in other protocols. This protocol is dedicated to breeding and housing mice for future experiments and between active experimental approved IACUC protocols.

Other:

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| **Section 3: Justification of Species Selection** |

1. List the species of animals to be used on this breeding protocol in the table below. The strain and number of animals needed will be recorded under “animals’ in the PM.

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| Species or Strain |
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1. Justify the selection of species (check all applicable boxes).

This is a new model.

A large database exists for this species, which will allow comparisons with previous data.

The anatomy, genetics, physiology, or behavior of the species is uniquely suited to the study.

This is the phylogenetically lowest species with adequate size, tissue, or anatomy for the study.

The results will be directly applicable to the health or care of this species.

Other (please describe):

1. Explain why a “lower order species” or non-animal alternatives cannot be used to achieve the desired results. Please check at least one of the below:

The mouse is the smallest vertebrate mammal model with brain blood vessel morphology and physiology appropriate for translational research to humans. In addition, the availability of transgenic mice provides a unique opportunity to verify the contribution of specific proteins to the processes of blood vessel growth and repair in the brain.

Other:

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| **Section 4: Justification of Number of Animals** |

A key principle in the ethical use of animals in research, testing, and teaching is that the number of animals used in each project is the minimum necessary to obtain valid and meaningful results. In determining the numbers of animals required, which of the following are applicable:

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|  | A statistical assessment (power analysis) was performed. Describe the statistical method/test used to determine appropriate group sizes/animal numbers, and state if a statistician was consulted to assist in the determination: |
|  | The numbers of animals or group sizes have been established by federal guidelines/requirements. |
|  | This is a pilot study that uses the minimum number of animals required to provide meaningful, but not statistically significant, data. |
|  | Other (please describe): |
|  | **Numbers proposed are based on the experimental needs of my experimental protocol, and may be increased by amendment to accommodate future protocols. Specific statistical analyses are provided in the IACUC approved experimental protocols. As an example: Breeder numbers are based on average litter size of 6 pups/litter for the C57BL6 background strain and 30 breeder pairs (60 mice) replaced every 6 months.** |

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| **Section 5: Pain Category** |

Please indicate which pain categor(y/ies) your study will be in, based on the descriptions below. List all pain categories animals may experience on this protocol.

**Pain Category Descriptions**

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|  | B: Breeding or holding animals only; no research conducted. For example: wild type mice. |
|  | C: Use of procedures that cause no or slight/momentary pain or distress (e.g., observational studies; injection of non-irritating agents; blood collection from peripheral vessels; collection of cells or tissues following euthanasia). For example: transgenic tail snipped by ≤16 days of age. |
|  | D: Use of procedures that would cause more than slight/momentary pain or distress, but are performed using appropriate anesthetics, analgesics, or tranquillizers to relieve pain (e.g., minor or major surgical procedures [survival or non-survival] performed under anesthesia; collection of cells or tissues prior to euthanasia; painful procedures performed under anesthesia [retro-orbital blood collection in rodents]). For example: transgenic mice requiring anesthesia for tail snips after 16 days of age. |
|  | E: Use of procedures that cause more than slight/momentary pain or distress, but that cannot be performed using appropriate anesthetics, analgesics, or tranquilizers without adversely affecting the study (e.g., toxicity and lethal disease studies in which the animals are allowed to die without intervention and mortality is the endpoint). Mechanical restraint may, depending upon duration and type of restraint, be considered a category "E" procedure. **Approval to conduct a Category E study requires detailed justification.** |

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| **Section 6: Evaluation of Alternatives** |

The Federal Animal Welfare Act and PHS Policy require that researchers evaluate the existence of alternatives when procedures cause more than slight or momentary pain or distress for the animal. Examples of alternatives include less sentient animal models, computer models, audio-visual training programs, and refinements to proposed procedures.

**The Virginia Tech IACUC requires that an alternatives search be conducted for studies in pain categories D and E. If applicable, complete the following questions:**

1. Which database sources or methods were used to evaluate the existence of viable alternatives? You must **include two or more databases** in your search. The IACUC Protocol Literature Search website (<https://www.research.vt.edu/iacuc/submission-process/iacuc-protocol-literature-search.html>) includes more information on how to formulate a database search.

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|  | AGRICOLA |  | Biological Abstracts |
|  | Current Contents Connect |  | PubMed (Medline) |
|  | VetCD (Index Veterinarius) |  | Other(s) (please identify): |

1. Give the key words used in conducting the database search and number of hits, i.e. anesthesia and mouse and lupus number of hits 512, etc. Example Key Words: tail snip, alternative, dna sampling, ear tags, toe snips

1. Date(s) when the search was conducted:

1. Years included in the search criteria:
2. What alternatives (replacements, reductions, and refinements) were identified? Please check any that apply below.

NOTE: the use of pain-relieving drugs for surgical and other procedures is considered a refinement.

Refinement: anesthesia will be used during tail snipping after day 16.

Alternatives identified that will not be used: blood sampling for dna instead of tail snipping

Alternatives identified that will not be used: each punches for dna instead of tail snipping

Alternatives identified that will not be used: ear tags for identification instead of ear notching

Alternatives identified that will not be used: microchipping for identification instead of ear notching

Alternatives identified that will not be used: tattooing for identification instead of toe snipping

Other:

1. If alternatives were identified but will not be used, please provide justification. Please check any that apply below.

To determine if genetically-engineered mice carry a gene of interest, tail snips are commonly performed to obtain DNA samples over taking blood samples. Blood sampling may require longer restraint of the animal and may lead to dehydration and anemia, side effects which outweigh the momentary distress of tail snipping.

Ear Notching (the simplest and most widely used method of identification) is preferred over ear tags or microchips. Ear tags, which are often large and heavy, can be lost during grooming or fighting and it is possible that infection can occur at the tag site. Microchips are too large for use with neonates, require extensive preparation of the area where chips are implanted and there have been some reports of tumors caused by microchips in laboratory mice and rats.

Other:

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| **Section 7: Animal Well-Being & Harm/Benefit Analysis** |

1. Explain how you will minimize expected animal pain and distress and enhance animal well-being (e.g., use of sedatives, tranquilizers, or anesthetics; familiarization/conditioning of the animal; enrichment opportunities). Please check at least one below:

Tail tips of genetically modified pups will be collected from mice in accordance with the [ARCD Rodent Genotyping Policy](https://internal.research.vt.edu/system/files/512.00%20-%20Rodent%20Genotyping%20Policy.pdf). Tail tip biopsies will be performed without anesthesia when mice are ≤ 16days of age. If biopsies are performed when mice are > 16 days of age the mice will receive appropriate anesthesia and analgesia in accordance with the policy. No research will be conducted on this protocol; only breeding or holding mice. Mice will be socially housed except when older males show aggressive behavior or if timed pregnancies are necessary or harem-bred females are separated before parturition. Environmental enrichment will also be provided per facility standards, usually nesting material and huts. Any dams experiencing dystocia (difficult labor and delivery) will be examined by veterinary staff and treated appropriately or euthanized, if necessary.

Other:

2. Please assess whether the "harm" caused to the breeding animals is justified, please explain the benefits to humans or animals or both from the proposed protocol activity outweighing the "harm". Please check at least one below:

The harm expected is limited to temporary pain from tail snipping or rarely dystocia. The benefits of this protocol are manifested in the findings in our experimental protocols.

Other:

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| **Section 8: Breeding Method and Design** |

Describe the breeding design of this protocol. Please include the following for each breeding method and design if you have more than one on this protocol: \*Strains: Please note there are two options for providing the strains of mice that will be used on this protocol. 1. Add each strain individually under the online “Animal” section or 2. Add only “mouse” under the “Animal” section answer questions in the “animal box”, for special instructions state “see attached spreadsheet under Supporting Documents” then attach a spread sheet under “Supporting Documents” with the following information for each strain: total number, ages, sex, any special housing/husbandry/phenotype.

1. Breeding Method (monogamous, trio or harem (must be scientifically justified)):

2. Total number of animals to be used, given as an equation, to include breeding animals and all pups produced even if they will be euthanized ex. (4 strains\* x 5 breeding animals/year x 2 animals/pair) + (200 pups produced/strain/year x 4 strains) = 840 total animals/year x 3 years = 2520 total or 840 strain/over three years:

3. Please give a general timeline for the breeding animals per cycle and approximately how many cycles they will go through until euthanized:

Describe any additional breeding designs following the above “format”:      

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| **Section 9: Breeding Protocol Procedures** |

1. Please list and describe all procedures identified in Section 8 and give any possible adverse effects they may have on animal health or well-being. \*For surgical procedures please only give possible adverse effects and say see appendix B for description. \*Attach any monitoring sheets Under Supporting Documents on the PM that will be used on this protocol. All injections will be performed per the approved IACUC guidelines on rodent injections. Check all that apply below.

**Breeding (Must follow the** [VT IACUC Guidelines to Prevent Mouse Overcrowding](https://internal.research.vt.edu/system/files/Guideline%20-%20Mouse%20Overcrowding_0.pdf)

Monogamous breeding: Mice will be used in monogamous breeding pairs from the age of 6-8 weeks through up to 18 months for some males and identified by cage cards. Pups will be weaned before a second litter is born or by no later than 28 days if there is no second litter. Mouse chow (dry or slightly moistened) may be placed on the cage bottom at the time of weaning to ensure food procurement by young mice.

Trio breeding: Mice will be used in a trio breeding scheme with one male and two females. Females will be separated into individual cages after breeding or before the birth of pups. Pups will be weaned before a second litter is born or by no later than 28 days if there is no second litter born. Mouse chow (dry or slightly moistened) may be placed on the cage bottom at the time of weaning to ensure food procurement by young mice.

Timed pregnant females: Female mice will be checked for vaginal plugs early each morning. Plug checks may require the use of fine-tipped forceps cleaned with 70% ethanol to gently open the vagina to facilitate vaginal plug detection. Tissue bruising may occur as a result of aggressive plug checking with fine forceps, and to avoid this, personnel will be trained in appropriate technique.

Harem breeding: Scientific justification is required. Please describe and give justification and/or historical data of using this breeding within your colony. Females will be separated into individual cages after breeding or before the birth of pups. Pups will be weaned before a second litter is born or by no later than 28 days if there is no second litter. Provide justification:

Other:

**Genotyping & identification**

Tail tips of genetically modified pups will be collected from mice in accordance with the[ARCD Rodent Genotyping Policy](https://internal.research.vt.edu/system/files/512.00%20-%20Rodent%20Genotyping%20Policy.pdf). A sharp and sterile or disinfected scalpel blade, scissors, or single edged (safety) razor blade will be used to remove the most distal region of the disinfected tail. The first tail tip biopsies will remove 2-3mm of tail tissue. If multiple tail biopsies are required, the total amount of tail tissue removed will not exceed 5mm. The instrument used will be disinfected between animals. This procedure will be performed without anesthesia when pups are < 16 days of age. If biopsies are performed after 16 days of age mice, local or general anesthesia is required and 24 hours of post-procedural systemic analgesia is recommended. If biopsies are performed after 28 days of age, general anesthesia and 24 hours of post-procedural systemic analgesia are required. Adverse effects may include bleeding of the tail when excessive tissue is removed or loss of identification when ear punches are not properly located. Tail tip hemostasis will be aided, if necessary, by applying direct pressure, styptic powder, or other acceptable means.

Ear pinna tissue will be collected from mice ≥14 days of age in accordance with the [ARCD Rodent Genotyping Policy](https://internal.research.vt.edu/system/files/512.00%20-%20Rodent%20Genotyping%20Policy.pdf). A commercially available ear punch device or small, sharp surgical scissors will be used to remove a 2-3mm section of ear tissue. Anesthesia or analgesia are not required. The instrument used will be disinfected between animals.

Toe clipping: Scientific justification is required. Please describe and give justification and/or historical data of using this identification method within your colony. Toe clipping will be performed in accordance with the [ARCD Rodent Genotyping Policy](https://internal.research.vt.edu/system/files/512.00%20-%20Rodent%20Genotyping%20Policy.pdf). Toe clipping involves amputation of the 3rd (most distal) phalynx severed at the most distal joint, is limited to one toe per foot, and should be limited to the hind paws. When performed on a front paw, it will not involve the first (most medial) digit (toe). Anesthesia is not required when performed at ≤ 7 days; however, general anesthesia is required when performed at > 7 days of age. Please uploading the numbering scheme that will be used to Section 9: Supporting Documents in PM. Provide justification:

Other:

**Euthanasia:** Described in Section 13.

**Additional Procedures**

1. Sedation/Anesthesia/Euthanasia/Analgesia Agents: Please complete the following table in order of when the agents will be used during the protocol. Under purpose please state if the agent will be used for pre-anesthetic, sedation, etc. \*\*Please see the table has been started; remove items that are not needed for this protocol, add items that are needed for this protocol and review all doses for accuracy.

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| **Agent** | **Dose (mg/kg)** | **Route of Administration** | **Frequency of Administration** | **Purpose** |
| Isoflurane | to achieve a surgical plane of anesthesia | inhaled by face mask or in a chamber | once | before mechanical method of euthanasia |
| CO2 | Flow rate: 30-70% volume/min, to achieve unconsciousness or death | inhaled in the home cage or a chamber | once | unconsciousness or death before mechanical method of euthanasia |
| Ketamine  Xylazine | 80-120 mg/kg  8-12 mg/kg | SQ; maximum volume of 200 ul | once | general anesthesia before mechanical method of euthanasia |
| Ketamine  Xylazine | 100-200 mg/kg  8-12 mg/kg | SQ; maximum volume of 200 ul | once | euthanasia |
| Pentobarbital | 150 mg/kg | IP | once | euthanasia |
| Isoflurane |  |  |  | general anesthesia for genotyping |
| Bupivicaine |  |  |  | local anesthetic for genotyping |
| Lidocaine |  |  |  | local anesthetic for genotyping |
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1. Please refer to the information below to determine if your study involves multiple major survival surgeries, and mark the appropriate response.

A major surgery is defined as one that penetrates and exposes a body cavity (e.g., abdomen, thorax, or skull) or produces substantial impairment of physical or physiologic functions. Multiple survival surgeries involve completion of a first surgery from which the animal recovers from anesthesia, and then a subsequent surgery from which the animal recovers from anesthesia.

This study involves multiple major survival surgeries. *Respond to question 4, and then go to question 5.*

This study does not involve multiple major survival surgeries. *Go to question 5.*

1. If your study involves more than one major survival surgery, provide a scientific justification for this need. Please note that the IACUC can approve multiple major survival surgeries only if there is an adequate scientific justification for the surgeries, the surgeries are required as part of routine veterinary care, or the attending veterinarian has determined that the surgeries are necessary to protect the health or well-being of the animal.

1. Indicate if death will be used as an endpoint in this study. Use of death as an endpoint refers to situations in which an animal is allowed to progress to death as a required experimental outcome. It does not refer to situations in which an animal is euthanized at an established humane endpoint.

Death will be used as an endpoint in this study.

Death will not be used as an endpoint in this study.

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| **Section 10: Humane Endpoints** |

1. Even if pain or distress are not anticipated in the breeding protocol, you must establish humane endpoints at which animals will be removed from the protocol, treated, or euthanized should unexpected complications arise. Examples of appropriate criteria might include a weight loss limit as a percentage of initial or expected body weight, allowable durations of anorexia, the presence of health problems refractory to medical intervention, and severe psychological disturbances. Describe the humane endpoint criteria that will be used for determining when sick animals will be euthanized or otherwise removed from the breeding protocol. \*Attach any monitoring sheets that will be used on this protocol under Supporting Documents on the PM. A editable monitoring sheet template is available in Supporting Documents. Check all that apply.

Any mice showing signs of illness or abnormal behavior will either be euthanized or checked by the veterinary staff and treated or euthanized as necessary.

Transgenic mice are not expected to show clinically apparent phenotypes or ill health as a result of their genetic differences from wild-type. However, if clinical signs arise they will either be euthanized or checked by the veterinary staff and treated or euthanized as necessary.

Other:

1. The IACUC requires the PI or a designated staff member to monitor animals that have developed significant signs of illness or toxicity **every four hours, 24 hours per day, including weekends and holidays**. Please provide contact information for the personnel who will be responsible for monitoring the condition of the animals.

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| Name | **Virginia Tech PID**  *(Typically the part of an official VT e-mail address that precedes @vt.edu.)* | Phone Number |
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| **Section 11: Study Personnel and Ordering** |

1. If any personnel do not have experience with the exact species and procedures indicated, please describe how they will be trained. Include information about the qualifications of the person providing the training. Please check at least one below.

Minimally all training will occur as follows: the trainee will observe the procedure three times or until comfortable, the trainee will perform the procedure three times or until the trainer feels they are competent to perform unsupervised. The trainer will be either the PI or an individual experienced and competent in the procedure. The training must be documented. Examples of documentation: [template one](https://internal.research.vt.edu/system/files/Training%20documentation%20template%201.doc) and [template two](https://internal.research.vt.edu/system/files/Training%20documenation%20template%202.doc).

Other:

1. Who will order the animals for this protocol?

ARCD (includes purchases for FBRI, LS1, ILSB, CVM, etc.)

Other:

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| **Section 12: Animal Housing and Husbandry** |

1. Indicate if the animals involved in this breeding protocol have been or will be used in other IACUC approved protocols. (Check all that apply.)

The animals involved in this breeding protocol have not been and will not be used in other IACUC approved protocols. *Go to question 3.*

The animals involved in this breeding protocol have been used in prior IACUC approved protocols. *Respond to question 2, and then go to question 3.*

The animals involved in this breeding protocol will be used in concurrent IACUC approved protocols. *Respond to question 2, and then go to question 3.*

The animals involved in this breeding protocol may be transferred to another IACUC approved protocol. *Respond to question 2, and then go to question 3.*

1. Please describe prior, concurrent or future use of these animals, and what measures will be taken to prevent overuse. Check all that apply.

Animals are being bred under this protocol specifically to be transferred to other IACUC approved protocols.

Retired breeders and excess progeny may also be transferred to IACUC approved training protocols, or euthanized under this protocol and tissues provided to other PI’s.

Other:

1. Are special or unusual housing or husbandry conditions required for the animals involved in this study?

Special or unusual housing or husbandry conditions are required. *Respond to questions 4 and 5, and then go section 13.*

There are no special or unusual housing or husbandry requirements. *Go to section 13.*

1. Please indicate what type of special or unusual housing or husbandry conditions are required:

Food restriction

Feeding on the floor

Water restriction

Single housing of social animals

Longer than brief restraint

Environmental temperatures outside of established ranges

Captive housing of wild-caught species

Other; *describe in question 5*

1. Please describe the necessity for special or unusual housing or husbandry requirements, and how maximum possible animal comfort will be assured under the conditions. Check all that apply.

Single housing: At times, male breeders and pregnant females may need to be housed alone for short periods of time. Single housed mice are required to be given two forms of environmental enrichment.

Pups will receive food on the cage floor at weaning to ensure procurement of food.

Other:

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| **Section 13: Animal Disposal** |

1. Will animals be euthanized during or at the conclusion of this breeding protocol? (check all that apply)

Animals will not be euthanized. *Respond to question 2.*

Animals will be euthanized. *Go to question 3.*

1. Describe how animals will be disposed of at the conclusion of this breeding protocol (e.g., transferred to another approved IACUC protocol). Check all that apply.

Mice will be transferred to other IACUC approved protocols.

Other:

1. Will animals undergo treatments or procedures prior to euthanasia?

Animals will not undergo treatments or procedures prior to euthanasia (e.g., tissue harvesting).

Animals will be euthanized after experimental treatments or procedures.

1. Describe the method of euthanasia that will be used. Please note that chemical methods of euthanasia are preferable to physical methods. If you plan to use a physical method of euthanasia, you will need to provide adequate justification for this choice (include published references). For injectable agents, include information on dose and route of administration. Check all that apply.

Use of CO2 followed by cervical dislocation or decapitation will be per the current AVMA guidelines: Compressed CO2 gas in cylinders is the recommended source of CO2 because gas inflow to the chamber can be precisely regulated. An optimal flow rate for CO2 euthanasia systems should displace 30 to 70% of the chamber or cage volume/min. Prefilled chambers are unacceptable. If euthanasia cannot be conducted in the home cage, chambers should be emptied and cleaned between uses.

Anesthesia with isoflurane followed by cervical dislocation or decapitation.

Anesthesia with ketamine/xylazine followed by cervical dislocation or decapitation.

Pentobarbital: used at 150 mg/kg IP with a 23-27 gauge needle in a volume not to exceed 0.5 ml.

Other:

1. Describe how the personnel performing the euthanasia will confirm that death has occurred. Examples include: thoracotomy under anesthesia, cervical dislocation under anesthesia, exsanguination under anesthesia, prolonged exposure to carbon dioxide (greater than five minutes), and observation of vital signs for five minutes. Check all that apply.

Cessation of vital signs for a minimum of five minutes.

Observation of vital signs and using a secondary mechanical (cervical dislocation or decapitation) means of euthanasia to ensure death.

Other:

1. How will animal carcasses be disposed of (e.g., rendering, landfill)? Check all that apply.

Regulated medical waste due to rDNA-containing transgenic mice

VT EHS disposal

Other:

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