BACKGROUND

The University of Rhode Island (URI) Institutional Animal Care and Use Committee (IACUC) has established the following guidelines for mouse breeding and cage density expectations for animal colonies. The guidelines are based on the national standards and recommendations contained within the 8th Edition of the Guide for the Care and Use of Laboratory Animals (NRC 2011).

The breeding of rodents in a new and renewed IACUC protocol is not be permitted unless 1) the protocol is a study of the effects of some variable on the outcome of breeding of the rodents or 2) the strain of rodent being bred is so novel and rare that is not available for purchase from any commercial vendor. Existing IACUC protocols that involve the use of breeding for any purpose may remain in place unaltered until the three year renewal. The IACUC has the responsibility to determine if proposed breeding falls within the two exemptions above. Additionally, the IACUC will consider other reasons for proposed breeding based on a written justification provided by the Principal Investigator (PI) in the IACUC protocol.

The URI IACUC requires that all Animal Use Protocols follow one of the common breeding schemes below when breeding laboratory mice (Mus musculus). When preparing an IACUC protocol that involves breeding, reference this policy. If a PI would like to follow a different breeding scheme, it must be specifically approved by the IACUC.

DEFINITIONS

- **Mouse Pup** – 0-28 days of age, prior to weaning.
- **Adult Mouse** – All mice after weaning. Must be able to eat solid food and reach water source.
- **Post-partum Estrus** - estrus that occurs 14-24 hours after delivery
- **Environmental Enrichment (EE)** – all nesting materials, additional feed items, housing domes/huts, chews, or exercise devices used to enhance the psychological and/or physiological welfare of the animal.
- **Spot Change** – removal of soiled bedding with addition of new bedding, or complete change of the cage unit.

PRINCIPAL INVESTIGATOR (PI) RESPONSIBILITY

1. Know and understand the breeding capabilities and characteristics of their IACUC approved rodent strain. This includes gestation length and appropriate breeding age.
2. Ensure a communication mechanism is in place to be able to receive timely communications from animal care staff regarding mice (e.g. voicemail that is routinely checked and/or a number that is routinely attended).
MATING SCHEMES

1. **Monogamous / Paired Mating**
   One (1) adult male paired with one (1) adult female. The pair is housed together permanently or for the duration of breeding as specified by the PI. This is the preferred method of breeding for production of offspring that need to be genotyped.

   **Advantages:**
   - Post partum estrus
   - Continual production
   - Easy to track pedigree
   - Easy to assess performance

   **Disadvantages:**
   - Restricts male to one female

2. **Trio Mating**
   One (1) male paired with two (2) females.

   **Advantages:**
   - Female mice may participate in co-parenting
   - Advantageous for strains with known low litter numbers

   **Disadvantages:**
   - Must remove male one week prior to expected delivery (parturition).
   - Must remove male or use a bigger cage
   - Not able to always tell maternity of pups
   - Should not be more than one litter per cage, unless under 10 pups. Refer to animal housing regulations.

3. **Harem Mating**
   One (1) male paired with three (3) females in which the females are removed and single housed before parturition.

   **Advantages:**
   - Rapid colony production

   **Disadvantages:**
   - Males must be single housed when removed from the female cage to prevent male -male fighting.
   - Increased monitoring
   - Greatest level of maintenance.
   - Risk of over-crowding
   - Additional labor of separating pregnant females
   - Additional cages needed and is more labor intensive
IACUC POLICY

Mouse Breeding and Cage Density

- No post-partum estrus
- Can increase stress of male

WEANING

- Weaning age for mouse pups is routinely 21 to 25 days of age. In the case of some genetically modified or mutant strains, it may be advantageous to allow the pups to remain with the female for 28 days.
- To extend nursing time past 28 days, the Attending Veterinarian (AV) must be notified. Extending weaning beyond 35 days requires IACUC approval (e.g., must be justified in the IACUC Protocol)
- Weaning may be delayed for pups unable to eat solid food or unable to reach water source. Use a “Please Take Notice” cage card to flag any runted pups left with nursing mother. Contacting the animal care staff is recommended to assess runted pups.
- Provide water and nutritional support during the first week of weaning with use of a Hydrogel cup for water and moist food. Dough diet may be added if needed for nutritional support. This support improves pup transition during weaning and is provided by the animal care staff.
- Use of pups prior to day 19, must be approved by the IACUC.
- Nursing females with a second litter are considered overcrowded. The older pups must be weaned when safe to do so (generally when new litter is 3 – 5 days old) when dam gives birth to a new litter.

RECORD KEEPING

The following information must be maintained in the breeding log and other documents.

- Date of birth of breeders
- Date of introduction
- Date of birth of litter(s)
- Date of weaning
- # of litters per each female
- # of offspring in each litter
- Genotype if applicable

OVERCROWDING

Typically, more than four (4) adult mice in standard static caging, more than five (5) adult mice in ventilated caging, or more than ten (10) adult mice in large caging (150 sq. inch floor) is considered overcrowded. For more information on minimum housing requirements, refer to Table 3.2 in the 8th Edition of the Guide for the Care and Use of Laboratory Animals (NRC 2011).

If overcrowded cages are identified by animal care staff, the cage card contact from the laboratory will be notified and there will be 24 hours granted to separate the overcrowded cage. If the overcrowded situation is not corrected within 24 hours after notification, animal care staff will separate the cage and charge the PI a $45 fee.
At the discretion of the Attending Veterinarian’s (AV) or animal care staff, and following AV communication with the PI, the animal care staff may separate any cage that has been determined to be an immediate concern to the welfare of the animals housed within the cage. Typically, this situation is a result of noncompliance with the breeding schemes outlined in this Guideline.

HUSBANDRY PRACTICES
To refine breeding and to add to the comfort of dams and new litters alterations in husbandry is encouraged.

- **Nesting** – All breeders and dams with litters must be provided with nesting materials. It is highly encouraged that nesting material is added additional times aside from normal cage changing. The use of more than one nesting material is preferred.
- **Cage changing** – for mice newly paired it is recommended to decrease cage changing. Cages with mice ready to give birth and cages with newborn litters should be delayed for at least 5 days or if the cage is not more than 25% wet. Spot changing is permissible.
- **Supplements** – high nutrient supplements are required by the AV to replace nutrients lost during the birthing process and weaning (or in certain situations to reduce stress). Use of supplements must be documented. Contact AV for recommendations for specific supplements.

COLONY MANAGEMENT
To maintain a successful mouse colony, ensure the following:

- Ensure that there will be enough mice available to replace breeders.
- When pairing up breeders, always add the female to male cage or set up an entirely new cage to reduce fighting.
- Cull unsuitable breeders. Examples of unsuitable breeders including those that have not had a litter in 60 days or more, those that have not successfully weaned in 2-3 litters, older mice, and those that have already had 6-7 litters.

RESOURCES
Animal care staff and the AV are available to assist the PI in identifying the appropriate breeding scheme.

REFERENCES
- Jax Mice, mouse models, tech support, [http://www.jax.org/index.html](http://www.jax.org/index.html)
- AALAS- (American Association for Laboratory Animal Sciences), [https://www.aalas.org/](https://www.aalas.org/)