IACUC Guideline
Mouse Breeding and Cage Density Guideline

The Institutional Animal Care and Use Committee and University Laboratory Animal Resources (ULAR) have established the following guideline for mouse breeding and cage density expectations for animal colonies at the University of Pennsylvania. The biomedical research community must comply with the national standards and recommendations contained within the 8th Edition of the Guide for the Care and Use of Laboratory Animal (NRC 2011) when establishing mouse cage density criteria.

It is the Principal Investigators’ responsibility to manage their own mouse colonies and to:
1. Oversee appropriate colony management, timely weaning of litters, and prevention of overcrowded cages;
2. Ensure a communication mechanism is in place to be able to receive timely communications from husbandry and veterinary staff regarding mice (e.g. voicemail that is routinely checked and/or a number that is routinely attended);
3. Ensure that the name and telephone number for the cage card lab contact person is current and accurate;
4. Ensure that overcrowded cages are separated and the available space for new cages is allowed within the ULAR facilities. The Principal Investigator should not set up breeding animal cages unless there is available space for the cages of weaned animals. If adequate vacant spaces are not available within the same housing room, the investigator must discuss options with ULAR Facility manager and/or the ULAR Associate Director for Husbandry Care and Vivarial Facilities.

DEFINITIONS:
Adult mouse: Any mouse that is of weaning age or older and able to eat solid food and reach the water source.
Post partum estrus: Female mice may undergo fertile estrus 14-24 hours following delivery of a litter of pups. Females that are housed with a male when they give birth to a litter can therefore become pregnant and give birth to a second litter around the time of the weaning date of the first litter, which can lead to overcrowding.
Delayed weaning: There may be instances where a litter has passed its weaning date but is unable to be weaned due to small size or inability to access food or water. This may be due to a failure to thrive and should be reported to the ULAR veterinary staff. If pups are verified by the veterinary staff to require delayed weaning, beyond weaning dates outlined in the ‘Mouse Breeding and Cage Density Policy’ due to expected phenotype, then an exemption to this policy must be approved by the IACUC prior to continued breeding.
Mouse pup: Term for any neonatal or young mouse up to ~21 days of age; a grouping of pups from the same mother is called a litter of pups.

BREEDING (please review included tables)
In order to provide for appropriate welfare of mouse colonies and to allow investigators the opportunity to independently manage mouse production, several breeding schemes are permitted. The investigator should choose the best breeding scheme for each particular project.

• Monogamous or Pair Breeding of ONE (1) adult male and ONE (1) adult female.
  - UNIVERSITY OF PENNSYLVANIA’s preferred method to prevent overcrowding
  - Offspring generated by this breeding scheme must be weaned by 28 days.
  - Offers an extended nursing time for inbred strains known to be small and slow growing

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- Maximizes productivity of females by utilizing post-partum estrus; allows for the identification of the dam for the litter

• Trio Breeding of ONE (1) adult male and TWO (2) adult females.
  - Pups born into the cage with the male must be weaned at 21 days by investigators’ staff in order to prevent excessive pup numbers after 3 weeks.
  - If one of the pregnant females is separated, pups born to this female must be weaned by 28 days.

**Up to two litters, and no more, are permitted per cage; any older litters must be weaned when new litters are delivered to avoid potential injury to neonates.**

- Harem Breeding with ONE (1) adult male and up to FOUR (4) adult females in a standard cage.
  - Pregnant females must be removed and placed into another cage before delivery of any litters; thus, there may be no litters born into cages with harem breeding.
  - For special circumstances of breeding in large mouse cages (floor space ~ 150” sq) harem breeding may be best to meet the needs for intensive mouse production. These large cages are designed for a maximum of TEN (10) adult mice. The capacity to maintain large mouse cages is only available in a limited number of facilities and must be coordinated with ULAR management for available space options.

<table>
<thead>
<tr>
<th>Housing scheme</th>
<th># Males*</th>
<th># Females</th>
<th>Benefits</th>
<th>Additional Considerations</th>
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<tbody>
<tr>
<td>Monogamous or Pair Breeding</td>
<td>1</td>
<td>1</td>
<td>*UPenn’s preferred breeding scheme</td>
<td>*Less efficient use of the male</td>
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<tr>
<td>Trio Breeding</td>
<td>1</td>
<td>2</td>
<td>*More efficient use of males than monogamous breeding</td>
<td>*Requires monitoring to avoid overcrowding</td>
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<td></td>
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<td></td>
<td>*Females may become pregnant within days of delivery of pups due to post-partum estrus</td>
<td>*Pups not permitted to stay with parental pair beyond 21 days of age</td>
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<td>*Most efficient use of the male</td>
<td>*May require allocation of reserve housing for placement of separated pregnant females</td>
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<tr>
<td>Harem Breeding</td>
<td>1</td>
<td>Up to 4; each female then separated into own cage or a cage with one other female once pregnant</td>
<td>*Most productive housing scheme for maximizing numbers of offspring</td>
<td>*Requires intensive monitoring to avoid overcrowding and delivery of pups into breeding cage</td>
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<td>*Inability to take advantage of post-partum estrus</td>
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<td>*Requires allocation of large amount of reserve housing for placement of separated pregnant females</td>
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<td>*No litters may be born into cages with harem breeding</td>
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</table>

*Only one male mouse is allowed per breeding cage at any time, regardless of breeding scheme*
Overcrowded cage and notification: Any standard mouse ventilated or static cage (67-75″ sq floor) containing more than FIVE (5) adult mice or any large cage (150″ sq floor) containing more than TEN (10) adult mice. If overcrowded cages are identified by ULAR husbandry 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, ULAR husbandry staff members are expected to separate the cage at a fee-for-service.

Grossly overcrowded cage and notification: Overcrowded cage wherein the magnitude of overcrowding is severe enough that there is 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. At the Attending Veterinarian’s (AV) discretion, and following AV communications with the investigator, the clinical vet staff may be directed to separate these cages as soon as possible for animal welfare reasons.

If incidences of overcrowding within a mouse colony become excessive (>10 overcrowded cages/month) for a particular laboratory, please contact the Office of Animal Welfare staff for assistance.

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