TITLE: 
Rodent Breeding Policy

PURPOSE:

The purpose of this SOP is to ensure that rodent breeding is conducted efficiently and that the Public Health Service policy and federal regulations governing housing requirements are met. The contents of this SOP apply to all NINDS personnel who are involved in the breeding of rodents. Personnel from other NIH institutions housing rodents in NINDS/AHCS managed animal facilities are encouraged to follow this SOP.

GENERAL INFORMATION:

A. General Breeding Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mouse</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puberty age</td>
<td>Female: 4-5 weeks</td>
<td>Female: 40-49 days</td>
</tr>
<tr>
<td></td>
<td>Male: 5-7 weeks</td>
<td>Male: 40-60 days</td>
</tr>
<tr>
<td>Optimum Reproductive Age</td>
<td>2-10 months</td>
<td>2-9 months</td>
</tr>
<tr>
<td>Retirement</td>
<td>Female: after 5-6 litters or up to 12 months of age; Male: after 1 year</td>
<td>Up to 12 months of age</td>
</tr>
<tr>
<td>Estrous Cycle Length</td>
<td>4-5 days</td>
<td>4-5 days</td>
</tr>
<tr>
<td>Onset of Fertile Postpartum Estrus</td>
<td>Yes (12-18 hours after parturition)</td>
<td>Yes</td>
</tr>
<tr>
<td>Duration of Pregnancy</td>
<td>19-21 days (pregnancy may be visibly verified on day 16)</td>
<td>21-23 days</td>
</tr>
<tr>
<td>Weaning Age</td>
<td>21 days (may be delayed up to 28 days by veterinary directive or if approved in the ASP)</td>
<td>21 days (may be delayed up to 28 days by veterinary directive or if approved in the ASP)</td>
</tr>
</tbody>
</table>

B. Estrus: when a female will accept the male for breeding. Postpartum estrus: female can be impregnated up to 48 hours after giving birth. Pup: <21 days old.

C. Individual adult breeders should be identified by ear tag, tattoo, transponder, or some other means of permanent identification.

D. If males and females are housed separately, it is recommended to put the female in the male’s cage for breeding. Female mice can become territorial which may become a barrier to successful mating.

E. Rodents breed most frequently overnight. If checking for a vaginal plug to confirm mating, females should be checked early in the morning because the plugs typically fall out within 12 hours, refer to SOP 3482. The Disposition Instructions Form for the relevant animal study proposal (ASP) must be complete with up-to-date information on how to reach the person responsible for colony maintenance (e-mail, phone numbers) to facilitate communication in case of emergency (e.g. dystocia, orphaned pups).

F. Whoever places a male and female(s) rodents together into a single cage is responsible for following this SOP.
G. Maintaining good breeding records is critical for successful colony management.

H. The facility staff has limited resources to provide technical support to PIs. Ultimately, it is the responsibility of the PI to setup and retire breeder animals, weaning of litters and maintain records of their breeding colonies.

I. Technical requests for limited breeding assistance need to be submitted to the AHCS at least 48 hours in advance of requested services at the following website: http://ahcs.ninds.nih.gov/AHCS_pages/vet.html.

PROCEDURES:

A. Procedures for Setting up Breeder Cages and Documenting Litters Born.
   1. For continuous (e.g. permanent monogamous pair) breeder set-ups, 1 male and 1 female may be permanently housed in a cage.
   2. Harem or one-time breeder set-ups:
      i. Up to 3 females may be temporarily housed with 1 male in a cage; however, females must be moved to separate cages as soon as they are visibly pregnant.
      ii. Harem breeding must be included in the ASP.
      iii. Two or more pregnant females must not be housed in the same cage for full gestation. This is to reduce overcrowding when both females give birth. The presence of two litters in the same cage is a violation of cage space regulations (Refer to SOP 3201 Reporting and Resolving Cages Requiring Attention for Issues Other Than Health Concerns) and is unacceptable unless detailed in the approved ASP or approved as part of veterinary care.
   3. Write the date the breeding is set-up on the cage card.
   4. Place a “Breeder Cage” flag if the cage contains a breeder pair, harem breeding group, pregnant female, or female with pups. Refer to the Cage Flag and Card System for Identifying Animals in the NINDS AHCS MAF posted at the front of each animal holding room. The AHCS staff will place two colored sticker dots on the date of (DOB) and the date of wean (DOW) on the flag.
   5. If “High Fat Chow” is desired, check the box on the “Breeder Cage” flag. The AHCS staff will provide this type of feed at cage change.
   6. Remove the “Breeder Cage” flag when the breeder pair has been separated. For pregnant females or females with pups, the flag should be removed when the female is no longer pregnant and no pups are present in the cage.
   7. When a breeding animal is removed from the cage, record the sex and ID (if applicable) of the animal removed as well as the date of removal on the cage card.
   8. For records and weaning purposes, please ensure that the strain name and/or the offspring name are clearly written or printed on the parent’s cage card.
   9. During daily health checks the AHCS staff will record new litters on the back of the cage card with the date, LB (Litter Born), and their initials. In addition, the (DOB) is written on the left colored sticker dot and the DOW is written on the right dot of the “Breeder Cage” flag. The DOW date is 21 days post-parturition.
   10. For more information regarding breeder cages, refer to Attachment 3480A Rodent Breeding Cage Flagging and Cage Card Notations.

B. Records
   1. Regardless of the type of record system maintained, we strongly encourage the following information be recorded:
i. Strain information be provided and written/printed clearly. If the male and female are different strain, indicate the desired strain name for the offspring.

ii. If genetically engineered animals, phenotypic characteristics (e.g. malocclusion, ataxia, etc.).

iii. Identification information for both the female and male.

iv. Date of birth and number of live births.

v. Weaning date and number of animals and sex weaned.

vi. Pedigree records (who is related to who).

vii. Mating date.

viii. Litter intervals.

REFERENCES:
1. The U.S. Government Principles for the Utilization and Care of Vertebrate Animals used in Testing, Research and Training
2. Guide for the Care and Use of Laboratory Animals (Revised 2011)
4. Breeding Strategies for Maintaining Colonies of Laboratory Mice (The Jackson Laboratory)
5. Laboratory Animal Medicine 2nd ed.
6. AALAS Learning Library (www.aalaslearninglibrary.org)
7. SOP 3201 - Reporting and Resolving Cages Requiring Attention for Issues Other Than Health Concerns.
8. SOP3202 Cage Flag and Card System for Identifying Animal Cages in the NINDS AHCS Managed Animal Facilities.

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Attachments:
1. 3480A Rodent Breeding Cage Flagging and Cage Card Notations.

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Updates and/or Changes:

3/5/15:
- Updated Section A rat gestation was 20-23 days and changed to 21-23 days.
- Updated Section E to read rodents vs mice and clarified the timing for plug checks.
- Removed Section B1 as it was repeated.
- Added more detail to the strain information in Section B1i.
- Updated and removed old or renamed SOP hyperlinks.
PI sets up breeding cage and hangs “Breeder Flag”

- Daily Health Checks: Note New litters on cage card with date, LB, and initials
- Add two colored dot stickers on the breeder flag
- DOB (left dot) and DOW (right dot) is written by the person that finds the new litter
- “Breeder Cage” flag stickers are removed when pups are no longer present in the cage. The person weaning or removing the last pups from the cage must remove the stickers.
- PI removes “Breeder Cage” flag when:
  1. Breeder pair is separated
  2. No pups remain with a singly-housed female

No “Breeder Flag” present on a cage with:
- Breeders
- Pregnant females
- Females with pups

- ACT hangs an “Attention Required” flag on cage and completes the log
- VT will contact PI and request PI to put flag on cage
- If animal(s) is FD, record date on back of cage card with initials

If the female is FD and the cage contains nursing pups report this to a VT or HM immediately

If breeder is FD, record sex of animal and date on cage card with initials

- Complete mortality report and email the PI when:
  1. Adult breeder FD
  2. Last pup in a litter FD
  3. Entire litter is FD

Abbreviations:
- DOB – date of birth
- DOW – date of weaning
- FD – found dead
- LB – litter born

If animal(s) is FD, record date on back of cage card with initials