



# STANDARD OPERATING PROCEDURE 701 MOUSE BREEDING COLONY MANAGEMENT

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## 1. PURPOSE

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This Standard Operating Procedure (SOP) intends to describe guidelines for managing mouse breeding colonies.

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## 2. RESPONSIBILITY

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Principal investigator (PI) and their research staff.

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## 3. MATERIALS

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- 3.1. Caging and environmental enrichment
  - 3.2. Cage cards
  - 3.3. Ear punch or ear tags
  - 3.4. Breeding records, BGU Preclinical Management System
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## 4. CONSIDERATIONS

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- 4.1. Generally, laboratory mice will reach sexual maturity at approximately six weeks, although females may have their first estrus as early as five weeks.
- 4.2. On average, the reproductive lifespan of mice is between 7 and 12 months.
- 4.3. The duration of the estrous cycle is 4–5 days. Mice are polyestrous and breed year-round; ovulation is spontaneous. Sperm production can vary by strain and may last 2 to 4 hours up to 1 week.
- 4.4. Gestation period: 19-21 days
- 4.5. Weaning age: 21-28 days; when pups are 7g or larger
- 4.6. Mating is usually nocturnal and may be confirmed by a copulatory plug in the vagina up to 8 hours post-copulation. Refer to section 5.4.
- 4.7. Pregnancy may be confirmed by gentle abdominal palpation after gestation day 12 or weight gain during the last trimester.
- 4.8. A fertile postpartum estrus occurs between 14 and 24 hours following parturition, and simultaneous lactation and gestation may prolong.
- 4.9. Fertility and litter size vary by strain. The success rate may also be dependent on the age of the breeders.

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## 5. PROCEDURES

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- 5.1. Breeding cages should be observed daily for newborn animals, litters ready for weaning, separating of females, and a general check of the animal's health and condition. The maintenance of good breeding records is essential.
- 5.2. To optimize breeding performance:
  - 5.2.1. Begin breeding mice early, preferably at approximately 6-8 weeks of age.
  - 5.2.2. Female mice should be placed in the male mouse's cage for mating.
  - 5.2.3. Pairing young females with older males can improve breeding performance.
  - 5.2.4. Replace breeders:
    - 5.2.4.1. Before their reproductive performance declines, breeding success decreases if the mice are older than eight months old.
    - 5.2.4.2. If no litters have been born for 60 days after mating or 60 days after weaning of the last litter and the female is not pregnant.
      - 5.1.1.1. If several litters have been born, but no pups have been weaned.
      - 5.1.1.2. If a significant decrease in litter size is noted, e.g., 1-2 pups born per litter when the average litter size was 8-9.
  - 5.2.5 Do not replace all breeding animals simultaneously; it is best to have breeding animals of various ages in the colony.
  - 5.2.6 Provide adequate environmental enrichment.
  - 5.2.7 Handle breeding cages gently and place them in a low-traffic area of the housing room. Avoid handling cages with newborn litter.
  - 5.2.8 If breeding difficulties persist in the colony, consult a veterinarian as soon as possible, as fertility decreases with age.
- 5.3 Breeding schemes:
  - 5.3.1 Monogamous pair
    - 5.3.1.1 One male and one female are housed together for mating.
    - 5.3.1.2 The mice are not separated when the female becomes pregnant or delivers the pups.
    - 5.3.1.3 It takes advantage of postpartum estrus and allows the female to become pregnant and nurse simultaneously.
    - 5.3.1.4 Litters are born approximately 21 days apart.
    - 5.3.1.5 The 3-week-old litter should be weaned prior to the birth of the new litter.
    - 5.3.1.6 For strains requiring pups to be weaned after 21 days, the female should be separated to avoid postpartum estrus (two litters) and overcrowding.
  - 5.3.2 Trio breeding
    - 5.3.2.1 One male and two females are housed together for mating.
    - 5.3.2.2 Only acceptable for strains with average litter sizes of 7 pups per litter or less.
    - 5.3.2.3 Both lactating females may be left in the same cage (+/- the male) only if each female has a litter of 7 pups or less.
    - 5.3.2.4 Pups should be weaned at 21 days of age, prior to the birth of new litters.
    - 5.3.2.5 For strains requiring pups to be weaned after 21 days, both females must be separated to

avoid postpartum estrus (two litters) and overcrowding.

### 5.3.3 Harem breeding

- 5.3.3.1 Due to the increased risk of overcrowding and impact on animal welfare, harem breeding is only permitted under specific circumstances and must be justified in the animal use protocol.
- 5.3.3.2 One male and up to 4 females are housed together for mating.
- 5.3.3.3 Pregnant females must be separated into another cage before parturition to avoid overcrowding. No litter should be born into cages with harem breeding.
- 5.3.3.4 In this breeding scheme, there is no advantage of the postpartum estrus.

### 5.4 Timed matings:

- 5.4.1 Used when a special day of mating is required, e.g., when embryos or fetuses of a specific gestational age are required.
- 5.4.2 One male and up to three females may be housed together for mating.  
**However, a 1:1 or 1:2 male: female ratio gives optimal results.**
- 5.4.3 Breeding cages for timed mating should be set up in the late afternoon, as mice usually mate during the dark cycle.
- 5.4.4 After mating, a vaginal or copulatory plug is formed by the secretions from the coagulating and vesicular glands of the male. The plug is white or cream-colored and generally fills the female's vagina and persists for 8 hours after breeding.
- 5.4.5 The presence of a vaginal plug does not guarantee a pregnancy.
- 5.4.6 The absence of the vaginal plug does not rule out pregnancy.
- 5.4.7 To check a female for the presence of a vaginal plug:
  - Look for a plug as early into the light cycle as possible. Otherwise, the plug may become dislodged or dissolved.
  - To see the plug, lift the female by the base of her tail and examine her vaginal opening for a whitish mass. Use a cotton-tipped swab or a blunt metal probe to open the vagina gently.
- 5.4.8 If a vaginal plug is found, separate the female. You may confirm pregnancy by weighing the female on the day of the copulation and at gestational day 8. Pregnant females will generally take two or more grams of body weight. Pregnancy may also be confirmed by abdominal palpation after gestational day 12.
- 5.4.9 If there is no plug, leave the female with the male and check her each morning until you see a plug. If after 14 days there is no vaginal plug, replace the breeders.
- 5.4.10 The first day of gestation is after the vaginal plug is observed.

### 5.5 Weaning:

- 5.5.1 Weaning refers to removing a pup from its home cage (rather than when a pup stops nursing and starts eating exclusively solid food).
- 5.5.2 Generally, laboratory mice are weaned between 21 and 28 days of age. Most strains are weaned when they are 21 days old. Pups with low growth rates can be weaned around 10-12 g.
- 5.5.3 Pups should be weaned if the same female gives birth to a new litter, and a female cannot nurse two litters simultaneously.
- 5.5.4 Weaning age may vary depending on weanling size, weight, and maturity; some strains benefit from being weaned later than 21-28 days of age. The growth of pups can be supported by placing a dish at the bottom of the cage containing moist powdered food with or without

powdered milk formula.

- 5.5.5 For colonies where mice are routine to be weaned after 21-28 days of age, the female must be separated from the male before giving birth to avoid postpartum estrus, overlapping of gestation and lactation, and overcrowding.
- 5.5.6 Upon weaning, pups may be separated as follows:
  - 5.5.6.1 Male and female pups are separated by sex into cages housing a maximum of 5 mice.
  - 5.5.6.2 Male and female pups are separated into cages housing up to 8 juveniles. When the juveniles reach a maximum weight of 16 g or are a maximum of 6 weeks of age, they must be separated by sex into cages housing a maximum of 5 mice.
  - 5.5.6.3 Only at weaning can males of different litters be grouped. After weaning, males of different litters should not be placed together as it will resolve in fighting. Grouping females together after weaning is generally acceptable, as fighting is less probable. It is preferable not to wean a single mouse alone.
- 5.5.7 A small amount of food may be provided to the weanlings at the cage's bottom.
- 5.5.8 If the pups are small at weaning, a long sipper tube should be placed.
- 5.6 Identification and Recordkeeping:
  - 5.6.1 Identify breeders by ear punching or ear tags.
  - 5.6.2 Identify cages of breeding animals with the appropriate cage card or with the BGU Preclinical Management System application, and include the following information:
    - 5.6.2.1 Identification of breeders
    - 5.6.2.2 Strain (using proper nomenclature)
    - 5.6.2.3 Mating date
    - 5.6.2.4 Date of birth and expected date of weaning for all litter
  - 5.6.3 Maintain breeding records that include:
    - 5.6.3.1 Parents identification numbers
    - 5.6.3.2 Date of breeding
    - 5.6.3.3 Date litter is born
    - 5.6.3.4 Litter size
    - 5.6.3.5 Number of mice that have been weaned
    - 5.6.3.6 Gender frequencies
    - 5.6.3.7 Interval between litters
    - 5.6.3.8 Phenotype
    - 5.6.3.9 Number of animals euthanized

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*Approved by the BGU Animal Policy and Welfare Oversight Committee*