
1. PURPOSE

This Standard Operating Procedure (SOP) describes guidelines for the management of mouse breeding colonies.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff, animal care staff and veterinary care staff.

3. MATERIALS

- 3.1. Caging and environmental enrichment
- 3.2. Cage cards
- 3.3. Ear punch or ear tags
- 3.4. Breeding records

4. CONSIDERATIONS

- 4.1. Generally, laboratory mice will reach sexual maturity at approximately 6 weeks of age, although females may have their first estrus as early as 5 weeks of age.
- 4.2. The reproductive lifespan of mice is on average 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 from 2 to 4 hours up to 1 week.
- 4.4. Gestation period: 19-21 days
- 4.5. Weaning age: 21-28 days
- 4.6. Mating is usually nocturnal and may be confirmed by the presence of a copulatory plug in the vagina up to 24 hours post-copulation. Refer to section 5.4.
- 4.7. Pregnancy may be confirmed by gentle abdominal palpation after gestation day 12.
- 4.8. A fertile postpartum estrus occurs between 14 and 24 hours following parturition, and simultaneous lactation and gestation may prolong gestation.
- 4.9. Fertility and litter size vary by strain. The success rate may also be dependent on the age of the breeders.

5. PROCEDURES

- 5.1. Breeding cages should be observed daily for newborn animals, litters that are ready for weaning, separating of females, and a general check of the animals health and general 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 weeks of age.
 - 5.2.2. Ideally, 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 begins to decline. Breeding success decreases if the mice are older than 8 months old.
 - 5.2.4.2. If no litters have been born 60 days after mating or 60 days after weaning of the last litter and female is not pregnant (90 days for strains known to have low fertility).

- 5.2.4.3. If several litters have been born but no pups have been weaned.
- 5.2.4.4. If a significant decrease in litter size is noted, e.g., 1-2 pups born per litter when previously average litter size was 8-9 pups.
- 5.2.5. Do not replace all breeding animals at the same time. 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 in a low-traffic area of the housing room. Avoid handling cages with newborn litters.
- 5.2.8. If breeding difficulties persists in the colony, consult with 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. Takes advantage of postpartum estrus and allows the female to become pregnant and nurse at the same time.
 - 5.3.1.4. Litters are born approximately 21 days apart.
 - 5.3.1.5. The 3-week old litter must be weaned prior to the birth of the new litter.
 - 5.3.1.6. For strains that require pups to be weaned later than 21 days of age, female must be separated to avoid postpartum estrus 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 must be weaned at 21 days of age, prior to the birth of new litters.
 - 5.3.2.5. For strains that require pups to be weaned later than 21 days of age, both females must be separated to avoid postpartum estrus and overcrowding.
 - 5.3.3. Harem breeding
 - 5.3.3.1. Due to 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 litters should be born into cages with harem breeding.
 - 5.3.3.4. Does not utilize post-partum estrus.
- 5.4. Timed matings:
 - 5.4.1. Used when the precise 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 four females are housed together for mating. A ratio of 1 male: 1 female or 1 male: 2 females gives optimal results.
 - 5.4.3. Breeding cages for timed matings 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 to 24 hours after breeding.
 - 5.4.5. The presence of a vaginal plug does not guarantee pregnancy.

- 5.4.6. 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. If necessary, use a cotton-tipped swab or a blunt metal probe to gently open the vagina.
- 5.4.7. 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 2 or more grams of body weight. Pregnancy could also be confirmed by abdominal palpation after gestational day 12.
- 5.4.8. 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.
- 5.4.9. The first day of gestation is considered to be the day after the vaginal plug is observed.

5.5. Weaning:

- 5.5.1. Weaning refers to removing a pup from its home cage (rather than to the time 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 rate can be weaned around 10-12 g.
- 5.5.3. Pups should be weaned if the same female gives birth to a new litter. 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 at 21-28 days of age. 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 routinely to be weaned after 21-28 days of age, the female must be separated from the male prior to giving birth as to avoid postpartum estrus, overlapping of gestation and lactation, as well as overcrowding.
- 5.5.6. Upon weaning, pups may be separated as follows:
- 5.5.6.1. Male and female pups separated by sex into cages housing a maximum of 5 mice.
 - 5.5.6.2. Male and female pups separated into cages housing up to 8 juveniles each. When the juveniles reach a maximum weight of 16g 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 together. After weaning, males of different litters should not be placed together as it will resolve in fighting. It is generally acceptable to group females together even after weaning as fighting is less probable. It is preferable not to wean a single mouse alone. If that cannot be avoided, wet food in a dish at the bottom of the cage needs to be provided for the first week.
- 5.5.7. A small amount of food may be provided to the weanlings in the bottom of the cage.

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, 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 litters
- 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

6. REFERENCES

- 6.1. Breeding Strategies for Maintaining Colonies of Laboratory Mice - A Jackson Laboratory Resource Manual, 2009: http://jaxmice.jax.org/manual/breeding_strategies_manual.pdf
- 6.2. Danneman, P.J., Suckow, M.A. & Brayton, C.F. (2000). *The Laboratory Mouse*. Boca Raton, FL: CRC Press.
- 6.3. Guide to the Care and Use of Experimental Animals Vol. 1 (2nd ed). Canadian Council on Animal Care, Canada, 1993: http://www.ccac.ca/Documents/Standards/Guidelines/Experimental_Animals_Vol1.pdf
- 6.4. Heyne G.W., Plisch E.H., Melberg C.G., Sandgren E.P., Peter J.A., Lipinski R.J. (2015), A Simple and Reliable Method for Early Pregnancy Detection in Inbred Mice. *JAALAS* 54(4):368-71.

SOP REVISION HISTORY

DATE	PREVIOUS VERSION	NEW VERSION
2016.03.16	4.6. Mating is usually nocturnal and may be confirmed by the presence of a copulatory plug in the vagina up to 24 hours post-copulation. Refer to section 5.4. The success rate will vary with the strain and the age of the breeders.	4.6 Mating is usually nocturnal and may be confirmed by the presence of a copulatory plug in the vagina up to 24 hours post-copulation. Refer to section 5.4. The success rate will vary with the strain and the age of the breeders.
2016.03.16	4.9 Fertility and litter size vary by strain.	4.9 Fertility and litter size vary by strain. The success rate may also be dependent on the age of the breeders.
2016.03.16	5.1 Breeding cages should be observed daily for newborn animals, litters that are ready for weaning, separating of females, and a general check of the animals health and general condition.	5.1 Breeding cages should be observed daily for newborn animals, litters that are ready for weaning, separating of females, and a general check of the animals health and general condition. The maintenance of good breeding records is essential.
2016.03.16	5.2.4.4 (no text)	5.2.4.4 If a significant decrease in litter size is noted, e.g., 1-2 pups born per litter when previously average litter size was 8-9 pups.
2016.03.16	5.3.3.1. (no text)	5.3.3.1. Due to increased risk of overcrowding and impact on animal welfare, only permitted under specific circumstances and must be justified in the animal use protocol.
2016.03.16	5.4.2. One male and up to four females are housed together for mating.	5.4.2. One male and up to four females are housed together for mating. A ratio of 1 male: 1 female or 1 male: 2 females gives optimal results.
2016.03.16	5.4.7. If a vaginal plug is found, separate the female. You may confirm pregnancy by abdominal palpation after gestational day 12.	5.4.7. 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 2 or more grams of body weight. Pregnancy could also be confirmed by abdominal palpation after gestational day 12.
2016.03.16	5.5.5.3. (no text)	5.5.5.3. Only at weaning can males of different litters be grouped together. After weaning, males of different litters should not be placed together as it will resolve in fighting. It is generally acceptable to group females together even after weaning as fighting is less probable. It is preferable not to wean a single mouse alone. It that cannot be avoided, wet food in a dish at the bottom of the cage needs to be provided for the first week.
2016.03.16	5.6.3.1. (no text)	5.6.3.1. Parents identification numbers
2018.11.12	5.3.2.2. Optimal for strains with small litter sizes (<8 pups per litter).	5.3.2.2. Optimal Only acceptable for strains with small average litter sizes (<8 pups per litter) of 7 pups per litter or less.
2018.11.12	5.3.2.3. Both lactating females may be left in the same cage (+/- the male) only if both females have small litters (6-8 pups per litter).	5.3.2.3. Both lactating females may be left in the same cage (+/- the male) only if both females have small litters (6-8 pups per litter) each female has a litter of 7 pups or less.
2018.11.12	5.5.3 (NO TEXT)	5.5.3. Pups should be weaned if the same female gives birth to a new litter. A female cannot nurse two litters simultaneously.
2018.11.12	5.3.2.4. Pups must be weaned at 21 days of age, preferably prior to the birth of new litters.	5.3.2.4. Pups must be weaned at 21 days of age, preferably prior to the birth of new litters.
2018.11.12	5.3.3.1. Due to increased risk of overcrowding and impact on animal welfare, is only permitted under specific circumstances and must be justified in the animal use protocol.	5.3.3.1. Due to 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.

How many animals in a standard mouse cage? Combien d'animaux par cage à souris standard?



5 adults
5 adultes

OR
OU



OR
OU



* pups must be removed when they reach 21 days of age
* les souriceaux doivent être séparés lorsqu'ils sont âgés de 21 jours

OR
OU



Up to 8 juveniles (3-6 weeks of age)
Jusqu'à 8 juvéniles (âgés de 3-6 semaines)



Your Veterinarian
Votre Vétérinaire