
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. Identification method
- 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. Generally, weaning occurs at 21 days. In some cases, e.g., slow growing strains or genetically modified strains with developmental impairment, weaning can be delayed up to 28 days, depending on the breeding scheme.
- 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. Gestation can also be confirmed by weight gain of 2 g at 8 to 10 days post-copulation or weight gain of 20-30% at 10-12 days post-copulation. Gestation may also be confirmed by ultrasound, when available.
- 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.
- 4.10. In case of breeding difficulties, consult with a veterinarian as soon as possible, as fertility decreases rapidly with age.

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, particularly with strains that are difficult breeders.

- 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. After 6 months of reproduction.
 - 5.2.4.3. If clinical signs of poor health of undesired phenotypes are observed.
 - 5.2.4.4. 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).
 - 5.2.4.5. If more than 2 litters have been born but no pups survive to weaning age.
 - 5.2.4.6. 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. Refresh breeders every 6 to 10 generations to avoid genetic drift. This can be accomplished by purchasing new breeders from a vendor or by backcrossing to the background strain.
- 5.2.7. Provide adequate environmental enrichment, nesting material is essential in breeding cages.
- 5.2.8. Handle breeding cages gently and place in a low-traffic area of the housing room. Avoid handling cages with newborn litters.
- 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 can continue to be housed together when the female becomes pregnant or delivers the pups.
 - 5.3.1.3. When male and female are housed together continuously, allows to take advantage of the postpartum estrus and allows the female to become pregnant and nurse at the same time.
 - 5.3.1.4. Litters are born approximately 19-21 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 expected 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, the male 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. Ideally, males and females are housed separately for 4-7 days before mating.
 - 5.4.4. Breeding cages for timed matings should be set up in the late afternoon as mice usually mate during the dark cycle.
 - 5.4.5. 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.
 - 5.4.6. The presence of a vaginal plug signifies copulation occurred but does not guarantee pregnancy.
 - 5.4.7. To check a female for the presence of a vaginal plug:
 - 5.4.7.1. Look for a plug as early into the light cycle as possible. Otherwise, the plug may become dislodged or dissolved.
 - 5.4.7.2. To see the plug, lift the female by the base of her tail and examine her vaginal opening for a whitish or cream-colored plug. If necessary, use a cotton-tipped swab or a blunt metal probe to gently open the vagina.
 - 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 2 or more grams of body weight. Pregnancy could 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 for a copulatory plug each morning. If after 7 days there is no vaginal plug, remove the male.
 - 5.4.10. The first day of gestation is considered to be the day after the vaginal plug is observed, 0.5 days post-coitum (dpc).
- 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. Avoid separating weanlings alone.
 - 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 result in fighting. It is generally acceptable to group females together even after weaning as fighting is less probable.
 - 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 as per Rodent Identification SOP.
 - 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. Danneman, P.J., Suckow, M.A. & Brayton, C.F. (2000). *The Laboratory Mouse*. Boca Raton, FL: CRC Press.
- 6.2. Canadian Council on Animal Care ([CCAC](#)) [guidelines: Mice](#), August 2019.
- 6.3. 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. *Journal of the American Association for Laboratory Animal Science : JAALAS*, 54(4), 368–371.
- 6.4. Wong, M. D., van Eede, M. C., Spring, S., Jevtic, S., Boughner, J. C., Lerch, J. P., & Henkelman, R. M. (2015). 4D atlas of the mouse embryo for precise morphological staging. *Development (Cambridge, England)*, 142(20), 3583–3591. <https://doi.org/10.1242/dev.125872>
- 6.5. Chow, K. H., Yan, Z., & Wu, W. L. (2016). Induction of Maternal Immune Activation in Mice at Mid-gestation Stage with Viral Mimic Poly(I:C). *Journal of visualized experiments : JoVE*, (109), e53643. <https://doi.org/10.3791/53643>

SOP REVISION HISTORY

DATE	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.
2016.03.16	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. The maintenance of good breeding records is essential.
2016.03.16	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. 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. 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 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. 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. Parents identification numbers
2018.11.12	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) each female has a litter of 7 pups or less.
2018.11.12	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.
2018.11.12	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.

2019.06.19	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 https://www.research.uci.edu/forms/docs/iacuc/JAX-breeding-strategies.pdf
2023.06.09	3.3. Ear punch or ear tags Identification method
2023.06.09	4.5. Generally, weaning occurs at 21 days. In some cases, e.g., slow growing strains or genetically modified strains with developmental impairment weaning can be delayed up to 28 days, depending on the breeding scheme.
2023.06.09	4.7. Pregnancy may be confirmed by gentle abdominal palpation after gestation day 12. Gestation can also be confirmed by gain weight of 2 g 8 to 10 days post-copulation.
2023.06.09	4.10 If In case of breeding difficulties persist in the colony, consult with a veterinarian as soon as possible, as fertility decreases rapidly with age.
2023.06.09	5.2.3. Pairing young females with older males can improve breeding performance, particularly with strains that are difficult breeders.
2023.06.09	5.2.4.2. Breeders should be replaced after 6 months of reproduction.
2023.06.09	5.2.4.3. If clinical signs of poor health of undesired phenotypes are observed.
2023.06.09	5.2.4.5. If several more than 2 litters have been born but no pups have been weaned survive to weaning age.
2023.06.09	5.2.6. Refresh breeders every 6 to 10 generations to avoid genetic drift. This can be accomplished by purchasing new breeders from a vendor or by backcrossing to the background strain.
2023.06.09	5.2.7. Provide adequate environmental enrichment; nesting material is essential in breeding cages.
2023.06.09	5.3.1.2. The mice are not separated can continue to be housed together when the female becomes pregnant or delivers the pups.
2023.06.09	5.3.1.3. When male and female are housed together continuously, allows to take advantage of the postpartum estrus and allows the female to become pregnant and nurse at the same time.
2023.06.09	5.3.1.4. Litters are born approximately 19-21 days apart.
2023.06.09	5.3.2.2. Only acceptable for strains with average expected litter sizes of 7 pups per litter or less.
2023.06.09	5.3.2.5. For strains that require pups to be weaned later than 21 days of age, both females the male must be separated to avoid postpartum estrus and overcrowding.
2023.06.09	5.4.3. Ideally, males and females are housed separately for 4-7 days before mating.
2023.06.09	5.4.6. The presence of a vaginal plug signifies copulation occurred but does not guarantee pregnancy.
2023.06.09	5.4.7.2. To see the plug, lift the female by the base of her tail and examine her vaginal opening for a whitish mass or cream-colored plug . If necessary, use a cotton-tipped swab or a blunt metal probe to gently open the vagina.
2023.06.09	5.4.9. If there is no plug, leave the female with the male and check for a copulatory plug each morning. If after 14 7 days there is no vaginal plug, replace the breeders remove the male.
2023.06.09	5.4.10. The first day of gestation is considered to be the day after the vaginal plug is observed, 0.5 days post-coitum (dpc).
2023.06.09	5.5.6.2. Avoid separating weanlings alone.
2023.06.09	5.5.6.3. 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.
2023.06.09	5.5.6.4. 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.
2023.06.09	5.6.1. Identify breeders by ear punching or ear tags as per Rodent Identification SOP.
2023.06.09	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
2023.06.09	6.2. Guide to the Care and Use of Experimental Animals Vol. 1 (2nd ed). Canadian Council on Animal Care, Canada, 1993: http://www.ccaac.ca/Documents/Standards/Guidelines/Experimental_Animals_Vol1.pdf
2023.06.09	6.2. Canadian Council on Animal Care (CCAC) guidelines: Mice, August 2019.
2023.06.09	6.4. Wong, M. D., van Eede, M. C., Spring, S., Jevtic, S., Boughner, J. C., Lerch, J. P., & Henkelman, R. M. (2015). 4D atlas of the mouse embryo for precise morphological staging. Development (Cambridge, England), 142(20), 3583–3591. https://doi.org/10.1242/dev.125872
2023.08.15	4.7. Pregnancy may be confirmed by gentle abdominal palpation after gestation day 12. Gestation can also be confirmed by weight gain of 2 g at 8 to 10 days post-copulation or weight gain of 20-30% at 10-12 days post-copulation. Gestation may also be confirmed by ultrasound, when available.
2023.08.15	6.5. Chow, K. H., Yan, Z., & Wu, W. L. (2016). Induction of Maternal Immune Activation in Mice at Mid-gestation Stage with Viral Mimic Poly(I:C). Journal of visualized experiments: JoVE, (109), e53643. https://doi.org/10.3791/53643

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