I. General Guidance

This guidance provides murine breeding colony management guidance to investigators and breeding colony personnel and ensures compliance with the *Guide* regarding space requirements for the animals used in breeding activities.

II. Definitions

1. Adult mouse: Any mouse that is 21 days or older and able to eat and drink on its own.
2. Weaning extension: The IACUC expects pups to be weaned at approximately 21days of age. If pup development is delayed, weaning may be delayed based on the breeding technician’s professional judgement.

III. Breeding Management

1. Management
   1. Breeding animals must be established based on need and managed according to principles of animal reduction such as cryopreservation.
   2. Depending on the experimental plan for the animals, breeding mice may be set up in pairs (monogamous breeding), trios or polygamous breeding groups.
   3. No more than 1 male and 4 females may be housed in the same cage for breeding purposes.
   4. Female mice must never be housed in the same cage with more than one male as this may result in fighting and injury to the animals.
   5. At the request of the PI, setting up breeding cages, documenting date of birth of litters on breeding cards and weaning is the responsibility of the ARC team. Weanlings must be removed at appropriate times to prevent overcrowding that could impact reproductive performance and animal welfare.
2. Breeding Schemes
3. Recommended breeding strategy: Use of monogamous breeding systems has been shown to reduce pup mortality, result in less disturbance of the cage (i.e., to remove pregnant females when more than one is present), and is less labor intensive than other breeding schemes.
4. Alternative breeding strategies**:** Trio breeding (Two females/one male) or polygamous breeding (1 male, 3-4 females). Use of this breeding scheme requires intensive oversight and management to prevent social stress and cannibalism of newborn litters.
   1. If multiple females are housed with a male, all females but one must be placed into separate cages once they are visibly pregnant to prevent cage overcrowding. Pregnant females must be separated into a new cage prior to parturition to ensure only 1 litter of pups is present at a time. A male may be left in the same cage with 1 breeding female and their litter.
   2. No more than 2 adults (1 female/1 male or 2 adult females, see c.i.) may remain in the cage after pups are born. To summarize, trio and polygamous breeding is acceptable, but trio and polygamous birthing is not.
   3. On the rare occasion that a pregnancy is missed and 2 females with litters are in the same cage the following action must be taken:
      1. If the litters are < 3 days of age of each other and no more than 12 pups are present in the cage, the male will be removed from the cage and the females may remain together with their pups until the pups are weaned. If more than 12 pups are present in the cage, one female and her pups must be placed into a separate cage, at the discretion of the ARC staff.
      2. If the litters are >3 days of age of one another, the female with the older litter must be placed into a separate cage.
5. In the event that a female becomes pregnant during the post-partum estrus cycle, pups must be weaned prior to delivery of the next litter to prevent loss of the younger litter due to trampling and food competition.
6. Additional Information from the *Guide for the Care and Use of Laboratory Animals*
7. Founding populations of outbred lines should be large enough to ensure the long-term genetic heterogeneity of breeding colonies. To facilitate direct comparison of research data derived from outbred animals, genetic management techniques should be used to maintain genetic variability and equalize founder representations (Hartl 2000; Lacy 1989; Poiley 1960; Williams-Blangero 1991). Genetic variability can be monitored with computer simulations, biochemical markers, DNA markers and sequencing, immunologic markers, or quantitative genetic analyses of physiologic variables (MacCluer et al. 1986; WilliamsBlangero 1993).
8. When inbred animals or their F1 progeny are used, it is important to periodically monitor genetic authenticity (Festing 1982; Hedrich 1990); several methods of monitoring have been developed that use immunologic, biochemical, and molecular techniques (Cramer 1983; Festing 2002; Groen 1977; Hoffman et al. 1980; Russell et al. 1993). Appropriate management systems (Green 1981; Kempthorne 1957) should be designed to minimize genetic contamination resulting from mutation and mismating.
9. Genetically modified animals (GMAs) represent an increasingly large proportion of animals used in research and require special consideration in their population management. Integrated or altered genes can interact with species or strain-specific genes, other genetic manipulations, and environmental factors, in part as a function of site of integration, so each GMA line can be considered a unique resource. Care should be taken to preserve such resources through standard genetic management procedures, including maintenance of detailed pedigree records and genetic monitoring to verify the presence and zygosity of transgenes and other genetic modifications (Conner 2005). Cryopreservation of fertilized embryos, ova, ovaries, or spermatozoa should also be considered as a safeguard against alterations in transgenes over time or accidental loss of GMA lines (Conner 2002; Liu et al. 2009).
10. Generation of animals with multiple genetic alterations often involves crossing different GMA lines and can lead to the production of offspring with genotypes that are not of interest to the researcher (either as experimental or control animals) as well as unexpected phenotypes. Carefully designed breeding strategies and accurate genotype assessment can help to minimize the generation of animals with unwanted genotypes (Linder 2003). Newly generated genotypes should be carefully monitored and new phenotypes that negatively affect well-being should be reported to the IACUC and managed in a manner to ensure the animals’ health and well-being. Accurate recording, with standardized nomenclature when available, of both the strain and substrain or of the genetic background of animals used in a research project is important (NRC 1979b).
11. References

Health Research Extension Act of 1985 and Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals

*Guide for the Care and Use of Laboratory Animals*, National Academy of Sciences, 2011