Overview

The establishment of a rodent breeding colony may be necessary to develop an animal model that is not commercially available, or produce young animals with specific ages or weights that cannot be provided by a commercial breeding colony. Investigators developing a new spontaneous or induced mutant animal model might also need to maintain their own breeding colony because there is no alternative source for the animal model.

From a regulatory perspective, tracking all animals utilized in animal research protocols is closely scrutinized by extramural accrediting and oversight bodies. Breeding colonies receive particular attention, and require careful consideration of the justifications for the animal numbers used. Record keeping and colony management practices must demonstrate efforts to utilize animal subjects in ways that are not wasteful.

Investigators maintaining colonies simply to preserve a genetic line of rodents should consider other conservation strategies such as cryopreservation of ova, sperm and embryos.

Permission to establish a breeding colony is granted on a case-by-case basis, with the most acceptable reasons for requiring a breeding colony listed below:

- Experiments involving pre-natal work at various days of gestation. If pre-natal age-matched controls are also needed, these may also be bred.
- Breeding transgenic lines not available commercially
- Creating transgenic, knock-out or other genetically modified animals. It may be necessary to generate stock lines for breeding, creating these transgenics or controls.
- Breeding rare inbred lines not available commercially.
- Production of eggs for molecular studies

Requirements

IACUC Review

All activities involving the breeding of rodents at the University must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). Breeding of animals must be scientifically justified, and, with a few exceptions, all breeding activities must be associated with a research protocol. The production of animals specifically for “cost saving” purposes is not permitted, since the actual costs involved include many significant overhead expenses that are subsidized by the University, and not recovered in per diem structures.

Some rodent strains are protected by patents which only permit the reproduction of these animals under formal contracts or licenses.

The IACUC reviews proposed breeding colonies to assure proper colony management, including appropriate breeding schemes, weaning ages, and methods for identification of individual animals.

Large numbers of animals may be required to maintain a breeding colony. The number of animals can only be approximated in advance because it is impossible to predict the exact number and sex of offspring. There also can be confusion about whether an estimate of number of animals distinguishes between breeders, young that cannot be used in experiments because they are of the wrong genotype or sex, and animals that are actually subjected to experimental manipulations. The National Academy of Sciences’ Institute for Laboratory Animal Research Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003) includes useful information for determining the total number of animals produced in order to obtain the number of animal needed to perform the research.

The IACUC pays special attention to the production of animals in colonies, and requires that the number of unusable animals must be minimized to the extent possible. Researchers should work with DLAR staff to make unusable animals available to other research activities, whenever feasible.

Breeding protocols should be separate and apart from the research protocol(s) which they supply. The exception to this is a project in which the breeding and research are very closely linked, e.g. the breeding colony supplies only one research project and the PI of the breeding protocol is the same as the PI of the research protocol. In this situation, if there were separate protocols, animals would always be transferred to the same protocol. Thus, animals produced in a combined breeding/research protocol are not intended for transfer to other protocols of the same investigator or to other investigators’ protocols.

If animals are being produced which are likely to experience clinical diseases (e.g., hypertension, neoplasia, diabetes, neurologic disorders) the anticipated problems should be described, to enable
an evaluation by the IACUC, and be known to husbandry and health personnel who will be caring for them. Unanticipated phenotypic disease manifestations should be reported to the IACUC and DLAR.

Summary

The purpose of this guideline is to: 1) Conserve valuable animal holding space for primary research activities; 2) Manage the administrative/compliance burden of tracking breeding colony management; 3) Operate the animal facilities in a cost effective manner; 4) Reduce activities that tend to promote opportunities for infectious disease transmission; 5) Assure the appropriate utilization of genetically defined strains of rodents in research, and 6) Assure that breeding activities are justified and well managed.

Transferring Animals

Animals produced in breeding protocols may be expeditiously transferred to a related research protocol, as needed, by completing a brief animal transfer form. The transfer of animals to other protocols not specified in a breeding protocol must be pre-approved by DLAR and specified in the protocol form.

This transfer process reassigns animals to the research protocol. Animals may be transferred the same day by the PI’s staff without administrative delay, when transferring within the same PI as specified in the protocol. New cage cards or existing cage card modified with a transfer label must be made with the relevant protocol number.

Animal movements within the vivarium should always be consistent with programs to curtail health problems within the facility, and require close cooperation between DLAR.

Operational Guidelines for Breeding Colonies

All Principal Investigators must have a current, approved IACUC protocol in order to breed laboratory animals. Individuals conducting breeding programs must be adequately trained by attending the DLAR Breeding Colony Training Class.

If the animals requested for breeding are from a non-commercial vendor, it will be necessary to complete a Materials Transfer Agreement. The health status of animals must be screened prior to shipment in order to perform a risk assessment. Animals with undesirable health histories will not be accepted into UT facilities. Animals with incomplete health histories may require special quarantine and further testing at the sending institution to ascertain their current health status.

Mating Systems:
The two most common systems are paired mating (a male left with one female) and harem mating (one male housed with 2-5 females). Due to housing density requirements, when using the harem system, each female must be moved to a single cage when pregnancy is confirmed. At no time can more than one female with a single litter be present in a single cage.

With paired housing, it is important to note that the female can get pregnant on the delivery date, when the male is housed with her during her post-partum estrus. If this occurs, the next litter
will be born 21 days later. Therefore, weaning of each litter promptly at 21 days is required (the
day of birth is considered “day 1” when determining weaning age).

Weaning:
This will vary by species. However, most rodents will be weaned at 21 days. Exceptions to this
can be made by special request in the IACUC protocol or an IACUC amendment. DLAR will
keep a written document of those who have exceptions. Some examples of valid reasons for
extending the 21-day weaning are dwarf or runted mice, transgenic and mutant strains that have
weak pups requiring extended maternal care. Regardless of weaning age, at no time can more
than one female with a single litter be present in a cage. Special precautions should be taken
when weaning animals to prevent health concerns. (Moist food and gelatinized water placed in
the bottom of cage, lixit moistened, bottle at weaning, etc.)

Documentation:
Cage cards for breeding animals should include the date set-up, date plugged (if following), date
of birth of offspring, number born, date weaned and number weaned. Optional information can
include ID numbers of parents and reference number of offspring for ease of tracking. The post-
weaning cage cards should include strain, sex, date born, date weaned, if positive for transgene
or mutation and intended use (research or new breeder). Lab notes must have detailed breeding
information for inspection when necessary.

Lab Records:
Records should be maintained in such a manner as to make tracking offspring simple. Information
necessary includes sire/dam number, date and number born, date and number weaned and how offspring are used. This should include if they are euthanized (reason and date), died (reason and date), used in an experiment, replacement breeders or transferred to another protocol or investigator. Disposition of all post-weaning animals should be noted in the records. The records can be maintained in a notebook or on an Excel spreadsheet. During the semi-annual compliance inspections conducted by the Institutional Animal Care and Use Committee, and during accreditation site visits, investigators are commonly asked to explain their record keeping activities.

Reporting:
The number of viable births regardless of genotype must be reported to DLAR by use of the
Pups List Form. The form should be turned into DLAR on or before the 7th seventh of the
following month.

Any animal(s) transferred to another protocol or investigator must be reported via a transfer
request.

Genotyping:
The generation of transgenic mice requires collection of tissue samples for genetic analysis. Acceptable methods are tail snip, ear punch, and peripheral blood sample or saliva analysis. The collection of samples through any of the above means should be documented in the IACUC approved protocol. Any deviations from this or additional samples being required should be approved through an amendment prior to collection.
Ear punching: This is commonly used as an identification method in rodents. It is done using an instrument that removes a small circular section of tissue from the ear pinna. Collection of the small tissues produced during ear punching may generate enough tissue to allow DNA analysis by PCR.

Peripheral Blood Collection: Following training in blood collection, this can provide a method for genetic analysis.

Saliva Analysis: Genetic analysis of oral epithelial cells collected in saliva of mice has been described and offers an alternative and noninvasive method of genetic analysis. It involves collection of saliva from mice by oral wash using a plastic pipette followed by nested PCR analysis.

Tail Clips: General anesthesia is not required for mice <21 days of age if less that 3mm of the tail is excised. Topical anesthetic via ice-cold ethanol for 20-30 seconds is required. General anesthesia is required for mice <21 days for excision of more than 3mm or if repeated excision of tail is required. General anesthesia is also required of animals ≥ 21 days undergoing a tail clip. Hemostasis should be complete when performing tail snips, prior to returning animals to the cage. This can be achieved with a cautery agent, such as silver nitrate stick, or tissue closing glues such as Vet Bond or Liquid Bandage. These agents must be noted in protocols.

Rodent Identification:
Acceptable methods of animal identification include ear punching, micro-tattooing, micro-chip and ear tagging. Ear punching is described above. DLAR has punches available for use and/or purchase. A universally accepted numbering scheme for ear punching is included at the end of this document. Micro-tattooing is a permanent mark made with a specially designed forceps for rodents. This requires anesthesia and training by DLAR staff. DLAR has the equipment available for use.

Micro-chipping is the injection of a small chip between the shoulder blades and is read with a transponder. This is ideal for large colonies as it is safe, reliable and easy to apply. Ear-tagging is a metal tag with an ID# attached to one ear of the animal. Young rats may also be skin tagged on the neck with numbered metal tags. These are usually applied at the time of genotyping or weaning. The disadvantage of ear tagging is that tags may fall out if not properly applied. Ear tags and microchip equipment must be supplied by the Investigator.

Cage Housing Density:
Rodent housing densities are described in the regulatory guidelines and MUST be followed. The typical small shoebox cage can only contain 4 mice weighing greater than 25 grams. NOTE: JAG 75 shoebox cages can house up to 5 mice per cage. When a litter is present only the mother should be present with them.

For rats, the small shoebox will only house 2 rats.

It is important to follow these guidelines when weaning animals. Cages exceeding these guidelines will be marked by DLAR staff. Research staff will have 48 hours to split the animals
up. Following the 48 hour period, marked cages will be separated by DLAR for a significant recharge.

Sexing Mice:
The male rodent has a larger gap between the genitals/anus than does the female. When sexing the animals, lift gently by the tail. Allow the front feet to stay on the floor. Evaluate the ano-genital distance. It is sometimes helpful to notice a side-by-side comparison of a number of animals until proficiency is obtained. Juvenile mice are much harder to perform sexing via this method. An option for juvenile animals is to look for nipples on 1.5 -2 week old fuzzy animals. Just as the fur starts to grow in, a “cyclone” appearance forms around the nipples. Only the females will have obvious nipples at this point. Once the fur is fully grown in (>18 days), this method is not always effective. Finally, animals can be placed in an empty cage (without bedding) so that they can be relaxed. When the cage is picked up and looked at from the bottom, the testicles of older male mice will drop and be easily identified.

Reviewed July 25, 2011