Mouse husbandry

This document describes several items related to mouse husbandry and colony maintenance including training, mouse guidelines, germline test mating, breeding strategies, and databasing.

Keywords: weaning | strains | mice | germline | breeding

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Training:

1. Employees working with mice must complete all required training, orientation, obtain card access approval, and be listed on/added to an active IACUC protocol.
2. Standard Operating Procedures (SOPs) vary from facility to facility; therefore, employees must schedule their orientation in the appropriate facility.
3. Movement (in-house transfers, outgoing mouse shipments, and in-coming shipments) of all mice is coordinated by the Division of Animal Care (DAC). Mice cannot be moved by anyone other than an approved DAC employee.

Mouse guidelines:

1. General characteristics, such as life span, litter size, fecundity, overall health, and behavioral traits vary from strain to strain. For example, outbred mice (such as ICR) are robust and produce large litters, while some inbred strains (such as 129S6), are less robust and produce smaller litters. These characteristics must be taken into account when planning breeding strategies of a particular strain. If the stain produces small litters the number of matings may need to be increased, while if the strain produces litters of 10 - 15, fewer matings may be necessary. Plan carefully to avoid generating unnecessary animals and to save cage cost.
2. The average life span of a mouse is about 2 years; however, fecundity may be reduced after the age of about 6 months and continue to decline over time.
3. The number of mice generated in a particular strain depends on the needs of the study. If, for example, one is trying to generate a large number of animals of a particular genotype, matings may need to be set up frequently; however, if the strain is in "line maintenance" mode, mice can be mated less frequently. When simply maintaining a line it is recommended that you do not let your youngest animals exceed 6 months of age (about 26 weeks) or you may put the line in jeopardy. In general, fertility begins to go down after 6 months of age although this varies greatly depending on the strain. Thus, if you do not want to risk losing a line, you should either 1) have it cryopreserved, 2) maintain multiple cages of mice that are no more than 6 months of age, or 3) maintain fewer cages (at least 2) but breed them every 12 - 16 weeks.
4. Cryopreservation of mouse embryos is an excellent way to preserve a mouse strain for a minimal cost. These services are provided by the TMESCSR and are described here.
5. Mice reach sexual maturity at around 6 weeks of age, but quite often do not breed until they are 8 - 10 weeks old. In order to set up a mating, place the desired male and female in a cage and wait until the female is visibly pregnant. Mice gestate between 19 - 21 days; therefore, it is not necessary to separate pregnant females prior to day 17 or 18. By leaving the couple together you may save cage cost.
6. The maximum number of adult mice allowed per cage is 5; therefore, you may mate one male with up to 4 females. If multiple females are placed with one male, careful monitoring is essential as the female must be removed before she delivers her pups. It is a violation of the SOP to have multiple females plus a litter in one cage, and it is difficult to determine the mother when the pups are born in a cage with other females. Remove all pregnant females prior to giving birth in a cage with other mice and house them in individual cages.
7. Male mice that have been separated from their brothers for breeding (or any other reason) cannot be returned to the cage with their male siblings. Male mice that have been separated and older males housed with their brothers will often fight; therefore, place any male that has been separated from its brothers in a cage by himself. He must live a solitary existence, unless used to breed again. Individually housed males can greatly impact the cost of housing mice; therefore, plan carefully in order to minimize individually caged animals.
8. Once a pregnant female is identified, remove her from the breeding cage and place her in a new cage. Provide 5015 chow to all pregnant and nursing female mice. Place a "5015" sticker on the cage card (this will inform the animal care technician which food to place in the cage). 5015 chow is higher in fat and will aid milk production. Provide the pregnant female with a cardboard hut for

nesting. Monitor the cage for pups and record the date of birth (dob) on the cage card and in the mouse database (described later in this document).

9. Mice may be weaned at 20 - 21 days of age. To wean mice, obtain clean cages for the pups. Weanlings (and adult mice) may be housed up to 5/cage; therefore, if there are 6 males in a litter, place them in two cages (preferably 3 in one cage and 3 in another). Similarly, if there 7 females in a litter place them in two cages (3 in one cage and 4 in another).
10. At weaning mice are: sexed, numbered, and separated from their mother. Generally a tissue biopsy is required for genotyping. DNA may be extracted from the ear punch tissue, or a tail biopsy may be taken. Follow the SOPs for these procedures set out by the DAC and IACUC.
11. Once the weanling is sexed and numbered, place it in a cage with its same sex siblings. Record the required information for each animal on the cage card. This may include, but is not limited to, the mouse number, sex, color, line name, and parent numbers. Feed weanlings standard chow (5001).
12. The mother mouse may be housed with her female offspring (up to 5 mice/cage) or be returned to another cage containing other female mice. It is best not to mix mice from different lines in the same cage; therefore, try to place female mice in a cage with mice from the same line. If none are available, place her in a new cage and provide her with a roommate from the same line.
13. Mice 6 weeks to 6 months may be used for breeding.
14. Mice are nocturnal and their breeding behavior is dependent on the light cycle. Mouse rooms are generally set up for 12 hours of light (beginning at 6:00 AM) and 12 hours of dark (beginning at 6:00 PM). Based on this schedule, mice breed (in theory) at midnight, therefore at 12:00 noon the next day, the embryo is considered to be 0.5 days post coitus (dpc). Do not expose mice to light during their dark cycle, as this may interfere with their breeding.

Germline test matings:

1. Background: Chimeras generated from the injection of gene targeted mouse embryonic stem cells (mESCs) into a wild type mouse blastocyst (3.5 dpc mouse embryo) must be test mated to wild type mice in order to determine whether the gene target of interest is passed in the germline. TL1 mESCs are male in origin; therefore, only male chimeras are of interest. Degree of "chimerism" may be determined by the coat color of the chimera. TL1 cells were derived from 129S6 (which codes for agouti) embryos and the wild type blastocysts were harvested from C57Bl/6 (which codes for black) mice. Both 129S6 and C57Bl/6 are inbred strains. In this situation, the greater the degree of agouti coat color, the more likely the gene target will be passed in the germline of the chimera. The reverse is also true; less agouti, less likely to achieve germline transmission. Percentage of agouti coat color is an indication of what percentage of the chimera was generated from the mESCs and what percentage is from the host blastocyst. Degree of chimerism is a subjective assessment; an estimation of what percent of the chimera has agouti vs. black hair. If the chimera appears to be 80 - 100% agouti, he is considered a good germline candidate. If, however, the chimera only has a few spots of agouti coat color, it is unlikely that the gene target will be passed in the germline. The range of agouti coat color is a subjective indication of potential germline transmission.

   Note: Coat color is mESC and host blastocyst dependent. Some mESC lines code for coat colors other than agouti and this must be considered when choosing strains for test matings.

   Given this, only agouti offspring in the first litter of a male chimera X wild type female can be positive for the gene target and because chimeras are heterozygous for the gene target, only 50% of the agouti offspring will carry the target. If agouti pups are born from a male chimera X wild type female mating it is a good indication that the male will be germline; however, the agouti offspring must be genotyped for the gene target in order to confirm germline transmission. Black offspring from this type of mating may be sacrificed as it is not possible for them to be positive for the gene target.

   Once a germline chimera has been identified and the offspring are genotyped, the positive offspring may be used to establish a line of mice. The positive agouti offspring should be mated to the desired strain of wild type mice. Because genes can segregate during cell division, the agouti gene may segregate from the targeted gene in this generation; therefore allowing positive offspring to have a coat color other than agouti. DO NOT SACRIFICE NON-AGOUTI PUPS FROM ANY GENERATION OTHER THAN THE ORIGINAL CHIMERA MATED TO A WILD TYPE (GERMLINE TEST MATING) UNTIL ALL OFFSPRING ARE GENOTYPED.
2. Once you receive the chimeras determine which animals to mate based on sex and percentage of agouti coat color. Any male that is 80 - 100% agouti is a good candidate; however, it is not necessary to test mate more than 6 animals. Save all of the male chimeras until germline transmission is achieved in the event that one of the chosen breeders may be sterile.
3. Assign a number to each chimera and mate each chimera with 2 wild type C57Bl/6 females. Allow breeding to continue for at 4 -

6 weeks. If no pups are born, separate and save the females. Select another high percent chimera and set up a mating with 2 other wild type females. (Note: Plan ahead. If you anticipate 5 test matings you will need 10 wild type breeding age females available at the time you obtain the chimeras.)
4. Remove any pregnant females and follow the guidelines outlined above (Mouse guidelines, item #8).
5. It takes about one week to determine the coat color of pups. Once coat color is evident, record the number of agouti pups and total pups. Sex, number, and genotype the agouti pups at 3 weeks of age. Sacrifice any non-agouti pups.
6. Positive offspring will be ready to breed between 6 - 8 weeks of age.

Breeding strategies:

1. Inbred vs. mixed strains: Germline chimera generated from TL1 mESCs are considered to be 100% 129S6. If this is the desired background strain, the germline chimera may be bred directly to 129S6 wild type mice in order to establish a 129S6 line. It must be noted however, that it is difficult to maintain a line on a 129S6 background. This strain tends to have small litters and is many times difficult to breed.
2. Alternatively, the offspring from the test mating may be backcrossed to the desired strain, for example C57Bl/6. The agouti pups from a chimera mated to a C57Bl/6 wild type are 50% C57Bl/6 (and 50% 129S6). Continuing to mate positive offspring to C57Bl/6 mice for 10 generations will result in a congenic line. In most instances, it is desirable to study animals that are genetically similar (congenic) vs. mice that are in a mixed background.
3. In some cases, gene targeted mice must be inter bred with other genetically modified animals in a different background and it may become impossible to keep the strain "pure." At a minimum, track the percent of each strain for each generation. Strain effect must be considered when describing phenotypes.
4. Mice generated from pronuclear injection of DNA (transgenic mice) are hemizygous. Since the DNA integration is random, mice should be maintained as hemizygous; there is no simple way to genotype mice from hemizygous X hemizygous breedings.
5. Transgenic mice are generally generated from a hybrid embryo (B6D2); therefore, wild type breeders should be selected based on the desired background strain. If a C57Bl/6 background is desired, mate the transgenic founder to C57Bl/6 and continue to backcross. Mice will be congenic (99.99%) C57Bl/6 after 10 generations.

Databasing:

1. Depending on the number of mouse lines and genetic backgrounds being tracked, it is advisable to maintain a database that includes descriptions, history, and activities of each mouse and mouse line. Several computer applications are available for this purpose including FilemakerPro, MouSeek, etc.
2. Assign read only and read/write privileges to individuals as needed. Update data daily and maintain information in "real time."

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Attachment

mouse_husbandry1.doc - Added on August 12, 2011 at 9:41 AM by Jennifer Skelton