Efficient Mouse Colony Management Guidelines (adapted from Baylor College of Medicine)

The goal of managing any mouse colony should be to maintain adequate numbers of animals in as little shelf space as possible, while adhering to the university's policies regarding health and well-being of the mice, and minimizing labor costs. What constitutes "adequate numbers" will, of course, depend on a number of factors, including:

- experimental needs,
- breeding characteristics of a given strain,
- genotypes and phenotypes (morbidity/mortality) of individual mice,
- limits of approved animal use protocol.

Therefore, these guidelines will address in detail only the minimum effort and cage space needed to keep a normal strain of mice "on the shelf." However, the principles of mouse numbering, culling, and replenishment can be utilized on any scale to minimize cage space.

Different strains of mice vary in fecundity, and certain mutant strains can be difficult to breed, due to a variety of factors such as small litter sizes, low fertility, poor mothering instincts, high rates of cannibalism of newborns, and higher morbidity or mortality resulting from the genetic mutation/alteration. When acquiring a new genetically modified strain, therefore, it is always a good idea to consult with someone who has direct experience with maintaining that strain.

- **Cages required to maintain a strain:** Simple strain maintenance generally requires no more than 2-3 mating cages and 3-4 additional cages to hold weaned pups that will be used to replace old breeders. To predict the cages needed to produce mice for experiments, figure about one litter per month from young breeders, and about 6-8 pups/litter, until experience proves otherwise. Maintaining these production levels requires consistent replacement of older breeders.

- **Breeding:** House breeders in pairs (one male and one female) or trios (one male, two females). Gestation lasts 19-21 days. Check cages at least twice each week to flag pregnant females and record approximate birth dates. Identification of pups can be accomplished prior to weaning by any of several methods (see below). Tissue for genotyping is usually collected at the same time that ID numbers are applied.

- **Weaning:** At 21-28 days of age, male and female pups are weaned to separate holding cages, with no more than 5 mice per cage. To minimize fighting, weaned males should be group-housed only with their littermates. Even littermates may have to be separated as they age to prevent fighting. Males that have been used as breeders should be housed singly, because they will kill other juvenile or adult males.

- **Replacement of breeders:** Replace breeders if:
  - they have not produced a litter in two months,
  - they are producing small litters (1-3 pups per litter),
  - they are killing their pups.

  New breeders should be 6-8weeks of age. Although both males and females can breed up to 8 months of age, most breeders are replaced after 6 months of age. Mice that have not

been allowed to breed prior to 4 months of age may never breed successfully. As breeders age, both litter size and the frequency of litters decrease. It may be wise to wait for new breeders to produce a litter before euthanizing old breeders if the strain presents any unusual breeding problems.

- **Cull older progeny:** Euthanize older offspring as new litters are weaned if the goal is to maintain the strain and minimize cage costs. It is easy to neglect weaned pups, since they are not breeding. Periodic evaluation of all cages is essential to minimizing cage usage. If progeny are more than 3 months old, you have new litters to replace them, and you don't need them for experiments, then take them off the shelf. The only reason to keep more than one or two weaned litters for a strain that is not being used in experiments is if that strain exhibits unusual morbidity or mortality, or is otherwise difficult to breed.

- **Pup identification:** The following table lists the common methods of pup ID, with pros and cons of each.

Method	pros	Cons
ear punching/notching	Simple, inexpensive, easy to read	Sometimes ambiguous, subject to tearing and healing, limited numbers
metal ear tags	Unique numbering, relatively inexpensive	Loss of tags, infections, hard to read
toe clipping	Simple, inexpensive, permanent	Must be performed at 7-10 days of age
tattooing	Relatively permanent, easy to read, may be done on newborns	More difficult and time-consuming, may fade with time if done improperly
microchips	Permanent, virtually unlimited numbers, can provide physiologic data	High cost per mouse, difficult to apply, requires expensive reader

An example of low-level maintenance

Week 1:

Maintenance cage 1	Maintenance cage 2	Holding cage	Holding cage	Holding cage	Holding cage
7 pups	6 pups	4 males	5 females	3 males	4 males
3 wks old	2 wks old	15 wks old	15 wks old	10 wks old	8 wks old

The progeny from mating cage 1 are ready to be weaned. Three females and four males are found. They are numbered and placed in two cages. The two cages of older progeny are discarded (shaded boxes). Result: no change in cage number.

Week 2:

Maintenance cage 1	Maintenance cage 2	Holding cage	Holding cage	Holding cage	Holding cage

8 pups	5 pups	3 males	4 males	4 males	3 females
newborn	3 wks old	11 wks old	9 wks old	4 wks old	4 wks old

The progeny from mating cage 2 are ready to be weaned. Two females and three males are found and numbered. The females are added to the cage of 3 females created in week 1, while the males are put into a new cage. The oldest cage of male progeny is discarded (shaded). Result: no change in cage number.

Week 3:

Maintenance cage 1	Maintenance cage 2	Holding cage	Holding cage	Holding cage	Holding cage
8 pups	No pups	4 males	4 males	5 females	3 males
1 wk old		9 wks old	5 wks old	4&5 wks old	4 wks old

The oldest male progeny can be discarded (shaded). No other action is necessary. Result: cage number reduced to five.

Dos and Don'ts of Mouse Breeding

- Do minimize the amount of noise and traffic in your mouse room. Try to always have the same person(s) take care of your mice. Ultrasonic noise, in particular, can disrupt normal mouse behavior (including mating behavior), and can be produced by something as innocuous as a dripping faucet.
- Do get to know your husbandry staff and maintain a good working relationship with them. They will usually be the first people to notice when something is wrong with your mice.
- Don't bang cages around or jostle the mice any more than necessary. Handle them calmly and quietly.
- Do allow male mice a little time (e.g., a day) to get used to a new cage before expecting them to mate successfully. Males like to mark their territory before "getting down to business."
- Don't let mice get too old prior to first mating if you are planning to breed them. It's best to give them at least some practice within 4 months of birth.

Don't expect breeding females to be productive after more than 8 months of age, although they can have litters well beyond that. Males are generally productive for at least a year, and often well beyond that. Maximize production of pups by replacing your breeders with younger mice on a regular schedule.

- Do cycle females through a male's cage to maximize production from a small number of males. The estrus cycle is 4-5 days long, so females should be left in the cage at least this long, unless you are checking for plugs.
- Don't cycle males through a female's cage - pheromones from new males can disrupt pregnancies initiated by previous males.
- Don't disturb gestating females any more than necessary, especially a few days before and after birth.
- Do leave a female with the same male continuously to take advantage of postpartum estrus. The female can get pregnant within 24 hours of giving birth and normal males will not harm their pups. If you have plenty of males, 1:1 matings are recommended to maximize production while minimizing workload.
- Do use dirty bedding from a male's cage to stimulate estrus in females. Females group-housed for long periods can go into anestrus, i.e., they will stop cycling normally. This is reversed by exposure to male pheromones present in the urine.
- Do check for vaginal plugs if you want to know exactly when a female has mated successfully. This is best done in the morning because the plugs will start to fall out after a few hours. The presence of a plug is not absolute proof of pregnancy, but it is a very reliable indicator. On the other hand, the absence of a plug is not a guarantee that the female isn't pregnant.
- Do communicate with the vivarium manager and husbandry staff about any special precautions for your mice, e.g., autoclaved water, cages, and bedding, and irradiated (or autoclaved) feed for immunocompromised mice. Special diets are available and those that aren't can be custom made by the manufacturer.
- Do give your mice some environmental enrichment. Nestlets are provided by vivarium staff, and should be transferred with the mice with each cage change.
- Do keep your mice on a regular light/dark cycle with at least 12 hours of light. The S&E1 vivarium is kept on a 14:10 hour light:dark cycle. The vivarium staff periodically checks that the lights actually come on and go off as scheduled. Avoid entering mouse rooms during dark periods. A red light can be used to minimize impact on the mice if you have to work in the room during dark hours.
- Don't transfer mice from one room to another without permission from the vivarium director. It is vivarium policy to have all animal transfers performed by vivarium staff.

Cross-fostering of Mouse Pups

Many factors affect the survival of newborn mice, including the mother's nurturing instincts, the number of pups in the litter (too many and too few are bad), amount of milk production, tendency for cannibalism, etc. Some problems can be overcome by moving the pups to the cage of another lactating female that will take better care of them. This is known as cross-fostering. Our protocol for cross-fostering is as follows:

1. Donor and recipient litters should be born within 2 days of each other, and neither litter should be more than 4 days old, for best results.
2. If possible, use a recipient strain with a different coat color from the donor strain for easier identification of the cross-fostered pups. The recipient strain is normally an outbred strain such as CD-1, ICR, or Swiss, because these tend to make the best mothers.

3. Remove the recipient mother from her cage. When you pick her up, try to get her to urinate on your gloves, and wipe the urine on the pups you are transferring. (Most mice will urinate if turned upside down.)
4. Remove some (or most) of the recipient's pups if she has a large litter. After cross-fostering, the total number of pups in her cage should be 5-10.
5. Mingle the transferred pups with the recipient's pups and rub them with dirty bedding to give them the same scent as the recipient's pups. Add nesting material if necessary.
6. Return the recipient mother to her cage and place the cage back on the rack.
7. Observe the recipient mother's behavior after a few minutes. If she settles down to nurse the pups, or is grooming them, she will probably accept the transferred pups. If she scatters them around the cage, it is unlikely she will take care of them.

Pheromone Effects

Mouse reproductive behavior is governed to a large extent by pheromones. Three important effects of pheromones are described here.

Lee-Boot Effect

Housing female mice in groups will result in synchronization of their estrus cycles. Prolonged absence of male pheromones results in a state of anestrus (lack of a normal estrus cycle).

Whitten Effect

Estrus can be induced in most group-housed females by adding male mouse urine (or dirty bedding from a male's cage) to their cage.

Bruce Effect

Pheromones from a strange male can prevent the implantation of embryos into the uterine walls of a recently bred female. This is why one should not move a female from one male's cage to another.

The Lee-Boot and Whitten Effects can be utilized to produce closely synchronized pregnancies. After group-housing females separately from males for some time, put them in a male's cage. Generally at least 75% of the females will become pregnant within 3 days.

Males prefer to have their territory scent-marked before they will breed efficiently. Therefore, it is better to leave males in their "home" cage and shuffle females in and out to maximize the production from a small number of males.