Aurora's Guide to Mouse Colony Management

-Aurora Burds Connor, 2006

Breeding mice in the laboratory is one method of ensuring an available supply of experimental subjects with desired characteristics. Although mice breed readily when left to themselves, it is helpful to have a working knowledge of reproductive physiology to obtain optimal results. In addition, accurate record keeping and a familiarity with standard terminology for the designation of mouse strains help ensure that the genetics of the research mouse was intended.

Contents

Handling of Mice

Mice are usually caught and lifted by the base of the tail. The tail should be grasped between the thumb and the forefinger about two-thirds of the way down from the tip. With this method, a mouse may be transferred to another cage and examined grossly; however, such restraint is not sufficient for treatment or a detailed physical examination. For more effective control, the mouse can be held by the tail and placed on a wire bar cage top or other surface that, preferably, the mouse can grasp, and the loose skin over the neck and shoulders grasped with thumb and fingers. If not done correctly the mouse is able to turn its head and bite, so regrasping of more loose skin or re-positioning may be necessary. The tail is then held between the fourth and fifth fingers of the same hand, resulting in good exposure for examination or treatment. The mouse must be held firmly but gently so it will not have difficulty breathing. Plastic restraint devices can also be used to hold mice and other rodents. Very young mice should be picked up by cupping the hands around the whole body, by grasping the skin across the shoulder blades with forceps, or by picking up a group of pups together with a small amount of nesting material.

Mouse Strains

Balb/C- Inbred strain. Albino, Passive, 8-14 pups per litter.
C57BL/6- Inbred strain. Non-agouti black. More aggressive (bite) and more sensitive to noise and smells. Tend to be poor mothers (eat their pups).

Average litter 6-9 pups.
129Sv- Inbred strain (with about 20 sub strains!), Agouti coat color (brown),
Average litter 7-10 pups, medium aggressive and sensitivity.
Swiss Webster (SW)-Outbred strain, Albino. Good mothers, large litters.

Mouse Breeding Basics

Sexual maturity (puberty): 4 to 7 weeks *
Estrous cycle for female fertility: 4 to 5 days*
Duration of estrous (peak fertility): 12 hours during the dark (overnight) part of each day
Ovulation: 2 to 3 hours after the onset of estrous
Gestation: 19 to 21 days*
Average litter size: 4 to 12*
Breeding lifespan: 10 to 12 months
Lifespan: 1 to 3 years
Weaning: 21 days*

* = Values can vary with mouse stock or strain

Breeding Systems

Monogamous - One male and one female are selected and paired together for the duration of their breeding life. This system simplifies record keeping and lends itself well to maintaining inbred or outbred colonies.
Polygamous - Also referred to as harem breeding, it is a system where one male is housed with two or more females (keeping in mind mouse housing density and overcrowding guidelines). This system results in the large number of young from the least number of breeding animals. It is the most economical method of laboratory animal production.
Inbreeding - Brother/sister or parent/offspring matings for a minimum of 20 generations. This type of system is used to produce animals that are very genetically similar. The reproductive performance and behaviors can vary depending on the strain.
Outbreeding - Also referred to as random breeding, this system avoids the mating of close relatives and produces the maximal amount of genetic heterogeneity and large litters. Animals of the same stock are mated, producing a more vigorous animal by maintaining genetic diversity. Accurate records are necessary in order to prevent breeding animals that are related to each other.
Line Breeding - In this system, the mating of animals by specific trait is performed. This is usually done to propagate mutant or transgenic lines, or because the trait is needed for research.
Cross Breeding - The mating of animals of different breeds or strains, Also called "hybrid cross."
Backcrossing – Breeding of successive offspring to pure (wildtype) mice generation after generation so that your mutation or phenotype is on a “pure” background. Backgrounds are not pure unless 20 generations of backcrossing has occurred. For example, each new generation of mice is bred to a pure C57B6 mouse.

Setting up Matings

Males are old enough to mate at 5 weeks of age. Once a virgin male is 2.5 months old, his plugging potential drops dramatically. By the time a virgin male is 3 months old, he will NOT plug well and should not be used to establish a new mating pair.

Females are often old enough to mate at 4-5 weeks of age. Be aware that is a female is not weaned early enough it is likely she will be plugged by her father or brothers. Females that become pregnant before 6 weeks are not physically developed enough to carry a litter well and may have a very small litter, may have difficulty during birth and will very often eat her pups because she is so hungry. For best efficiency, wait until a female is 6 weeks old before setting her up with a male. Female virgins can be mated at any age, but start running low on eggs after 6 months, especially C57B6.

When setting up a mating the following information should be written on the cage card: ID Number, Genotype and Wean Date of each parent. As well as the date the pair were "wed" or crossed. Subsequently, keep note on the cage card of the day each new litter is observed. If a pair does not successfully raise a litter within 2 months of their wedding, they should be sacrificed and if appropriate, a new mating set up.

There should be minimal variation in light cycle, room temperature, and humidity. A nutritious diet, with higher fat for lactation, and water should always be available free-choice. Handling should be avoided during the first two days post-partum, but at other times regular cage changes, with gentle handling, will increase fertility. Noise, rough handling, high population densities, and other forms of "stress" lead to decreased fertility and increased pup mortality.

Timed pregnancies/ Plugging

Timed pregnancies become necessary when you wish to look at specific embryonic stages. When mice mate, the male ejaculates a viscous substance that solidifies quickly and lasts for 12-14 hours. This vaginal plug acts as a barrier to prevent other males from mating with the female and also aid his sperm in reaching their target. The plug also provides an easy means by which to tell that a pair has mated. Based on the light/dark cycle, female mice ovulate between 11 pm and 1 am, thought they can mate hours earlier or later. Therefore, plugs are checked every morning between 9 am and noon. Checking plugs too early can disturb potential matings. If plugs are not checked by noon, hey will work their way out or dissolve, thought I have observed plugs as late as 2 pm. Plugs are often easily spottable by eye, but can require probing if they are small or deep plugs. We use capillary pipettes that have been rounded on end and store in 70% Ethanol to check plugs Noon on the day a plug is discovered is considered embryonic day 05. (EO.5)

When working with mutant animals, successful plugs can often result in no pups. When attempting to obtain staged embryos from timed matings, be sure to look at the mouse before it is brought upstairs. With young females, it is normally fairly obvious that they are pregnant by day 9.5 or 10.5 by a stereotypic rounding of the belly. If it is very difficult to catch plugs, but mice are getting pregnant, it is possible that the mice have a plugging phenotype. In such a case, try checking plugs early in the morning or late in the afternoon.

Characteristics of Mice from Birth to 4 Weeks of Age

	C57BL/ 6	129SV	Balb/C

Birth	Blood red skin color		
1 Day	Lighter red skin color. Milk visible in stomach		
2 Days	Lighter (pale pink) skin color. Ears flat against head.		
3 Days	Ear elevated about 45 away from head.		
4 Days	Ears elevated 90 away from head.		
5 Days	Skin thicker, with pigment. Milk no longer visible in stomach.	Lighter pigment in skin	No pigment in skin (white mice)
6 Days	Fur starts as a fine stubble over back.		
7 Days	Complete coat of fine fuzzy fur is visible.		
8 Days	Lower incisors visible, but not erupted.		
9 Days	Inguinal nipples visible in		
10 Days	Lower incisors erupted.		
11 Days	Upper incisors erupted.		
12-14 Days	Eyelids open. Slit-like palpebral opening.		
3 Weeks	Oval palpebral opening, fine soft fur, triangular shape to head.		

4 Weeks	Round palpebral opening, smooth fur, trapezoidal shape to head.		

Weaning

Pups are weaned at three weeks of age. At three weeks, they should be fairly active, eyes open, and eating pellet food. However, they may still be suckling. A good test for whether or not they're ready to wean is their reaction when you remove the lid from the cage. If they stay perfectly still, they're too young. If they poke around, they're old enough. Pups need to be weaned by the time they next litter is born in the cross, so that they new pups are not trampled and can feed. If pups are weaned too early, they will not survive, so it is pivotal that pups are weaned at an appropriate time. If pups are weaned late, they may be plugged by their father or brother or try to mate with a sister. This creates a mess when trying to track the background of the mice.

Weaning involved separating males from females into separate cages. Females of any age can be house together without difficulty. Males, however, must be housed only with other males of similar age who have never been mated. Housing males of separate ages together or housing any male with males that have bred will result in fighting and, often, death. However you can keep them from fighting by providing some hiding places (PVC pipe provided by DCM). The date of weaning should be noted both in the genotyping book and on the cage card next to each mouse's ID number.

Runted (or sickly) mice should be weaned late to ensure that they survive. Runted mice are often mice of the genotype we find most interesting and therefore the most valuable. Keeping runts in with their mother for an extra week or culling unneeded genotypes in a big litter can ensure their survival. Do not cull a litter to less than 3 or 4 mice, because the mother will stop lactating and the pups will starve to death. Similarly, if a mother has a small litter (>4 pups), they may need to be fostered. If a mother does stop lactating, move the pups in with a foster mother who has pups of similar ages. It is preferable that the pups have been toed and tailed before they're moved in with a foster mother, so we that we know the identity of their parents.

Tailing and Tagging

Always contact your local veterinarian and have all procedures approved, with training, before beginning any new procedure on a live animal!

Some labs perform ID/genotyping at day 10-14, but this should be approved with your local Animal Care Committee and receive specific training for manipulation of small mice.

Mice are tailed and tagged often at weaning (21 days old). One common method of ID is the ear tag, which looks like a flat, looped pierced earring with a number stamped into the metal. As a single procedure, tail tip amputation requires only brief anesthesia. Injectable agents such as Avertin (tribromoethanol) are commonly used, but the prolonged duration of anesthesia makes several alternatives more attractive. In particular, an inhalant agent such as isoflurane is safe in experienced hands and allows for a rapid recovery of the animal. When tailing, only a small sample is necessary

(~4mm or 1/4 inch). Cut the very tip of the tail and pick up the tail piece with clean forceps. Drop tail pieces into a labeled 0.5 mL eppendorf tube and touch mouse's tail to a paper towel to slow bleeding, then dip in styptic power. Return tagged & tailed mouse to cage. Forceps should be cleaned with Ethanol or Quatricide between litters or any time blood is observed. (Avoid Cross-contamination!!). If a tail sample is lost or misplaced among other cuttings, simply cut another piece of tail.

Every litter tailed and toed should be noted in your mouse book or database. List father's number sand genotype, mother's number and genotype, dated tailed and number of progeny.

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Tails should be stored at -20°C to avoid degradation of genomic DNA.

Prepping Tail DNA

After tails have been collected, the DNA must be extracted from them. Tails can be stored in a freezer for a few days until the DNA is extracted. Then DNA can be used right away, stored a few weeks at 4°C or stored longer at -20°C. Samples will lose DNA is freeze/thawed too many times. Here are 3 methods of DNA extraction, starting with the one that gives cleaner DNA to the dirtiest. Your PCR may be more or less finicky, and kits are also available for DNA extraction from tail samples. Proteinase K (20 mg/mL) can be stored, frozen in 1mL aliquots. Storage also OK in refrigerator for 3 months. Temperatures above 65c inactivate Proteinase K.

Method #1 –

100 mL Lysis Buffer:	5 mL	2M Tris pH 8.8
	1 mL	5M NaCl
	1 mL	500mM EDTA pH 8.0
	2 mL	10% SDS
	91mL	DNase-free MilliQ Water

For each tail sample, add 250 µL Lysis Buffer to 2.5uL Proteinase K (20 mg/mL). Incubate 55°C overnight. Spin tube briefly to collect hair to bottom. Transfer liquid (200 µL) to a new tube. Add an equal volume of isopropanol, invert, spin at full speed 10 minutes. Aspirate liquid carefully, let dry 10 minutes. Add 10mM Tris pH 8.0 and let sit 2 hours.

Method #2 –

10x Tail Digestion Buffer (10 mL)	3.35 mL	2M Tris pH 8.8
	1.66 mL	1M $(NH_4)_2SO_4$
	1.34 mL	500mM $MgCL_2$
	3.65 mL	DNase-free MilliQ Water

Add together fresh, 100 µl for each tail:

10 µL 10x Tail Digestion Buffer
1 µL Beta-mercaptoethanol (aka 2-ME or BME)
0.5 µL 10% TritonX-100
2 µL Proteinase K (20 mg/mL)
86.5µL DNase-free MilliQ Water

Incubate 55°C overnight. Heat Inactivate enzyme with 85°C - 95°C for 15min (hot block). Use 1-3 µL DNA per PCR.

Method #3 –

Prepare fresh 50mM NaOH each month. Weak NaOH is neutralized easily by exposure to air.

- Boil Tails at 95°C in 400 µL of 50mM NaOH for 10 minutes
- Add 40 µL of 1M Tris-HCl pH6.8
- Vortex tube for 5-10 seconds
- Spin at max speed for 6 minutes

- The swollen tail remains mostly intact throughout the procedure. Transfer 300 µL of liquid to new tube, Use 2-3 µL per PCR, store at -20°C

PCR Genotypes

Accurate genotyping is a pivotal part of research. If genotypes are unclear or seem incorrect, they must be repeated.

Prepare genotyping reactions on ice using barrier tips to prevent contamination from pipetman. Aliquot the genotyping mix into tubes and then add 1-5 µl tail sample individually (changing tips between samples). Completed genotyping PCRs should be stored at 4c until they are run on a 0.8%-1.0% agarose gel. Gels can be run as high as 120V, (bigger gels can run faster than smaller ones) allowing a gel to be done in 20-30 minutes. Photographing the genotypes is essential for keeping records. Make sure that all genotypes are clear on the photograph before throwing the gel into the disposal pail. Tails may be kept at -20°C once the genotypes have been recorded.

Contamination is a big issue whenever you're working with PCR. PCR is designed to amplify small amounts of DNA and if you contaminate a reagent, even only a little, it will affect your results. Contaminated genotyping reactions can set the lab back for months. Genotyping stock of primers, dNTPs, buffer, dH_2O, Taq, etc. should be kept separate from other PCR stocks to avoid contamination. Never stick a pipet tip into two different tubes of reagents and never reuse tubes for reagents. Using fresh tips and fresh tubes will ensure that genotyping PCRs do not crash.

A "no DNA" control is the most important sample you are running. Always include it as a negative control. Positive controls are also important. If possible, run a "no DNA" control every 10-12 samples and run a positive control for each gel if you have so many samples that you will need multiple gels.

Sacrificing Animals

Sacrificing (sac'ing) is often performed at the same time as weaning, but also becomes necessary at other times. Being stringent about keeping the colony small and sac'ing unneeded animals can save the lab $1000 of dollars. Animals are euthanized with CO_2 according to approved protocols.

Any animal over 6 months of age should be sac'd unless you are keeping it in a study, looking for tumors or other phenotypes. If a mating pair is over 6 months of age, but still producing litters, a new mating pair should be set up and allowed to raise its first litter. Once pups are 10 days old in the new mating pair, the old mating pair should be sac'd. This ensures that the influx of animals is kept constant, the lab is never left wanting for animals, and less useful matings are removed in a timely manner.

Any mating pair that has not produced or raised a litter in 2 months should be sac'd.

Any male that hasn't been mated within 3 months of birth should be sac'd.

Any animal of an unneeded genotype should be sac'd. The specific genotypes of animals that need to be saved is an ever-changing list. It is the duty of those in charge of the mouse room to remind mouse users that mice either need to be used promptly or sac'd.

References:

MIT DCM Lab Animal User's Handbook. Fourth Edition. 2006.

Manipulating the Mouse Embryo: A Laboratory Manual (Third Edition) By Andras Nagy, Marina Gertsenstein, Kristina Vintersten & Richard Behringer. © 2003 764 pp. (ISBN 0-87969-591-9) Available from Cold Spring Harbor Laboratory.