Aurora's Guide to Mouse Colony Management at MIT

-Aurora Burds Connor, Feb 2007

Maintaining a breeding colony of mice is one method of generating and then 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.

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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

Here is a brief summary of characteristics of commons strains and their breeding habits as observed in the MIT mouse rooms. Complete lists of all available wild-type strains with descriptions can be seen at these websites:
Taconic http://www.taconic.com/wmspage.cfm?parm1=579
Jackson Labs http://jaxmice.jax.org/list/cat481365.html
Charles River
http://www.criver.com/research_models_and_services/research_models/mice_a_b.html

Balb/c- Inbred strain. Albino, Passive, 8-14 pups per litter.

C57BL/6- Inbred strain. Non-agouti black. More aggressive (bite & jump) and more sensitive to noise and smells. Tend to be poor mothers (eat their pups). Average litter 6-8 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* pups
Breeding lifespan: 6 to 12 months
Lifespan: 1 to 3 years
Weaning: 21 days*

* = Values can vary with mouse stock or strain

Breeding Systems

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.

Cross Breeding - The mating of animals of different breeds or strains, Also called "hybrid cross."

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.

Line Breeding - In this system, the mating of animals by specific genotype or trait is performed. This is usually done to propagate mutant or transgenic lines, or because the trait is needed for research.

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.

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 different lines 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.

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.

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 rarely plug well and should not be used to establish a new mating pair unless you are desperate.

Females are often old enough to mate at 4-5 weeks of age. Be aware that if 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.

Mating Chimeras for Germline Transmission

ES cells containing your desired mutation are injected into blastocysts to generate chimeric mice. These mice are a mixture of cells differentiated from the injected ES cells and from the ES cells present in the recipient blastocyst. Most of the time, a researcher chooses to use recipient blastocysts whose ES cells will result in a fur/coat color that is a different color from the fur/coat of cells whose lineage is the altered, injected cells. In this way, the chimera's colors will often be patchy or striped. The percentage of the coat that is the color expected from the ES cells gives you an idea of what percentage of the mouse is made up of cells from your altered ES cells.

Ideally, you want your altered ES cells to have contributed to the production of sperm or eggs in your chimera so that you can build a colony of mice that contain your desired mutation. Here, again, coat color becomes useful in tracking. You can mate your chimera to wildtype mice whose coat color is recessive to the color produced by your ES cell, and then any offspring that have the ES cell color must have come from ES cell based gametes.

Here are the two most common situations at MIT:

1- Agouti (brown, from 129 background) ES cells are injected into black (C57B6) blastocysts. The chimeras are a mixture of black and brown. You mate chimeras to wildtype C57B6 mice. Some offspring are black. These are from the combination of a C57B6 gamete in the chimera with a C57B6 gamete in the mate. These mice are wildtype and will not have your desired alteration. Some of the offspring are brown. These are from the combination of a 129 ES cell-derived gamete in the chimera with a C57B6 gamete in the mate (the wt mate can only generate C57B6 gametes because it is not a mix). These brown offspring have 50% chance of getting the mutation you engineered into the ES cells. All brown pups should be tested for the desired alteration. This scheme works because the agouti color is dominant over black. You colony is now starting with a mixed background of 50% 129Sv and 50% C57B6. If you desire a pure C57B6 background, you will need to backcross 19 more times to C57B6 mates. If you desire a pure 129Sv background, you can breed a wt 129Sv mouse to the chimera who has proven itself to pass on your desired genetic alteration. All of the offspring will be agouti in color, and you will simply have to genotype everything to find the few who inherited the mutation.

2- Black (C57B6) ES cells are injected into white (Balb/c) blastocysts. This coat color combination is a bit more tricky. Balb/c skin releases an agouti pigment, which is masked and produces no color. However, when Balb/c skin cells are near C57B6 skin cells, the secreted agouti color is unmasked by the black pigment. So, a chimera that is a mix of C57B6 and Balb/c cells are often tri-colored with areas of white, areas of black, and areas of agouti in between the white and black. Do not be alarmed! These chimeras can be bred to Balb/c mates or to C57B6 mates. If bred to Balb/c, the desired offspring will be agouti in color (because each cell will contain one copy of the masked agouti color from the wt Balb/c gamete as well as the black, unmasking pigment from the altered, C57B6 gamete in the chimera). The wildtype offspring will be white. If the chimera is bred to a C57B6 mate, then the desired offspring is black (C57B6 gametes from the both the chimera and the mate) and the pups you do not want will be agouti (combination of Balb/c from the chimera and C57B6 from the mate). Breeding to a C57B6 mate means that your colony is starting off as 100%, pure background of C57B6, which can be very desirable for some experiments.

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, though 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, they will work their way out or dissolve, though I have observed plugs as late as 2 pm. Plugs are

sometimes spottable by eye, but usually require probing to detect small or deep plugs. We use capillary pipettes that have been rounded on end and stored in 70% Ethanol to check plugs. Some people prefer to use the FisherSci "seeker with bent end" # 08-995. Noon on the day a plug is discovered is considered embryonic day 0.5. (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.

Section 2 - From Birth-to-4 Weeks

Daily characteristics of newborns, Weaning

Characteristics of Mice from Birth to 4 Weeks of Age

	C57BL/ 6	129SV	Balb/c
Birth	Blood red skin color, pigment under eyelid	Same as B6	No eye pigment
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.	Similar 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.	May see lighter color than B6	
8 Days	Lower incisors visible, but not erupted.		
9 Days	Inguinal nipples visible in females Pups smaller than 129 or Balb pups	Pups larger than B6 pups	Pups larger than B6 pups
10 Days	Lower incisors erupted.	Clearly brown, not black fur	
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.		9g-13g
4 Weeks	Weight=12g-16g Round palpebral opening, smooth fur, trapezoidal shape to head.		12g-20g

weight information from http://jaxmice.jax.org/info/weight/

Weaning

Pups are weaned at three weeks (21+ days) 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, run around or jump about, they're old enough. Pups need to be weaned by the time the 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, new cages that have some food pellets placed on the floor of the cage for easy access. Females of any age can be house together without difficulty. Males, however, must be housed only with other males that are also being weaned that week. Housing males of separate ages together or housing any male with males that have bred will result in fighting and, often, death. If males do fight, you can reduce this by providing some hiding places (PVC pipe provided by DCM). The date of birth and date of weaning should be noted on the cage card.

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 that you know the identity of their parents.

Section 3 - Genotyping

Tagging and Tailing, Prepping Tail DNA, PCR Genotyping, Sacrificing Animals

Tailing and Tagging

Always prepare long in advance by checking which method of mouse ID is approved under your CAC protocol and make sure you are trained by DCM staff or by an experienced member of your lab before beginning any new procedure on a live animal!

Methods for IDing mice at MIT include the ear tag, ear punching, ear clipping, tail tattoo, toe tattoo and toe clipping. Any method you use must be included in your CAC protocol for approval. Toe clipping requires scientific justification for CAC approval. If you would like more information about any of these ID methods, please contact Jen Statile Kilpatrick at jstatile@mit.edu

Some labs perform ID/genotyping at day 8-14, but this should be approved with your 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) was 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. DCM has anesthesia/isofluorane machines available for this reason, and DCM training is required for their use. 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 piece into a labeled 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 mouse tailed and ID'd should be noted in your mouse book or database.

Tails should be stored at -20 ^{o}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 (or even weeks) until the DNA is extracted. Extracted DNA can be used right away, stored a few weeks at 4c or stored longer at -20c. The DNA will degrade somewhat if frozen/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.

Many MIT labs have found the genotyping services of DCM (contact Nate Rogers, nrogers@mit.edu) or Transnetyx (www.transnetyx.com) to be good options for outsourcing tail prep and genotyping tasks.

For the cleanest DNA, DCM and I both have found the Roche "High Pure PCR Template Preparation Kit" cat# 1796828 to be excellent. We modify the tail lysis buffer reaction a bit to dissolve the entire tail rather than needing to pulverize each tail manually for 1 minute: use 190uL Tissue-Lysis Buffer (in the kit)
40uL Proteinase K (in the kit)
5uL 10x Tail Digestion Buffer (see Method #2 for recipe)
5uL 10% TritonX-100
Follow all other directions included in the kit.

Proteinase K can be stored, frozen in 1mL aliquots. Storage also OK in refrigerator for 2-3 months. Temperatures at or above 65^{o}C inactivate Proteinase K.

Method #1 –

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

For each tail sample, add 250uL Lysis Buffer to 2.5uL Proteinase K (20mg/mL). Incubate 55 ^{o}C overnight. Spin tube briefly to collect hair to bottom. Transfer liquid (200uL) 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 (10mL)	3.35mL	2M Tris pH 8.8
	1.66mL	1M $(NH_4)_2SO_4$
	1.34mL	500mM MgCL2
	3.65mL	DNase-free MilliQ Water

Add together fresh, 100ul for each tail:

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

Incubate 55 ^{o}C overnight. Heat Inactivate enzyme with 85c-95c for 15min (hot block). Use 1-3uL DNA per PCR.

Method #3 –

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

- Boil Tails at 95 ^{o}C in 400uL of 50mM NaOH for 10 minutes
- Add 40uL of 1M Tris-HCl pH 6.8
- Vortex tube for 5-10 seconds
- Spin at max speed for 6 minutes
- The swollen tail remains mostly intact throughout the procedure. Transfer 300uL of liquid to new tube, trash the tube with the tail.
- Use 2-3uL per PCR, store at -20 ^{o}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 4 ^{o}C until they are run on a 0.7%-1.0% agarose gel. Gels can be run as high as 120V, (bigger gels can run at higher voltage 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. Tail DNA may be kept at -20 ^{o}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 stocks of primers, dNTPs, buffer, dH2O, 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.

Sacrificing Animals

Sacrificing/euthanizing of mice is often performed at the same time as weaning, but also becomes necessary at other times. Being stringent about keeping the colony small and sacrificing unnecessary animals can save the lab $1000s of dollars. Animals are euthanized with CO2 and confirmed by physical means according to approved DCM protocols.

General rules of thumb for keeping your colony manageable:

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 productive 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 without a desired 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.

Section 4 –More Info

References, Software, Links, and Training

References

MIT DCM Lab Animal User's Handbook. Fourth Edition. 2006. https://web.mit.edu/comp-med/Restrict/CAC/LAUH_link.html (requires MIT Certificates)

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.

Mouse Phenotypes: A Handbook of Mutation Analysis by Virginia E. Papaioannou and Richard R. Behringer (Spiral-bound - Nov 2004)

Software

The Jackson Laboratory's Colony Management System (JAX-CMS) is a multi-user relational database management system for managing animal colonies in a research environment. JAX-CMS is available with documentation to the academic scientific research community at no charge. http://www.jax.org/jcms/index.html

Links

UCI guidelines for Efficient Mouse Colony Management
www.memory.uci.edu/~tjf/**Colony**Guidelines.doc

Laboratory Animal Medicine and Science - Series II RATS AND MICE:
https://web.mit.edu/comp-med/Restrict/anr/Labanimal/data/ratsmice/9042/index.html (MIT Certificates Required) This is somewhat like a slide show that covers all you ever wondered about rat and mouse housing (cages/bedding/food/water), facilities (air/light/sanitation), handling (different hand holds and restrain devices), transportation (shipping to/from other facilities/quarantine/health screening), breeding, and euthanasia. If you click on "INDEX" at the top of the blue box on the left, then you see the other programs available on the site. They are also good, but this is most specific to colony management.

Training

DCM offers training for all mouse procedures at MIT (from mouse handling and determining sex to using biohazards, anesthesia and surgery). Descriptions of required training as well as additional training and training videos for download or loan are available. https://web.mit.edu/comp-med/Restrict/CAC/training_new.htm (MIT Certificates Required)

Wokshops and Courses at Jackson Labs
http://www.jax.org/courses/events/current.do

Good Luck!