Campbell Lab Rodent Breeding / House Protocol

**How do I handle mice?**

Believe it or not, most strains of mice are quite docile, and will not bite unless provoked. If the mice become upset, do something else and return to them to their respective until they have calmed down.

**Transferring mice from cage to cage:**

Gently pick up the mouse by the tail with a gloved hand. Grabbing the mouse by the base of the tail will give you greater control (and prevent tail from ripping). Relax and handle the mice gently. In most circumstances, if you are relaxed, if the mouse makes an attempt to bite you, it will be a gentle nibble that will not hurt.

**Handling mice for marking / injections and checking genital plugs, inspecting mice:**

Complete control of the mouse is required for marking, injections and gavage. Place the mouse on top of the cage bars, with the bars running left-right. Grab the tail with your right hand and then scruff the mouse with your left hand. Scruffing the mouse properly is the key to ‘success’. You want to immobilize the head of the mouse, and to accomplish this, all loose skin around the back of the neck must be scruffed. To get all this skin in your grip, start your scruffing motion low on the shoulders of the mouse, thumb on one side, and index finger on the other. The skin on the back of the mouse can be trapped between the remaining fingers and palm. For injections, the body of the mouse needs to be immobilized as well: stretch the body of the mouse by gently pulling the tail with your right hand and hook the little finger of your left hand over the tail. If you are not constrained to work on the mice at a particular time of day, you may find it easier to work on the mice in the morning to early afternoon. Mice are at their most active just before the lights go out and are often more difficult to handle late in the day. (This is a small thing we noticed along the way, may just be superstition)

**What are the acceptable effective ways to mark/ID mice? (Assuming you have IUCUC approval)**

Ear punching can be done without anesthesia (but is recommending using a small amount of isoflurane exposure). Ear punches is the most recommended method (link below to purchase) but can become difficult to read after several weeks because of healing and if the punch tears from fighting. However, we still recommend using ear punches for the first several weeks of life but if the study requires the mice to live longer then we recommend tattooing or ear tags (although these commonly fall out or rip off). As mentioned, Tattooing is an acceptable alternative, although it is less commonly used (link below to purchase). For all types of marking, we recommend using brief exposure to isoflurane to sedate the mouse and perform the marking prior to their return to consciousness.

For equipment, we recommend:

Ear punch - [Fisher: Ear Punch NC0264349](https://www.fishersci.com/shop/products/ear-punch-punch-diameter/NC0264349#?keyword=Ear%20Punch)

Ear Tag - [Fisher: Ear Tag Applier NC0038715](https://www.fishersci.com/shop/products/ear-tag-applicator-ss-each/NC0038715#?keyword=ear%20tag)

Tattoo - [Fisher: ATS-3 Tattoo System 14-370-133](https://www.fishersci.com/shop/products/aims-ats-3-general-rodent-tattoo-system-large-lab-animal-upgrade-package/14370133)

**How do I genotype the mice?**

An efficient way to manage your mice is to ear punch and genotype them 2 days prior weaning them but after 14 days of life. Genotyping by PCR is the most efficient. Ideally, the PCR primers are specific to the mutation. Please double check these primers with company you purchased them from and their recommended protocols. The extensive protocol used for the R403Q mutant strain can be found on LabArchives under Campbell Lab->Protocols->Genotyping. For genotyping, you must obtain tail snips (cut a 2-4 mm section from tip of tail); it is ideal to do this when you also perform the ear punch as the mouse will then only have to undergo isoflurane exposure once. We also recommend applying Kwik-Stop to stop any potential bleeding from the tail.

To stop tailing bleeding, we recommend [Kwik-Stop](https://www.amazon.com/Laboratories-Kwik-Stop-Styptic-Powder-Birds/dp/B000093HIQ/ref=pd_lpo_199_t_2/136-6733138-6875262?_encoding=UTF8&pd_rd_i=B000093HIQ&pd_rd_r=64d37a57-a555-4fbc-a2c6-a35711bdd65c&pd_rd_w=KZqRl&pd_rd_wg=oY3ID&pf_rd_p=3b5203d9-bdd0-47f6-97e5-387010fc3251&pf_rd_r=16X4BGRN91YMZ35J4JJD&psc=1&refRID=16X4BGRN91YMZ35J4JJD)

**How do I tell males from females?**

The distance between the external genitalia and the anus is greater in males than in females at all postnatal stages. Additionally, after about two weeks of age, the nipples of females are typically visible, whereas the nipples of males are not. It is easiest to sex newborn mice if the genital region of the mouse is fully extended: pick the mice up and gently bend the lower back slightly to stretch the genital region.

**How do I mate mice?**

If you are not in a rush to produce a lot of offspring, house a male mouse with one or two female mice. The mice can be left together until the pups are ready to be weaned if the cage doesn't get too crowded. If you need mice of predominantly of one sex, you can remove the unwanted sex a few days after birth (don't disturb the moms during the first 24 hours after birth). The remaining pups will grow faster. However, you should be aware that females are better mothers if they have at least 3 pups to care for, so don't discard too severely. If you need to expand a strain quickly, you can mate females in heat with the males every day and check plugs the next morning. House females of similar plug dates together through to weaning of the pups. For many strains, two pregnant females and their litters can be housed together until weaning. The IACUC guidelines for mice with litters limit the number of mice to 2 adults and no more than 20 pups. (check ‘**genetic background**’ for more info)

* DO NOT house breeder males with other males (male breeders should be singly housed with enrichment)
* DO NOT breed mice in the cage that contains multiple males
* We highly recommend mating moving the singly house male in the female cage for most optimal breeding
* We recommend giving the female 1-2 weeks rest after her pups have been weaned
* After birth, we recommend keep the sire or another non pregnant female in the cage until weaning
* A cool trick to time out your breeding is 1-2 days prior to breeding, move breeder male beading into the female’s cage stimulate/restart the estrus cycle. (Preparing them for breeding)

**How long is gestation?**

Gestation is 18 to 20 days, depending on the strain.

**How can I prevent mothers from cannibalizing their litters?**

Mice are social and care better for their young when they are housed with friends. House females continuously with the sire or house a pregnant female with a non-pregnant female. However, do not add mice to a cage just a few days before birth, as this will disturb them. First time mothers and very young females are less likely to raise a litter successfully than experienced mothers and more mature females. Mothers and their litters should not be disturbed the first day after birth. By the second day, mothers should have acquired full maternal behavior and will tolerate disruptions better. In difficult situations, you can foster the pups to a more maternal strain or cohouse a pregnant mouse of a maternal strain at the same or more advanced stage of pregnancy together with your problematic mom.

**When should mice be weaned?**

Mice should be weaned between 21 to 28 days after birth. Pups must be weaned if the same mom gives birth to a second litter. The pups should be robust, active, have open eyes, teeth and adult fur rather than the thinner fur of babies. They need to be able to jump up to the top of the cage to feed and drink. If they are too immature, let them go longer with their mom. In many strains, pups ready to wean will jump like "popcorn" when the cage lid is opened. We recommend added some food to the bottom of the cage and dripping some water on it to soften the food to help them through the first day or two post weaning.

**When do mice become sexually mature?**

Female mice become sexually mature at 6 weeks after birth and males at 8 weeks.

**What are the acceptable methods of euthanasia?**

Mice are narcotized by CO2 inhalation and then euthanized by cervical dislocation. Let the gas flow for 1 minute to fill the chamber and leave the chamber closed for 5 minutes. The narcotization of mice is rapid, so do not leave the chamber unattended. Death should be ensured by cervical dislocation. An alternative method of euthanasia is to anesthetize the mice with isoflurane before cervical dislocation. In a chemical fume hood (i.e. an explosion-proof hood vented to the outside), put a cap-full of isoflurane on tissues in the bottom of a small chamber, place the mouse inside and close the chamber. When the mouse is immobile, open the chamber and perform cervical dislocation. Cervical dislocation is performed by picking up the mouse by the base of the tail. Then grip the base of the skull firmly between thumb and index finger and pull the base of the tail away from the head until you feel the cervical vertebrae “pop”. 

**How are males and females housed? Don't the male’s fight?**

Females can be housed five to a cage and can be mixed with unfamiliar females without problems. Special attention must be paid to the housing of males because of their propensity for fighting. Males will generally not fight if they are housed together from before sexual maturity through to old age. After sexual maturity, males will fight when introduced to a new male. For example, males that have been housed alone will fight with any introduced male. Therefore, stock males from the same litter should be housed together from a young age to conserve space. Males that are used as breeder males are housed one per cage and are never placed into a cage with other males.

**What is a plug?**

Plugs are useful for obtaining timed matings. A plug is hardened semen blocking of the female vagina, and remain in place for about 12 hours after mating. Plugs are detected by visual inspection much how you would inspect for gender (above). Mating is assumed to occur at the midpoint of the dark cycle (midnight under a 12 hour on/off cycle starting at 6), and thus noon of the next day is 0.5 days of gestation.

**How do I tell if a mouse is in estrus (in heat)?**

Female mice in estrus will be receptive to mating. By picking females in estrus, you can maximize the breeding of your mice, or obtain multiple females mated at the same time. You should expect two-thirds to three-quarters of mice in estrus to mate, on average. Females in estrus have swelling of the lip of the vulva closest to the anus. Pick up the female by the base of the tail with thumb and index finger holding the tail, let the mouse grab the cage bar with forepaws and gently press down with the other fingers on the lower back and sacrum to tilt the genital-anal region up. In estrus, the vulva is swollen, but the vagina does not gape open. The estrus cycle is 4 to 6 days, so about 1 in 5 females on average should be in estrus at any time if the females are cycling randomly. However, females housed continuously together can cycle together, or can exit the estrus cycle. Young females (6 to 8 weeks) are less likely to have stopped cycling. Exposure to males will restart the cycle. Transfer of bedding from a sexually mature male's cage can be used to stimulate/restart the estrus cycle.

**My mice are not breeding. What can be done to promote reproduction?**

Mice breed best if they are less than eight months old, so keep track of the age of your mice. The amount of fat in the diet can have a significant effect on female fertility (more fat, more fertile), but increased fat can have a detrimental effect on sire performance. Sudden noises can have a detrimental effect on breeding, as can poor air quality. The light-dark cycle has significant effects on mouse reproduction. Ensure that your mice are on the appropriate cycle (12 hours light, 12 hours dark). In some cases, extending the light period (14 hours light and 10 hours dark) can improve reproductive success.

**Should I be concerned about the genetic background of my mutant mice?**

Genetic background can have a significant effect on mutant phenotype. For many mutants, you will want to have your mutation on a well characterized, robust, common inbred strain like C57Bl/6 or the 129S6. After your first generation (F1) of mice have been produced, you should breed a F1 mouse back to your original breeder mice (F0). This will “rescue” your strain and prevent mutations. You should only breed F0 to F0 or F1 to F0, if possible.

**How large a colony of mice should I maintain?**

The size of your mouse colony depends upon your needs. Given the costs of keeping mice, you should keep your colony as small as is practical. For strains that you are not currently using, a small breeding colony of several cages is sufficient (letting it get down to one cage is living on the edge and we don’t recommend this). Mice that don't breed are a ‘dead end’, so make sure that if you have reduced your holdings of a strain to a minimum, that the mice are fertile and young. Schedule breeding of replacement breeders so that breeders may be replaced when they are 6 to 8 months of age. The structure of a breeding colony will depend on your needs. For those who need timed mating, a set of sire males individually housed, and cages of non-pregnant females housed five to a cage are essential. For maintaining stocks by breeding, breeding pairs (male and female and litter) are typically part of the colony as well.

**How extensive should my breeding records be? What should I track?**

Your particular needs will determine the level of detail that you will need in your breeding records. With large colonies, detailed record keeping can consume significant resources. However, detailed records are essential to solving problems when they arise. Records can be kept in a combination of lab notebooks and cage cards, in excel docs, or in online databases (the Campbell lab uses [SoftMouse](https://www.softmouse.net/) and [RedCap](https://www.project-redcap.org/))

**What is SoftMouse?**

SoftMouse is a online database that allows you to sort cages by mousseline/groups, view billing costs based on the number of days cages are active, pool females together, and ensure that no cages are over-crowded or not in use. View each male and female breeding/cage history. All your data is inputted accurately and kept securely in one place for each lab member or PI to see, thus making it easier to publish your data and write reports. It allows you to input data collected for upwards of 50 mice at a time (very quickly too). You can also make time-sensitive decisions by viewing the desired data when you need and hiding parameters that are considered clutter. Your data is searchable, and you can keep historical data accessible when members leave the lab, as well as help new members get up to speed quickly on good colony maintenance practices. Detailed breeding records are crucial to evaluate the breeding performance of a colony. Allows you to always know who each pups DOB, Sire, Dam, and other crucial information.

Things you can store into SoftMouse include:

Physical Tag (Ear punch, Tattoo, Ear tag), Mouse SID (auto generated SoftMouse mouse ID), Sex, Date of Birth, Age, Strain, Mouseline, Offspring Generation, Genotype, Cage SID (auto generated SoftMouse Cage ID), Cage Number, Protocol, Litter SID (auto generated SoftMouse Litter ID), Sire and Dam information (SID, Tag, Mouseline, Genotype, DOB, Age, Generation, and Diet), Total litters, Coat Color, Tail / Tag Date, Wean Date, and Calendar Events.