**Campbell Lab Animals Info Page**

**Training**

**Breeding and Animal Handling**

Here we have a typed out protocol for Housing and animal breeding. This follows the guideline of DLAR. Also, the diet that DLAR uses for the mice can be found under this folder. Handling the mice properly is extremely vital. 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. So, when transferring or moving the mice, 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.

**Mouse Strains**

We have used the 129S6 strain as out background stain.

**R403Q Mouseline**

The R403Q mutation consists of a point mutation (arginine replaced with glutamine) at position 403 on the actin binding pocket of the myosin heavy chain molecule. This model consisted of transgenic mice that contained the R403Q point mutation with an additional amino acid deletion and insertion of non-myosin amino acids in the myosin heavy chain. This model was constructed to produce a mutant myosin that would have a strongly altered interaction with actin. The development of this model was based on the hypothesis that genetically linked hypertrophic cardiomyopathy is a disease of the sarcomere, whereby alterations in cross-bridge interactions can lead to further downstream effects that ultimately generate a hypertrophic phenotype. Only a small percent of hypertrophic cardiomyopathy contained the mutation (1-12%) but a similar histopathology to human patients is observed. By 12-14 weeks common pathologic features are apparent including hypertrophied cells, myocellular disarray and LV hypertrophy.

Another mouse model was developed that included only the R403Q point mutation. Mice homozygous for the mutation died at day 7; however heterozygous mice survived for 1 year. After ~15 weeks of age, the R403Q mice show similar pathology to R403Q human patients including myocyte disarray and interstitial fibrosis. In addition, these pathologies increased with age. However, the major difference in this model was the presentation of left atrial enlargement rather than LV hypertrophy observed in humans.

**Record Keeping for Mice**

Keeping breeding records is vital and 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 dos, or in online databases (The Campbell lab uses SoftMouse and RedCap)

**Genotyping**

The Campbell Lab has a Genotyping Protocol that was made originally for the R403Q mouse line but can be modified for other lines.

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" \o "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" \t "_blank)

**Genotyping Explained - R403Q**

Left side: **PCR Reaction only**

Right side:**PCR Reaction + AvaI Restriction Enzyme Digestion**

Samples 1 & 2 are loading control mice (Null)

Lanes 3 & 4 are R403Q Heterozygous mice of the 129S6 strain

Lanes 5,6,7, & 8 are Null mice of the 129S6 strain

**PCR: what’s happening?**

* Green GoTaq® Master Mix: amplifies target DNA that the primers attach to
* F. Primer: amplifying Exon 13 with specificity to 5'-GCTGGACAAAGGAATGGAGGTA-3'
* R. Primer: amplifying Exon 13 with specificity to 5'-CTGATGGTCTGAGTGGGTAGGTGAG-3'
* The forward primer attaches to the start codon of the template DNA (the anti-sense strand), while the reverse primer attaches to the stop codon of the complementary strand of DNA (the sense strand). The 5' ends of both primers bind to the 3' end of each DNA strand. In this case it is targeting exon 13 of the mouse a cardiac MHC gene. In between this start codon of the template DNA and the stop codon of the complementary strand of DNA is the AvaI binding site. The entire stand is 380 bp

**AvaI Restriction Enzyme Digestion: what’s happening?**

* AvaI Restriction Enzyme: is a restriction enzyme that targets/cleaves at the AvaI site.
* CutSmart Buffer: has over 215 restriction enzymes are 100% active
* Incubate at 37°C for 5 hours: to allow restriction enzymes to cleave DNA sequence (see below)
* 80°C inactivation step for 20 minutes: inactivates the AvaI restriction endonuclease

AvaI targets the C/YCGRG sequence that is located ~260/120 bp

5’… CÚ**Y**CG**R** G …3’

3’… C **R**CG**Y**ÙG …5’

**R** = A or G (purine) - **Y** = C or T (pyrimidine)

The R403Q heterozygous mouse lacks the AvaI site on one of its MYH6 alleles but contains it on the other. This explains why the heterozygous mouse has an non-cleaved band (380bp) and the set of two bands (260/120 bp). While the Null mice control un-mutated contains only the set of two bands (260/120 bp) as both of their MYH6 alleles contain the AvaI site.

**Analysis of R403Q mouse line:**

Exon 13 of mouse DNA was amplified, digested with Ava I, and fractionated on an agarose gel. (R403Q Heterozygous mice contain one mutated (lacking) and one normal allele of the Ava I site)

**Further Notes:**

The final concentration of glycerol in any reaction should be less than 5% to minimize the possibility of **star activity**. For example, in a 50 µl reaction, the total amount of enzyme added should not exceed 5 µl. Under non-standard reaction conditions, some restriction enzymes are capable of cleaving sequences which are similar, but not identical, to their defined recognition sequence. This altered specificity has been termed “**star activity**". It has been suggested that star activity is a general property of restriction endonucleases and that any restriction endonuclease will cleave noncanonical sites under certain extreme conditions

By definition, 1 unit of restriction enzyme will completely digest 1 μg of substrate DNA in a 50 μl reaction in 60 minutes. This enzyme : DNA : reaction volume ratio can be used as a guide when designing reactions. However, most researchers follow the "typical" reaction conditions listed, where a 5–10 fold over digestion is recommended to overcome variability in DNA source, quantity and purity. NEB offers the following tips to help you to achieve maximal success in your restriction endonuclease reactions.

**AvaI Restriction Enzyme:**

* AvaI is purified from a recombinant source.
* Cleavage with AvaI restriction enzyme may be blocked or impaired when the substrate DNA is methylated by CpG methylase.
* AvaI enzyme will digest unit substrate in 5-15 minutes under recommended reaction conditions, and can also be used safely in overnight digestions.
* AvaI Targets sequence: C/YCGRG
* AvaI has 100% activity in rCutSmart NE Buffer
* AvaI has a heat incubation of 37°C for 5 hours
* AvaI has a heat inactivation: 80°C for 20 minutes

**Methods for Tagging Mice**

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)

**Sexing Mice**

How do I tell males from females... well 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.

**Mouse Mating**

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)

**SoftMouse**

SoftMouse is an 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.

**Exercise Testing**

The Campbell has yet to do any exercise test but from protocols have been uploaded that include;

[Maximal Work Test](https://teams.microsoft.com/l/file/97E982BD-6670-494F-AD8F-E99DEED8368D?tenantId=2b30530b-69b6-4457-b818-481cb53d42ae&fileType=docx&objectUrl=https%3A%2F%2Fluky.sharepoint.com%2Fsites%2FCampbellLab%2FShared%20Documents%2FAnimals%2FExercise%20Testing%2FMaximal%20Work%20Test%20protocols.docx&baseUrl=https%3A%2F%2Fluky.sharepoint.com%2Fsites%2FCampbellLab&serviceName=teams&threadId=19:cf0b3a02d0f342f7ab42ede27bd05783@thread.skype&groupId=4e4675c3-ea35-4036-9b4c-2ace772cc6af)

[Setting Up Mouse Wheels](https://teams.microsoft.com/l/file/602AA6D7-AABC-41BF-AB53-B3E1917DCE7A?tenantId=2b30530b-69b6-4457-b818-481cb53d42ae&fileType=docx&objectUrl=https%3A%2F%2Fluky.sharepoint.com%2Fsites%2FCampbellLab%2FShared%20Documents%2FAnimals%2FExercise%20Testing%2FMouse%20Wheel%20Set%20up%20guide.docx&baseUrl=https%3A%2F%2Fluky.sharepoint.com%2Fsites%2FCampbellLab&serviceName=teams&threadId=19:cf0b3a02d0f342f7ab42ede27bd05783@thread.skype&groupId=4e4675c3-ea35-4036-9b4c-2ace772cc6af)