Working with Mice in the Laboratory:

A Mouse Handbook

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Table of Contents

I. Gaining Access to the Animal Facility

1. Obtain a unique BSD ID. This can be done at https://duke.bsd.uchicago.edu/uniqueid/

Steps 2-7 can be completed in any order.

2. Your principal investigator must add you to the existing Animal Care and Use Protocol (ACUP).

3. Attend General Orientation to Animal Use. These sessions are offered the first Tuesday and the third Thursday of every month in M-137 at 1:00pm. A calendar is available at http://www.localendar.com/public/uofcarc

4. Enroll in the Occupational Health and Safety program. More detailed instructions can be found at http://ors.bsd.uchicago.edu/ohs/
   Complete the quiz and submit to ORS, 5751 Woodlawn Ave., McGiffert Hall, 2nd Floor, Room 214 (or fax 4-0659)

5. Enroll in the Barrier Facility Orientation Course and complete the quiz. More detailed instructions can be found at http://arc.bsd.uchicago.edu/ARC_website/training/barrier_facility_directions.html

6. Complete the Health Questionnaire and return to UCOM in L-156. The link can be found at http://arc.bsd.uchicago.edu/ARC_website/facilities/facility_access.html

7. Schedule and attend a facility tour. Contact:

Facility	Phone	Pager
Carlson Barriers / Biosafety	4-3487	188-5139
General Carlson / Isolation	4-1812	188-7998
FMI / CLSC	2-3169	188-8559
CIS and Breeding facility	4-3553	188-3233

8. Complete a Request for Access to Animal Facility form and return to P110. The form can be found at http://arc.bsd.uchicago.edu/ARC_website/assets/pdf/forms/Fmadm022access.pdf

II. Entering and Exiting the GCIS Mouse Facility

Entering the Facility, Suite, and Preparing the Hood

1. Swipe your Chicago card (University of Chicago ID) through the reader to enter the mouse facility.

2. Enter the garbing area through the locker room.

3. Put on a hairnet, face mask, disposable lab coat, and a pair of shoe covers. The shoe covers should be put on as you step over the black and white line.

4. Swipe Chicago card to enter the suite.

5. Put on gloves before entering the mouse room.

6. *Special Quarantine Procedure*
   Put on a second pair of shoe covers when stepping into the mouse room.

7. Put on a pair of sleeves, which can be found next to the door.

Exiting the Suite and GCIS Facility

1. *Special Quarantine Procedure*
   Remove hair net, face mask, gloves, sleeves, disposable lab coat, and the outer pair of shoe covers when exiting the room.
   Outside the suite, put on a new hair net, face mask, and disposable lab coat.

2. Proceed to the garbing area. Remove shoe covers as you step over the black and white line. Take off hair net, face mask, and disposable lab coat.

3. Exit facility though the locker room.

III. Navigating the Mouse Room

Using the Hood

1. Spray the hood you will be working in with Clidox, then wipe dry.

2. Spray the hood with ethanol to react with any remaining Clidox, and wipe dry.

3. Turn on blower and lights on the hood.

4. When done using the hood, turn off the blower and lights on the hood and repeat steps 1&2.

Placing and Removing a Cage in the Rack

1. To remove a cage from the rack, pull the side lever, revealing the red dot.

2. Pull the cage straight out of the rack.

3. To place a cage in the rack, first make sure a water valve is installed.
    a. To install a water valve, push back metal and insert the valve.
    b. Test to make sure water comes out of the valve.
    c. Insert the cage into the spot on the rack and push until the cage clicks in and the red dot is no longer visible. If the red dot is visible, remove the cage and insert it again.

4. When inserting the cage, make sure no mice are around the water inlet because it is possible they could get stuck when the cage clicks in.

Cage Cards and Overlays to be Familiar With

Cards Used by Investigator

- Standard Cage Card (white): required on every cage in the mouse room; contains mouse tag numbers, strains, male or female, date of birth, as well as contact information
- Temporary Cage Card (white): to be used if there are no pre-printed standard cage cards available- fill out and drop of the copy in the facility drop box; contains all the information on a standard cage card, as a standard cage card will be generated from it
- Breeding Overlay (blue): indicates a breeding cage; each litter is recorded on the breeding cage card
- New Cage (darker hot pink): indicates a cage put in the rack by ARC staff; usually a cage released from quarantine or received from an approved vendor
- Post-Surgery (light green): indicates a cage with animals that are recovering from a procedure
- Requesting Bactrim (light blue: indicates a cage where the animals are to receive bactrim in their water, an antibiotic given to mice with weak immune systems
- Transfer (yellow): designates a cage to be transferred to another facility or mouse suite; place on the cage as soon as the Transfer Request is submitted
  *Transfers occur on Tuesdays and Thursdays before noon

Cards Used by ARC Staff

- Impending Overcrowding (orange): the pups in the cage must be separated within the next week (day 21-28) or the ARC staff will separate the cage on day 29
  *Investigators are charged every time the ARC staff separate a cage
- 24 Hour Overcrowding Notice (hot pink): pups must be separated within 24 hours or the ARC staff will separate the cage
  *Overcrowded Cage: more than 5 adult mice in one cage, more than 2 adults in the same cage as a litter of pups, and more than one litter of pups in the same cage; adult mice are older than 28 days
- Animal Health Problem (dark green): indicates that an animal in the cage has a health problem; the health problem is written on the card

Forms to be Familiar With

All forms can be found in the GCIS facility near the dropbox, in the ARC office (P110) or online at http://arc.bsd.uchicago.edu/ARC_website/forms/forms.html

1. Animal Euthanasia Request: use when requesting cages of mice be euthanized

2. Cage Card Request: use to request more cage cards.

3. Transfer of Ownership and Transport Form: use when a mouse is transferred to another PI or moved to another facility.

4. Cage Card Activation and Deactivation Form: use to deactivate a cage card when there are no mice left in a cage

5. Approved Vendor Form: used to order mice from approved vendors

6. Non-approved Vendor Form: used to order mice from a non-approved vendor; the purchased mice go into quarantine

7. Animal Bite/Scratch Report: used to report an animal bite or scratch

8. Special Services Request Form: used to alter the standard animal conditions, like diet, water, etc.

IV. Setting Up Cages

New Cage

1. Gather the necessary components: cage, wire food holder, food (in white garbage can), cage lid, cage card holder, and a new cage card. Gel food may also be necessary if weaning pups before day 21.

2. Fill wire food holder with food.

3. Place mice in the cage.

4. Place wire food holder on top of the cage.

5. Fill in all the necessary information: mouse tag numbers, sex of mice, date/s of birth, strain, genotype, and for newly weaned mice, the parents' tag numbers, and place in the cage card holder. *Record the information from the cage card in order to add the information to the mouse list in the lab*

6. Place the cage card holder on the edge of the cage.

7. Place lid on the cage.

Breeding Cage

1. Steps 1-7 above (setting up a new cage) all apply with a few additional steps.

2. Place a nestlet (cotton square) in the cage.

3. Record the date the breeding cage was set up and the animal tag numbers on a Breeding Cage overlay, and place it in the cage card holder.

V. Working with Mice

Picking Up Mice

1. Pick up a mouse by its tail.

2. Put the mouse down on the metal grating in the front of the hood, so the mouse can grab on to the grating.

3. Grab the mouse's neck, with fingers touching the shoulder blades.

4. Grab the skin on the mouse's back between your thumb and index finger. The mouse should have very limited movement of the head and front legs.

Determining the Sex of Mice

Male: the genital area (penis) is farther away from the anus than in females; there is a clear separation between the anus and penis

Female: the genital area (vagina) is closer to the anus than in males, usually about ¼ inch away; there is a line running from the vagina to the anus; has nipples but may be covered by fur

Tagging and Tailing Mice

Tagging and tailing of mouse pups should be done at 3 weeks of age.

1. Get all materials (including mice) and bring them to the hood. The tags and tagger are in a white box in a plastic container above the bags with new cages.

2. Cover the metal grating at the front of the hood with a few pieces of paper towel to prevent the tags or snipped tails from falling through the grating.

3. Take the lowest number tag and put it in the tagger. The tag should be positioned so that the opening in the tag is aligned with the indentation on the tagger.

4. Pick up a mouse and position tag inside the ear, as close as possible to the ear cartilage. Even number tags should go on the right ear, and odd number tags on the left ear.

5. Squeeze tagger until the tag is locked and release.

6. Still holding onto the mouse, use the scissors to cut off about 0.5 cm of the mouse's tail to use for genotyping.

7. Place the tail in the appropriate PCR plate well using the tweezers.

8. Return mouse to cage.

9. Record new tag numbers, parent tag numbers, and cage card number to add to the mouse list in the lab.

Weaning Pups (Separating form Parents)

Pups should be weaned between day 21 and 28. Depending on the results from the genotyping, not all the mice will be kept.

1. Males and females should be placed in separate cages. *Female mice from different litters can be placed in the same cage, however, male mice CANNOT*

2. Follow the directions for setting up a new cage.

3. If weaning pups before day 21, place a food gel in the cage.

4. Record all new cage information to add to the mouse list in the lab.

Euthanizing Animals

1. Place all animals to be euthanized in a cage. There can be more than the usually allowed number of mice in the cage, i.e. it can be an overcrowded cage.

2. Turn the cage card over and write SAC and the date on it.

3. Return the cage to the rack.

4. Fill out an Animal Euthanasia Request Form and drop it off in the facility drop box or fax it to the ARC.

5. If the animal must be euthanized immediately, 2 forms of euthanasia must be used.
    a. The carbon dioxide chamber must be used.
    b. A second form of euthanasia can be one of the following: cervical dislocation or opening the thoracic cavity.
    c. When euthanizing pups, decapitation must be used because pups are resistant to carbon dioxide. A second form of euthanasia is unnecessary.

6. Place the animal in a plastic ziplock bag. Take it to the mini-morgue located in the room next to the mouse room. There is a mini-fridge in that room that serves as a morgue.

VI. Genotyping

Extracting DNA

1. Place mouse tails in labeled wells, if they are not already.
2. Add 100µl of 50 mM NaOH to each well. (If extracting DNA from embryos, use 50µl NaOH.)
3. Cover with foil and roll to seal.
4. Centrifuge.
5. Run *Rapid Tail DNA Prep* on tails in the Thermocycler. The program heats the tails at 98°C for 20 minutes then holds at 8°C.
6. After removing the tails from the Thermocycler, centrifuge.
7. Add 10µl of 1M Tris (pH8)/10mM EDTA. (If extracting DNA from embryos, use 5µl of Tris/EDTA).
8. Cover with foil and roll.
9. Vortex and centrifuge.
10. DNA is ready to be used in PCR.

PCR Procedure

1. Get an ice bucket and fill with ice. The reaction must be kept cold.
2. Gather mix and controls for the specific gene you are testing for.
3. Label wells.
4. For each animal needing to be genotyped use 23.8µl of mix and 0.2µl of Taq polymerase. *Keep the Taq cold*
5. Mix the appropriate amounts of mix and Taq in an epi tube.
6. Vortex for about 10 seconds then centrifuge.
7. Pipet 24µl of mixture into each well.
8. Piper 1µl of DNA into each appropriate well.
9. Cover with foil and centrifuge.
10. Place in the Thermocycler, and run the program for the gene you are testing for.

Pouring an Agarose Gel

1. Measure 300 mL of 1X SB buffer, and pour into a flask. The flask used for pouring gels is located at the gel station by the microwave.

2. Add agarose: 3g for 1% gel, 6g for 2% gel, 9g for 3% gel

3. Swirl to dissolve the agarose.

4. Place flask in microwave, using beaker on top of the microwave to cover the top of the flask.

5. Prepare a gel tray.

6. Heat until agarose has dissolved, then swirl.

7. Heat until the solution boils. The agarose is completely dissolved when the solution bubbles when swirled.

8. Add 3-5µl of 10 mg/mL ethydium bromide per 100µl of agarose solution. Add 10µl of ethydium bromide to the 300µl solution and swirl.

9. Pour hot solution into the gel tray.

10. Add 4 1 mm combs, evenly spacing them.

11. Allow the gel to cool for 30 minutes.

Running a Gel

1. Obtain the correct percentage gel for the allele you are testing for.

2. Place it in the gel box and add enough 1X SB buffer to cover the wells with buffer.

3. Mix 2µl of 6X loading dye with 9µl of the PCR reaction for each well.

4. Load 10µl of the resulting mixture into each well of the gel. Also load DNA ladders.

5. Record the loading order, including DNA ladder markers.

6. Run the gel at 250 volts until the lower band has moved about half-way down the gel.

Developing a Gel and Taking a Picture

1. Place the gel on the tray in the BioRad machine.
2. Live/Focus
3. Make sure the iris is all the way open.
4. Zoom in so the gel fills the screen.
5. Adjust the focus so the bands/wells on the gel are clear.
6. Freeze
7. Auto Expose (or Manual Expose if Auto Expose doesn't produce an optimal image)
8. Zoom in on desired section of image using Zoom Box.
9. Transform
10. Invert the image and adjust contrast settings (High, Low, Gamma) until the bands on the gel are clear.
11. Export the image to JPEG with 100% quality.
12. Save in Lab Network Storage in the appropriate folder. The title should be in this format: MMDDYY-GENE-pic#
13. Print image and record in lab notebook.
14. Record mouse genotypes in the mouse list.