1. Checking Date of Birth (DOB) of New Litters

\*Day 0 = very bright pink, no milk spots

\*Day 1 = still pink, more than ½ of the litter has milk spots

\*Day 2 = duller pink

\*Day 3 = dull pink, backs start to darken, one ear has “popped”

\*Day 4 = both ears have “popped”

\*Day 5 = darker

2. Toeing/Ear Punch

\*If you are toeing, bring the grad student’s genotyping binder, a tub of ice, a 50mL centrifuge tube of 70% EtOH, and a tub/bag of 1.5mL tubes if the mouse room supply is running low

 \*\*Ask grad student for his/her genotyping binder

 \*\*Use empty containers labeled “He lab 1.5mL/2.0mL tubes” for ice

\*\*Ice room located down the hall as you walk to the north side elevator on the right hand side

\*\*50mL centrifuge tubes are in bags in the boxes on the shelves on the right side as you walk in the lab’s main door

\*\*70% EtOH container kept in the cell culture hood room

\*\*Take enough 70% EtOH to conveniently immerse the toeing surgical scissors

\*Toeing supplies are kept in a plastic bin in the storage room of the suite, labeled “He Lab Toeing Supplies”

\*Always check that the bin is properly stocked with a 1.5mL tube rack, a 50mL centrifuge tube rack, surgical scissors, and a bag of extra 1.5mL tubes

\*Pups can be toed as early as 7 days old but try to wait closer to 8 or 9 if the gender is hard to determine

 \*Generally it is early enough to toe once the toes of the pup are clearly separated

 \*Pups will be difficult to toe once their eyes have opened at 12-14 days and must be ear

Punched when they are weaned

3. Weaning

\*Pups can be weaned as early as 21 days old, but try to wait until closer to 24-26 days old

\*Mating cages containing pups older than 26 days will be marked for overcrowding (OC) - avoid that by weaning close to 26 days

\*If there is a very pregnant female or a new litter of pups in the mating cage (greater than 5 days apart), wean the older litter(s) IMMEDIATELY if they are 21 days old, or ASAP if they are not yet 21 days old

 \*Alternatively you can separate the pregnant female into another cage

Daily Routine:

\*Always complete the routine duties in this order: Date of Birth, Weaning, Toeing

 1. When you get to lab, gather all the toeing supplies and bring down to the mouse room

2. Pull out each mating cage and look at the bottom of the cage

3. If you can see pink pups and can tell old they are and how many there are in the litter, put the cage back and record DOB and number of pups in the litter in the “DOB” column on the manila mating cage card

4. If you can see some pink pups, or suspect that there are pups, or know that there was a very pregnant female in that mating cage, mark it by putting the manila card right-side-up in front of the identifying mating cage cards

5. Check the manila card for litters that are at least 7 days old and mark them for toeing by placing the manila mating cage card upside down in front of the identifying mating cage cards

6. Check the manila card for litters that are at least 21 days old and mark them for weaning by turning the manila card around

7. If a cage needs to be marked for 2 or more duties, mark for the duty that needs to be completed first (ie - if a cage needs to be checked for both DOB and be weaned, mark it for DOB)

5. Go through all mating cages and check for all 3 duties

a. As you go through all the mating cages, also check that each mating cage has a shepherd shack, cotton pads, enough Tek-Fresh bedding to make a thin layer throughout the cage bottom, and has sufficient food and water.

b. If a mating cage is missing any of these, mark it somehow and add the supplies afterward

6. Go back and check for DOBs

a. Pull out each cage marked for DOB that you could not see all the pups for one at a time and place under the hood

b. Disturbing the mice as little as possible, open the cage slightly and peek in to determine the age and number of the pups

c. Record the DOB and number of pups on the mating cage card, and return the cage to the rack

 7. Go back and check for weaning

 a. Take out the cage that needs to be weaned

b. Check if the pups are large enough to be weaned. If they look smaller than average for their age, wait a few more days

c. Check the litter cage cards that are behind the identifying mating cage card

d. Separate the males and the females and put them in the respective cages that correspond to the litter cage cards

e. Prepare food and water for the cage as well as moisture food

 8. Go back and check for toeing

 a. Take out the cage that needs to be toed and prepare an empty cage next to

the cage that needs to be toed

b. Place 2 paper towels on the edge of the hood’s working surface underneath the cages

c. Take out a 1.5mL tube rack, a 50mL centrifuge tube rack, a pair of surgical scissors, the tub of ice, a tub of 1.5 mL tubes, and the genotyping binder and place under hood

d. Carefully separate the male and female pups: the females should have 6 nipples showing and the distance from genitalia to anus should be less than that of a male. Place the males towards the top half of the empty cage and the females towards the bottom half. It helps to stack the pups on top of each other so they do not move around

e. Take out the appropriate number of 50mL centrifuge tubes and line them up on the rack corresponding to the number of males towards the top of the rack and number of females towards the bottom of the rack and label each tube accordingly (cage number, litter (odd or even), toe/tail) ie. 53m96-1-0 for first male litter, and tail

f. Rinse the surgical scissors in 70% EtOH and wipe dry with one of the towels

g. Toe each mouse accordingly by picking up the pups from the back with your nondominant hand and put the tail between your ring and pinky finger and the bottom left foot between your middle and ring finger. Rinse the surgical scissors in 70% EtOH and wiping it dry after each toe

h. Place each toe in respective 50mL centrifuge tube and group the set of toes together into the tub of ice once all the toes are collected

 i. If the cage is too old to be toed (pups eyes are open) then they must be ear

Punched. Proceed from step e. Rinse the ear puncher in 70% EtOH and wipe dry. Pick up the mouse by the tail using your dominant hand. Place the mice on a green cage top and orient the mouse so that it is facing you. Use the index finger and thumb on your nondominant hand to scruff the mouse by pinning it to the lid and grabbing as much fur as possible to limit movement. Put the tail between your ring and pinky finger and then use your dominant hand to hole punch the ear in the order of no hole, hole right ear, hole left ear, hole right & left ear, two hole right ear, and two hole left ear. Be sure to rinse and wipe hole puncher dry between each punch. After the hole has been punched snip a part of the tail off and put in 50mL centrifuge tubes and tub of ice.

j. Clean up the hood by wiping down the surface and turning off the fan and hood light

4. Saccing

1. Go back and check for the cages that need to be sacced

 a. When saccing mice make sure you have OLAC certification email or if you are

training make sure that your trainer is also certified to sac the mice.

b. Put cages that need to be sacced on cart and go to the necropsy room

c. When saccing each cage put the cage in a vacuum chamber. Remove all

excess contents from the cage so that all the mice are visible. Put food container

and cage lid aside. Put the lid of the vacuum on top of the cage making sure that

the two ventilation holes are within inside the boundaries of the cage.

d. Set the chamber to between 2-3 ppm CO2 and wait for the mice to cease breathing by watching for cessations (it usually will take around 2 to 3 minutes for each cage).

e. After cessations have ceased, lay a paper towel on top of the workstation and cull the mice by pulling the tail with one hand and having two fingers pressed around the neck (you should hear a popping sound).

f. Place the mice in a clear plastic bag and take up to the lab to be put in the freezer with all the other dead mice.

5. Other lab duties

1. Biohazard waste disposal- Once the bag of biohazard in the lab is full, replace the bag

(extra bags are in the cell culture hood room) and take the bag mice to first floor

biohazard room. (Once about every week to week and a half)

2. Gel Drying- Dry gel under the hood by placing wet gel from the styrofoam box located

by where all the chemicals are kept. Stack the gel on the racks. Gel should take about 1 to 2

days to fully dry.