RUTGERS UNIVERSITY Office for Research	CMR STANDARD OPERATING PROCEDURE	
TITLE: Colony Management of GF mice		
SOP Category: Gnotobiotics	**CMR SOP #: 4.31**	**Page: 1 of 9**
Effective Date: 2/4/25	**Approval: *LaTisha V. Moody, DVM, DACLAM***	
Revisions:1/23/25		

SCOPE:

This document describes the procedures to be followed when managing axenic rodent colonies. This SOP applies to all Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS), and Research Staff members (RS) at the Rutgers Gnotobiotic University facilities.

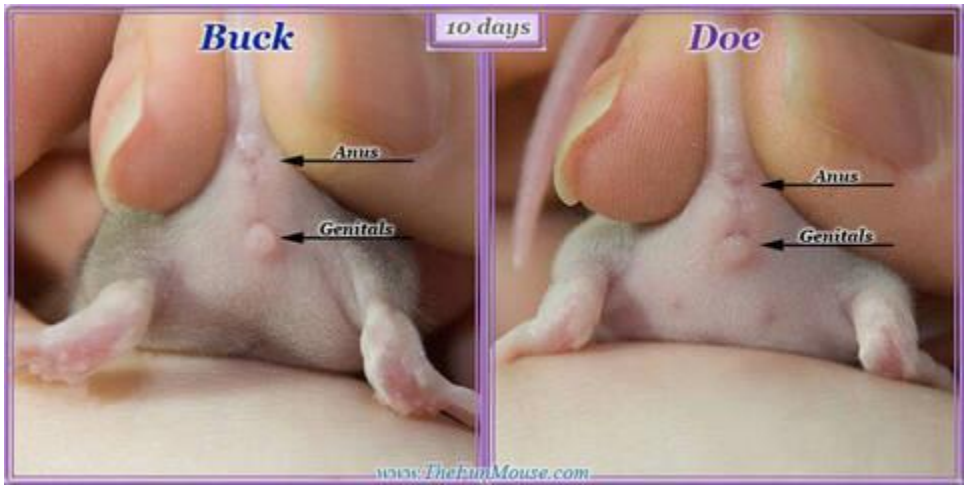
OBJECTIVE:

The objective is to describe how colony management is performed for axenic animals.

PROCEDURES:

Sexing and Weaning Mice

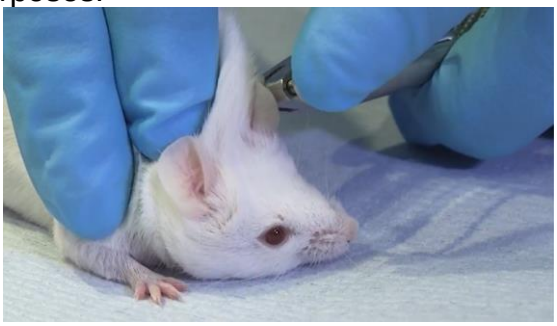
1. Mice can be accurately sexed by 10-14 days of age.
2. Weaning is typically performed at 21 days, however many GF strains may be small and delayed weaning is recommended (up to 28 days can be approved in the animal use protocol).
3. When sexing mice, the anogenital distance for males is always greater than in females postnatally. After 14 days of age, the females typically have visible nipples, whereas males do not have nipples.



4. Once mice reach weaning age, they are weaned into same sex groups in new cages separated from their parents. 5 mice can be group housed in the cages at time of weaning. If there is a single mouse in a cage, it can be combined with another mouse of the same gender that is the same age during weaning from the same investigator of the same strain.
5. Do not wean mice with congenital defects such as hydrocephaly & malocclusion, these mice are usually culled.
6. Small animals at the time of weaning can be given moistened chow in an autoclaved container. Use sterile diet and water from the home cage.

Ear punches for genotyping or Identification

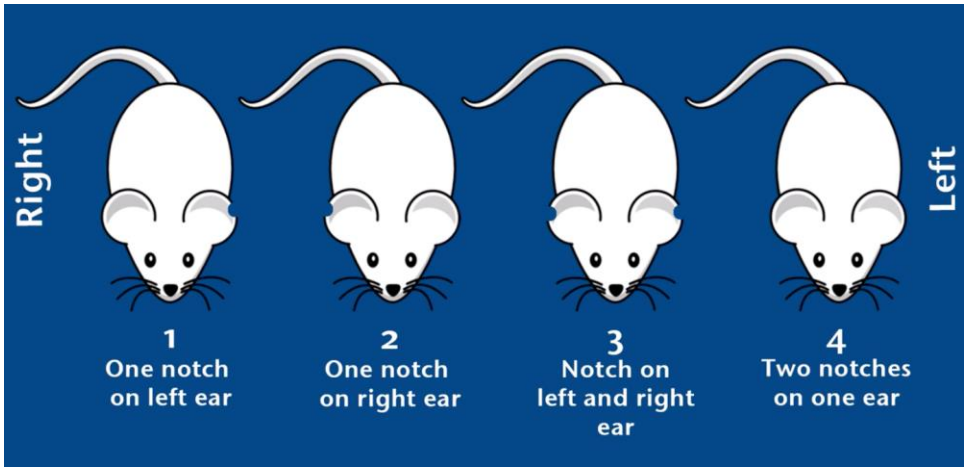
1. Genotyping of animals should be performed *prior* to weaning to reduce retention of animals of an undesirable genotype or sex and reduce potential pain and distress associated with collecting tissue samples at older ages. This method can be performed on mice once the ears have developed (>8 days old).
2. All instruments such as ear punches will be sterilized according to *SOP #4.18 Prepping and Sterilizing Supplies*. A list of items needed:
    a. An empty cage or beaker to place ear punched mice
    b. Ear punch & forceps
    c. Sterile drape (optional)
3. Ear punching may require a single operator or the 2-person method when working in a sterilized BSC following *SOP #4.22 Sterilization of BSC and Surfaces*. Both operators may be required to put on sterile PPE such as sterile gloves and a gown.
4. Using aseptic technique, the primary operator will restrain the mouse by gently scruffing the animal.
5. The sterile secondary assistant will collect the ear tissue by punching a deep notch piece of ear tissue on the distal edge and putting the tissue inside of a sterile collection tube. Each pup will need a collection tube for its own tissue. It is recommended that an ear tissue sample should be at least **2 mm** for genotyping purposes.



6. Samples can be stored at –80 before shipping.
7. The ear punch terminology is listed below.

Abbreviation	**Ear Punch Description**
LE	Left Ear

RE	Right Ear
LERE	Left Ear Right Ear
LELE	Left Ear Left Ear
RERE	Right Ear Right Ear
LELELE	Left Ear Left Ear Left Ear
RERERE	Right Ear Right Ear Right Ear
NN	No Notch



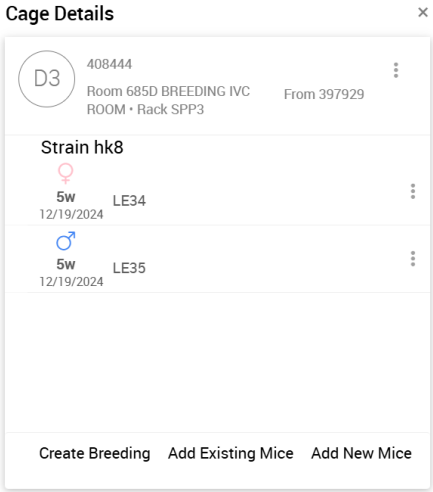
Ear punch video: https://www.understandinganimalresearch.org.uk/resources/video-library/1790

Setting up Mating's

1. Female mice reach sexual maturity at 6 weeks and males at 8 weeks.
2. Mice are typically paired together to form a monogamous breeding pair; however, trio breeding (2 females & 1 male) is also approved in the gnotobiotic protocol.
3. The gestation period is 21 days.
4. Mice are social animals; to prevent cannibalization it is best to house the dam continuously with the sire. There should be no more than two adults in the cage when there's a litter present to avoid overcrowding.
5. The pups should not be disturbed the first 24-72 hours after birth.
6. Some may take advantage of the 12-24 hours post-partum estrus that occurs in mice by leaving the sire with the female; however, the male must be removed and pups weaned before next litter drops to prevent overcrowded cages.
7. Breeder RFID cards must be used for all breeder cages.
8. Once male is removed, a normal RFID card must be used and can be singly housed to be used for additional breeding in the future.
9. Females should be retired from breeding after 7-12 months of age or when female is no longer able to produce litters greater than 2 pups.
10. Males should be retired from breeding after 1 year of age or when male does not successfully produce any litters after being paired with other females.

Setting up Mating's in Transnetyx

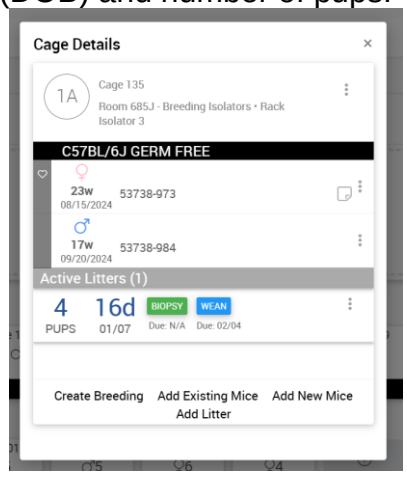
1. Login to Transnetyx https://www.transnetyx.com/
2. From the map view, drag the desired cages to the workbench and combine the animals by creating a new cage. Once the new cage is created then click on create breeding.



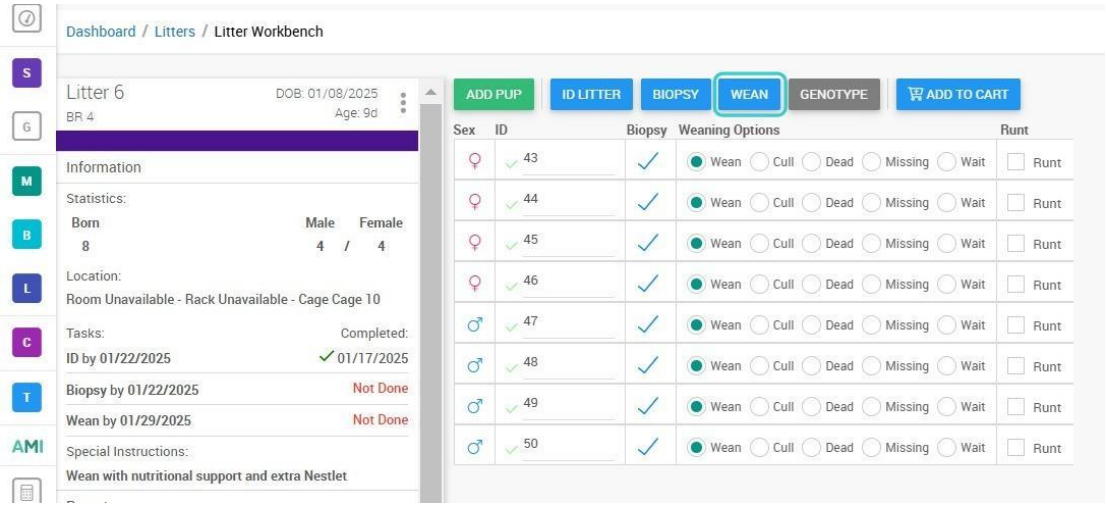
3. Ensure the breeding pair information is accurate. Click the NEXT button to continue.
4. Make sure the weaning information is correct according to the approved protocol.

Adding a litter and Weaning in Transnetyx

1. Add the litter by logging in to Transnetyx and from the dashboard click on the desired cage. Then click on add litter. Add the information of the pups such as date of birth (DOB) and number of pups.



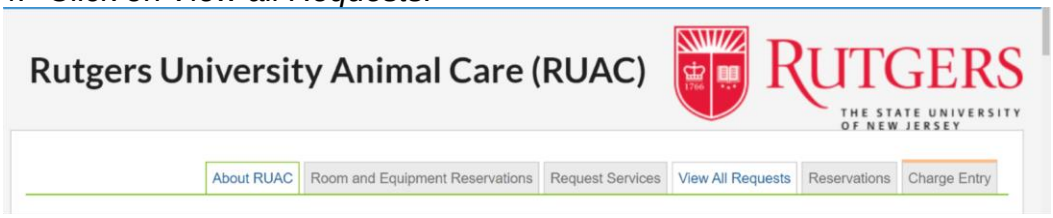
2. Transnetyx will send automated reminders of dates of cages that need to be weaned.
3. When weaning go to the dashboard and click on the cage. Make sure all information is correct then click WEAN.



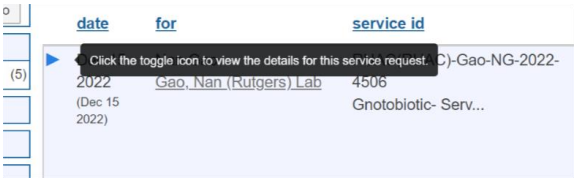
4. The weaned cages will automatically be created according to sex. Then click edit on the new cages and record the new cage number.

Using iLab to bill for Colony Management Services

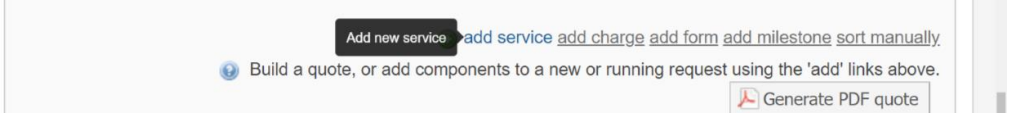
1. Setting up mating's, weaning's, ear punch for genotyping or anything related to colony management must be charged through iLab.
2. The research lab is responsible for placing all requests for colony management through iLab.
3. After performing the activity, login to iLab https://rutgers.ilab.agilent.com/landing/196
4. Click on *View all Requests.*



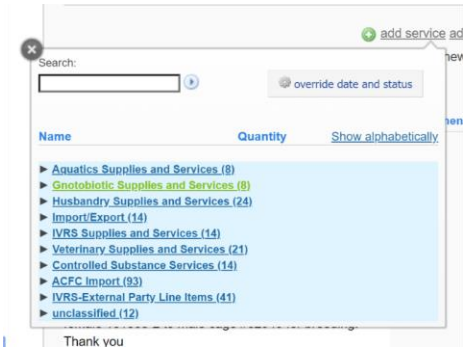
5. Click on *Gnoto View* to view all gnotobiotic service requests.
6. Click on the *blue triangle* for the lab you performed the activity for to expand the iLab form. The blue triangle will turn down when the form is expanded.



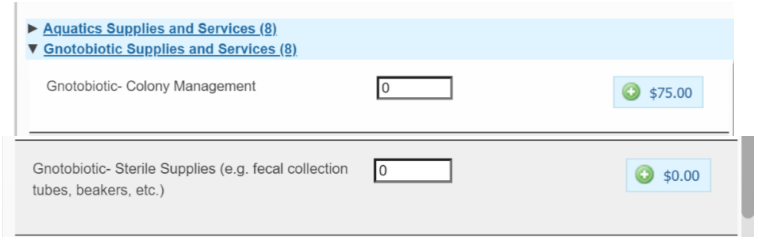
7. Scroll down the form and click on *add service* for each activity you have performed.



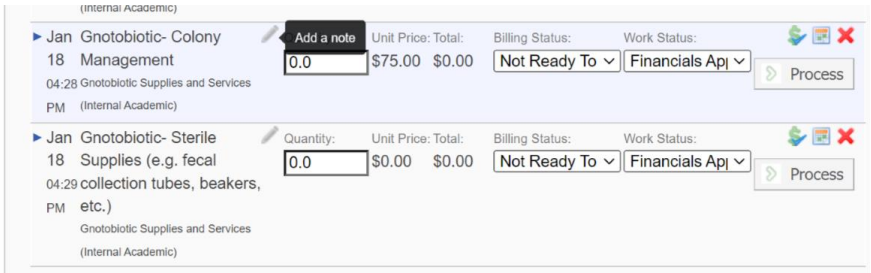
8. Under add service, click on the *Gnotobiotic Supplies and Services*. This will give you 8 options to choose from and each service you choose will add a new billable line item.

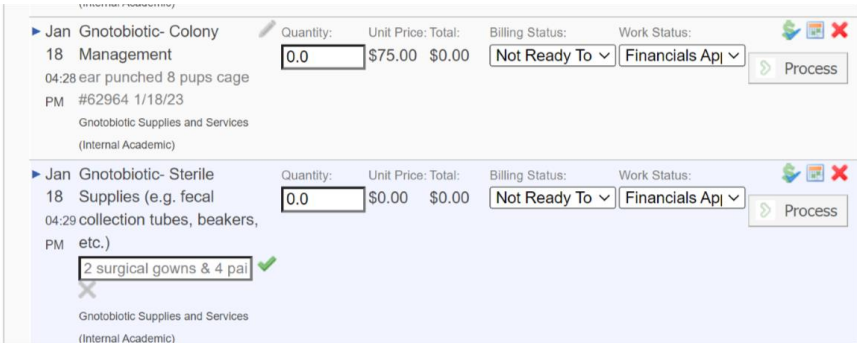


9. For example, the gnotobiotic technician took 1.5 hours to perform ear punches for genotyping and the technician used 2 sterile gowns and 4 pairs of sterile gloves. From the 8 options, click the green + sign next to *Colony management* to enter your tech time and click the green + sign next to *Gnotobiotic- sterile supplies*. Next click the “X” at the top left to close the screen.

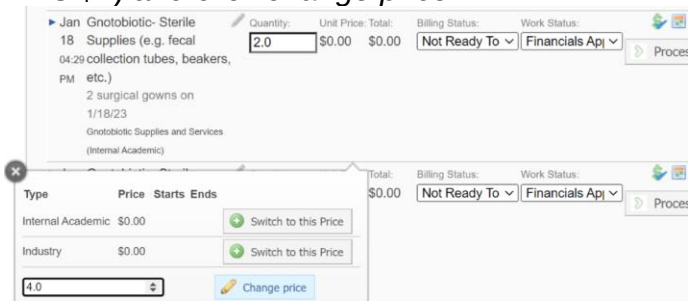


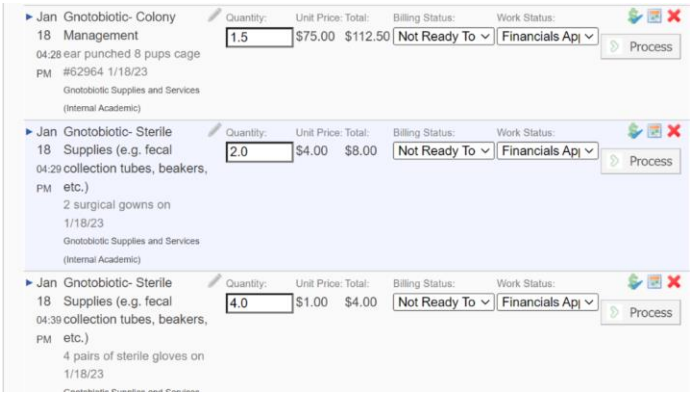
10. To edit the new line items (services), click on the pencil icon to *add a note*. Use the note to enter any pertinent information related to the colony management activity you performed and include the date of completion. Once your note has been typed into the box, click the green checkmark to <u>save</u> your note.



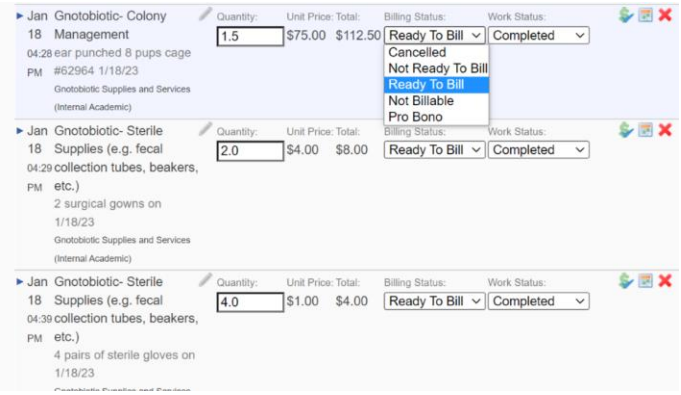


11. Enter in the tech time and the price of the items that were used for colony management. Click on the quantity to enter the amount of time (example: 1.5 hours) and the price will automatically update the total to be billed. For sterile supplies, the unit price must be manually updated; click on the appropriate quantity and click on the *unit price* to change it (example: 1 sterile gown is $4) and click *change price*.

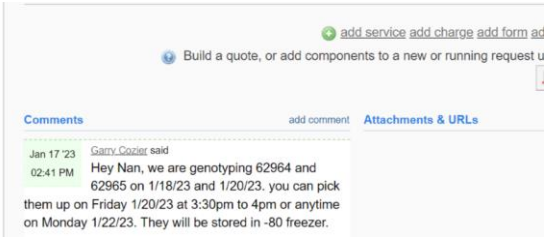




12. Once the activity has been performed and entered in iLab, under Billing status choose the option *Ready to Bill*. **All billable items and charges are due at the end of every month.**



13. Send any communication to the lab by *adding a comment*. Click send message, an email notification will be sent to all parties listed in the comment section.



Genotyping Form

Date: _____ **Parents ID: _____**
PI: _____

Cage #:
DOB:

ID number	Sex	Ear punch	Comments

Genotyping Form

Date: _____ **Parents ID: _____**
PI: _____

Cage #:
DOB:

ID number	Sex	Ear punch	Comments