



## DEPARTMENT OF LABORATORY ANIMAL RESOURCES

### Rodent Breeding Colony Training

#### Breeding colony resources

- Jackson Laboratory Colony Planning and Worksheet  
<https://www.jax.org/jax-mice-and-services/customer-support/technical-support/breeding-and-husbandry-support/colony-planning>
- Charles River  
<http://www.criver.com/products-services/basic-research/transgenic-colony-services/contract-breeding-aging>

#### Reasons to establish a rodent breeding colony:

1. To develop an animal model that is not commercially available
  2. Expanding transgenic, knock-out or other genetically-modified animal colonies and crosses.
  3. Experiments involving pre-natal work at various days of gestation. If pre-natal age-matched controls are also needed, these may also be bred.
- Note: Investigators maintaining colonies simply to preserve a genetic line of rodents should consider other conservation strategies such as cryopreservation of ova, sperm and embryos.
  - Some rodent strains are protected by patents which only permit the reproduction of these animals under formal contracts or licenses. Contact the UT Tech Transfer office for more information.
  - <http://www.utoledo.edu/research/TechTransfer/>
  - If the animals requested for breeding are from a non-commercial vendor such as another university, it will be necessary to complete a [Materials Transfer Agreement \(MTA\)](#).
    - UT Tech Transfer and DLAR will assist you with the import.
    - The health status of animals must be screened prior to shipment in order to perform a risk assessment. Animals with undesirable health histories will not be accepted into UT facilities. Animals with incomplete health histories may require special quarantine and further testing at the sending institution to ascertain their current health status.

#### Operational Guidelines for Breeding Colonies

- All Principal Investigators must have a current, approved IACUC protocol in order to breed laboratory animals.
- Individuals conducting breeding programs must be adequately trained. Breeding Colony Training occurs in the Vivarium Orientation Class but one-on-one training can also be setup by contacting DLAR.



## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE “IACUC”

### [IACUC's Web Page](#)

SUBJECT: Rodent Breeding and Breeding Cage Densities

DATE: February 14, 2018

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### **Rodent Breeding and Breeding Cage Densities Guidelines**

The establishment of a rodent breeding colony should be scientifically justified and not based on convenience. Overcrowding, as a result of breeding, can be a significant animal welfare issue and is in violation of federal and University guidelines on animal care, as well as the *Guide for the Care and Use of Laboratory Animals*. The purpose of this guideline is to address the health and wellbeing of mice and rats by ensuring safe breeding practices and breeding population densities. In all cases, cages should be regularly monitored to ensure the well-being of the neonates (e.g., size, age, and activity level), as well as characteristics of the cage environment and colony breeding performance. Exceptions to this guideline require IACUC approval.

#### Mice

When breeding, no more than 2 adults and 1 litter, regardless of the size of the litter, will be allowed in a 67 square inch (standard static mouse) cage or 75 square inch (standard ventilated mouse) cage.

1. The recommended breeding strategy for mice is monogamous pairs in either a standard ventilated or static mouse cage.
2. Breeding trios (1 male:2 females) or harem breeding (1 male:3 females) are allowable in a mouse cage only if all but one of the pregnant females are removed by the lab prior to parturition (birth) such that only one litter of pups and two adults remain in the cage after pups are born.
3. If post-partum estrus is used, the first litter must be weaned by 21 days of age to prevent the presence of two litters in a cage, i.e., no extended weaning is allowed if post-partum estrus is used.

#### Rats

When breeding, no more than 1 adult and 1 litter, regardless of the size of the litter, will be allowed in a 143 square inch (standard rat) cage. No more than 2 adults (1 male: 1 female) and 1 litter, regardless of the size of the litter will be allowed in a 268 square inch (standard guinea pig) cage.

1. The recommended breeding strategy for rats is monogamous pairs in a standard rat cage.
2. If post-partum estrus is used, the first litter must be weaned by 21 days of age to prevent the presence of two litters in a cage, i.e., no extended weaning is allowed if post-partum estrus is used.

#### References

1. *Guide for the Care and Use of Laboratory Animals*, 8<sup>th</sup> Edition. 2011. National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US).
2. *Guidelines for the Establishment and Use of Mouse Breeding Groups*. National Institutes of Health ARAC Guidelines, July 2012. [https://oacu.oir.nih.gov/sites/default/files/uploads/arac\\_guidelines/ breeding\\_groups.pdf](https://oacu.oir.nih.gov/sites/default/files/uploads/arac_guidelines/ breeding_groups.pdf)

*Contact the IACUC if you have any questions about the above specifications.*

# DEPARTMENT OF LABORATORY ANIMAL RESOURCES

## General Considerations for Breeding

### IACUC approved breeding:

- Breeding should be incorporated in your approved protocol. It's recommended to consolidate your research and breeding into one protocol as breeding and research are usually very closely linked, e.g. the breeding colony supplies the research project and the PI of the breeding protocol is the same as the PI of the research protocol.
- The IACUC reviews proposed breeding colonies to assure proper colony management, including appropriate breeding schemes, weaning ages, and methods for identification of individual animals. Please refer to and follow your PI's approved IACUC protocol.
- If animals are being produced which are likely to experience clinical diseases (e.g., hypertension, neoplasia, diabetes, neurologic disorders) the anticipated problems should be described, to enable an evaluation by the IACUC, and be known to husbandry and health personnel who will be caring for them. Unanticipated phenotypic disease manifestations should be reported to the IACUC and DLAR.
- The number of breeding animals and offspring must be justified. Record-keeping and colony management practices must balance the production of sufficient experimental animals while maintaining the lines but not producing excessive animals.
- The numbers of animal produced from breeding colonies must be reported monthly to DLAR to deduct them from the PI's total number of approved animals on the given protocol.

### Documentation:

- Cage cards for breeding animals should include the date set-up, date of birth of offspring, date weaned and number weaned.
- Optional information can include ID numbers of parents and reference number of offspring for ease of tracking.
- An Expecting Pups card should be placed behind every permanent cage card to notify staff of potential litters.
- The post weaning cage cards should include strain, sex, date born, date weaned, genotype and parents ID.
- Lab records should be maintained in such a manner as to make tracking offspring simple.
  - Information necessary includes sire/dam number, date and number born, date and number weaned and how offspring are used (breeding continuation or research protocol). This should include if they are euthanized (reason and date), died (date), if used in an experiment, replacement breeders or transferred to another protocol or investigator.
  - The records can be maintained in a notebook or on an Excel spreadsheet.
  - During the semi-annual inspections conducted by the IACUC, and during accreditation site visits, investigators are commonly asked to explain their record keeping activities.

PI	Protocol #
Month/Year	# of Pups/Fish
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	
31	
Total	0

### Counting offspring:

- All animals utilized in animal research protocols must be counted.
- The number of viable births regardless of genotype must be reported to DLAR by use of the [Breeding Record Form](#) monthly.
- DLAR aids in this process by sending an e-mail out to all PI's with approved breeding and the [Breeding Record Form](#) is attached to the e-mail.

## Breeding Guidelines

### Mating Systems

- **The recommended breeding strategy for rodents is monogamous pairs (1 male and 1 female).**
- Detailed records for breeding colonies are critical to the success of one's colony. [Example of a spreadsheet is on our website.](#)
- Harem breeding (1 male: 2-3 females) are allowable only if all but one of the pregnant females are removed by the lab prior to parturition (giving birth).
- For mice: No more than 2 adults (1 male and 1 female) with a litter regardless of the size of the litter is allowed in a standard static mouse cage or in a standard ventilated mouse cage.
- For rats: No more than 1 adult and 1 litter regardless of size of the litter is allowed in a standard ventilated rat cage.
- With breeding pairs, it is important to note that the female will have a post-partum (24 hrs. after giving birth) estrus and can become pregnant as soon as the day after the female delivers pups. If this occurs, the next litter will be born 21 days later. Therefore, weaning of each litter promptly at 21 days is required (the day of birth is considered "day 1" when determining weaning age).
- If post-partum estrus is used, the first litter must be weaned by 21 days of age to prevent the presence of two litters in a cage, i.e., no extended weaning is allowed if post-partum estrus is used.
- **At no time can two litters or more of pups be present in one cage.**

### Timed Mating Steps:

1. Weigh female, no more than 8 hours prior to mating
2. Put pairs together in the evening between 5:00-7:00p.m.
3. Always place female into male cage and male should have been in his own cage for 3-5 days prior.
4. Return the following morning approx. 6:30-7:30a.m. to check for a plug in the female. Separate non-plugged and plugged females. Mating plugs can fall out within 8-12 hours but could have fallen out sooner.
5. When plug is noticed, to assure pregnancy weigh the female 48, 72, 96 hours post plug/breeding. Continual weight gain indicates pregnancy, thus plug date would be pregnancy day 1.

### Mouse Breeding

#### Physiological Data:

- Body Temp: 35.8C -37.4C (96.6F- 99.7F)
- Heart Rate 328-780 beats per min
- Respiration: 90-220 breaths/min
- Weight: Newborn 1 gram, Weight at weaning: 10-15 g, Adult 25-40 grams
- Water Consumption: 4-7 ml, or 1.5 ml per 10g body weight per day
- Food Consumption: 3-6g, or 1.5 g per 10g body weight per day
- Feces: Firm rice size, dark brown in color
- Urine: clear with light yellow tint
- Life Span: 1-3 years

#### Reproductive data:

- Puberty: 5 weeks
- Sexual Maturity: 7 - 9 weeks
- Estrous Cycle: 4-5 days
- Estrus/sexual receptivity: 12 hours and within 24 hours after parturition
- Fertilization: 2 hours after mating
- Implantation: 4-5 days after mating
- Gestation: 19-21 days
- Litter Size: 6-12 pups
- Eating solid food: 11-12 days
- Weaning: Standard weaning is 21 days (depending on strain, size of litter and protocol) animals must not exceed 30 clays of age for weaning.
- Breeding span: this depends on the strain. Specialized strains may not breed past 4-6 months.
- Two breeding schemes commonly used for mice



- Monogamous or breeding pairs 1 male: 1 female
- Harem Breeding: 1 male: 2-3 females

## **Rat Breeding**

### **Physiological Data:**

- Body Temp: 35.9-37.4C (96.6-99.5F)
- Heart Rate 250-600
- Respiration: 66-144 p/min
- Weight: Newborn 5 g, Weaning 40-50g, Adult male 300-500g, Adult female 200-400g
- Water Consumption: 24-60 ml, or 10-20 ml p/ 100g body weight per day
- Food Consumption: per day 15-30g, or 5-6g p/100g body weight
- Feces: Firm , dark brown, elongated mass with rounded ends
- Urine: clear with light yellow tint
- Life Span: 2.5-3.5 years

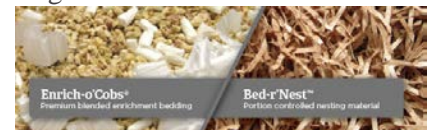


### **Reproductive data:**

- Puberty: 7 weeks
- Sexual Maturity: 8-10 weeks
- Estrous Cycle: 4-5 postpartum estrus and within 24 hours after parturition
- Gestation: 20-24 days
- Litter Size: 4-15 pups
- Eating solid food: 11-12 days
- Weaning: Standard weaning is 21 days (depending on strain, size of litter and protocol) animals must not exceed 30 clays of age for weaning.
- Breeding span: this depends on the strain. Specialized strains may not breed past 4-6 months.
- Two breeding schemes commonly used for rats
  - Monogamous breeding pairs 1 male: 1 female
  - Harem breeding: 1 male: 2-3 females


## **Problem-solving tips for unsuccessful breeders**

- Address non breeding pairs within 2 gestation cycles to offset any long term problems.
  - Some strains of mice only breed for 2-4 months.
- Ask DLAR to check light cycle to assure they are set at 12 hours light/12 hours dark.
- Cannibalism: The process where the adult parents kill and eat their young. This is not uncommon and the best practices are not to disturb a cage of newly born pups for 3 days, feeding breeder chow, which is higher in fat and protein to prevent cannibalism.
- Check food: successful breeding colonies commonly receive a breeding diet. You must check with the PI before changing or feeding any diet outside of what is currently on the cage feeder.
- Add the female(s) to the male's cage.
- Also insure nesting material is added, Nestlets ® or Bed-r-Nest ®
- If the nest is in good shape transfer it at cage change.
- Normally the male will be more active marking their territory for the first 24-72 hours after a cage change. Therefore it is essential for timed mating to add the female to the male cage about 3 days after cage changing.
- When separating the female near term, place in a clean cage at ~18 days gestation.
- If separating the male, remove the male and leave the female in the home cage to reduce stress on her.
- Use the same male(s) for rebreed.
- When there are no pups born within 30 days of mating, rule out male or female sterility. Mate the non-producing female with a successful breeding male.
- Males that remain on a breeder diet #2919 for a long period of time will become obese, thus becoming a poor breeder.
- Stud animals have a higher fertility rate when they receive a 5-7 day rest period each month.





## Expecting Pups and Weaning Guidelines

Front of Card:

Pups DOB: _____ Wean by: _____	
YOU'VE GOT PUPS! / WEANING NOTICE	
	
EXPECTING PUPS	
Postpone weaning of underaged pups. _____ New Wean Date: _____ Female cannot be postpartum bred. _____ Cannot postpone longer than 28 days of age. _____	

Back of Card:


New litter found on cage change day! Postpone Change To: _____ <i>Do not disturb new mother and litter for 3 days after birth!</i>	
Check Lixit for 7 days. From: _____ To: _____ * If supportive care is needed after 7 days submit a Vet Care Card	
	
Check Lixit for 7 days. From: _____ To: _____ * If supportive care is needed after 7 days submit a Vet Care Card	
	


### What to expect when you're expecting...pups!

- Following pairing of mice for breeding and/or successful mating each cage card of breeders must have an Expecting Pups card placed behind the permanent cage card.
- When a litter is born the DOB and Wean date must be filled out on the front of the Expecting Pups card.
  - Wean dates may vary depending on protocol specifications.
- If DLAR, at cage change, finds a new litter staff will flip the cage card over and fill out the 'New Litter Found' line to postpone cage changing.
  - **A new mother and her litter should never be disturbed for at least 3 days following birth.**
- If, prior to weaning, your lab has decided the pups need more maternal time (i.e. small pups) you may postpone weaning up to 28 days of age for mice and up to 30 days of age for rats.
  - If you have post-partum bred your animals, this option is not available. Pups must be weaned at 21 days of age.

### Weaning Procedures:

- Rodents are weaned as a standard at 21 days.
  - Always wean same sex groups and ideal 4 max in mouse cages.
  - Exceptions to this may be made if specified in the IACUC protocol.
  - Some examples of valid reasons for extending the 21-day weaning are runt or weak pups requiring extended maternal care.
  - If a strain is known to have small/runted pups, it's best to supplement for a week the parent's cage prior to weaning.
- Mice: Provide Boost and/or wet food and food on the floor (see description below).
- Rats: Provide wet food and food on the floor (see description below).
- Special precautions should be taken when weaning animals to prevent health concerns.
  - **Pelleted food and Boost placed in the bottom of cage and automatic watering lixit is activated for 7 days (each day) to allow for a drop of water to be on the end helping the mice/rat pups know where to drink.**
  - **On the back side of the Expecting Pups Card there is a blue and pink notification banner that can be torn away and placed on the newly weaned cages. This notification banner tells DLAR staff and Research staff to activate the watering valve in the back of the cage for 7 days post weaning. This is critical for newly weaned pups to learn to**

Check Lixit for 7 days. From: _____ To: _____ * If supportive care is needed after 7 days submit a Vet Care Card	
	

Check Lixit for 7 days. From: _____ To: _____ * If supportive care is needed after 7 days submit a Vet Care Card	
	

drink from the valve.

### DietGel® Boost

DietGel Boost is a high calorie supplement that provides energy for weanlings, post-surgical and debilitated and aging animals. Proven to increase survival rates in compromised animals, it is formulated from purified ingredients and provides 60% water, calories and minerals.

#### DietGel® Boost: for Mouse Cages

High calorie supplement that provides energy for weanlings, post-surgical and debilitated and aging animals.

- One Boost container will provide supplement for up to 3 cages.
- Split the container into 3 parts using the supplied petri dishes.
- Once split, place a couple pieces of the rodent chow on top of the Boost (push it into the Boost).
- Place petri dish of Boost and diet on the floor of the cages to allow the rodents easy access.



#### Diet and Water: for Rat Cages

Must be provided for weanlings, post-surgical and debilitated and aging animals.

- Place diet into the paper cup or crock and fill with water. When using a crock with smaller rats make sure to use enough diet to fill the crock.
- Place paper dish or crock of water and diet on the floor of the cages to allow the rodents easy access



#### Diet and Water: for mice cages unable to have Boost

Must be provided for weanlings, post-surgical, injured or ill and aging animals.

- Place diet in a petri dish and fill with water to moisten.
- Place petri dish in cage and provide dry diet on the floor.



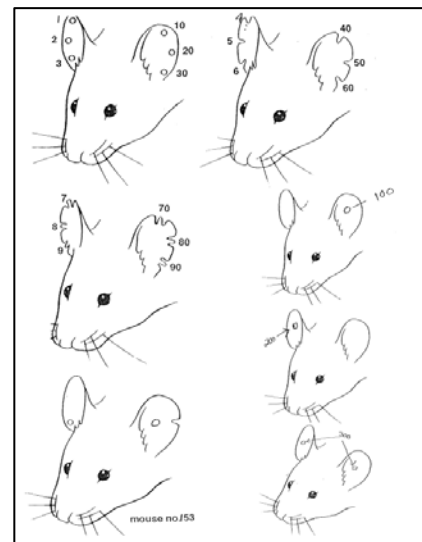
#### Determining Gender:

- The male rodent has a larger gap between the uro-genital opening and the anus than does the female.
- When sexing the animals, lift gently by the tail. Allow the front feet to stay on the floor. Evaluate the ano-genital distance. It is sometimes helpful to notice a side-by-side comparison of a number of animals until proficiency is obtained.
- Neonatal mice are much harder to determine sex. An option for juvenile animals is to look for nipples on 1.5-2 week old animals. Just as the fur starts to grow, a "cyclone" appearance forms around the nipples. Only the females will have obvious nipples at this point. Once the fur is fully grown in (> 18 days), this method is not always effective.



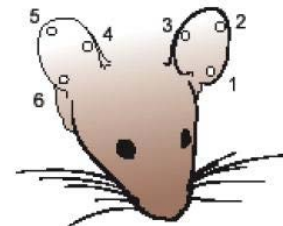
## Genotyping:

- The generation of mutant rodents requires collection of tissue samples for genetic analysis.
- Acceptable methods are ear punching, tail snips, peripheral blood or saliva sample analysis.
- The collection method will be specified in the IACUC approved protocol. Any deviations from this or additional samples being required should be approved through an amendment **prior** to collection.
- Ear punching: (Preferred and most humane way of genotyping)
  - This is commonly used as an identification method in rodents.
  - It is done using an instrument that removes a small circular section of tissue from the ear pinna. DLAR has ear punches available for use and/or purchase.
  - The ear tissue generated may be enough to allow DNA analysis by PCR.
- Tail Snips (1-2 mm of the distal tail):
  - General anesthesia is not required for mice <21 days of age if equal too or less than 2mm of the tail is excised.
  - Topical anesthetic via ice-cold ethanol is required.
  - General anesthesia is required of animals >21 days undergoing a tail snip.
  - Hemostasis must be complete when performing tail snips, prior to returning animals to the cage. This can be achieved with a cautery agent, such as silver nitrate stick, or tissue glue such as 3M Vet Bond or Liquid Bandage. These agents must be noted in the IACUC protocol.
- Peripheral Blood Collection:
  - Following training in blood collection, this can provide a method for genetic analysis.
- Saliva Analysis:
  - Genetic analysis of oral epithelial cells collected in saliva of mice has been described and offers an alternative and noninvasive method of genetic analysis.
  - It involves collection of saliva by oral wash using a plastic pipette followed by nested PCR analysis.



## Rodent Identification

- Acceptable methods of animal identification include ear punch, micro-tattoo, micro-chipping and ear tagging.
- Ear punching as described above (**See genotyping**).
- Micro-tattooing is a permanent mark made with specially designed forceps for rodents. This requires anesthesia and training by DLAR staff. DLAR has the equipment available for use.
- Micro-chipping is the injection of a small chip between the shoulder blades and is read with a transponder. This is ideal for large colonies as it is safe, reliable and easy to apply.
- Ear-tagging is a metal tag with an ID# attached to one ear of the animal. Young rats may also be skin tagged on the neck with numbered metal tags. These are usually applied at the time of genotyping or weaning. The disadvantage of ear tagging is that tags may fall out if not properly applied.
  - Ear tags and microchip equipment must be supplied by the Investigator.



## Transferring Animals

- Ideally breeding and research should be combined on a given protocol but in the event PI's separates breeding and research on two different protocols the animals produced from the breeding protocol will have to be transferred to a related research protocol to utilize these animals. This is accomplished by completing the Animal Transfer Form located on DLAR website.
- Animals may be transferred the same day by the PI's staff without delay when transferring within the same PI as specified in the protocol.
- Transfer labels are located in each animal room's 3 ring DLAR binder. Once the transfer form has been submitted to DLAR office the research staff must complete transfer labels and place these on each cage of animals that are being transferred to the new protocol. Never hand write or scratch out protocol numbers to represent a transfer.
- Animal movements within the vivarium should always be consistent with the established DLAR traffic flow within the facility (from cleanest to least clean zones).