

IACUC Policy: Rodent Breeding Colonies

I. IACUC Review Requirements

All activities involving the breeding of rodents at the University of Pittsburgh must be reviewed and approved by the IACUC. The IACUC carefully reviews breeding colonies to assure proper colony management, appropriate breeding schemes, weaning ages, and methods for identification of individual animals. A breeding colony may be necessary to develop an animal model that is not commercially available, or to produce young animals of a specific age or weight that cannot be provided by a commercial breeding colony. Investigators developing a new spontaneous or induced mutant animal model might also need to maintain their own breeding colony because there is no alternative source for the animal model.

In addition, if the species/strain is commercially available, the production of these animals should be carefully justified on scientific grounds. Cost savings **alone** is NOT a valid justification.

Investigators who wish to obtain approval for a breeding colony must complete the Rodent Breeding and Weaning Supplement that is part of the IACUC protocol Attachment 5 (see attached).

II. Colony Size must be Appropriate to the Research Program

The number of animals required to maintain a breeding colony can only be approximated because it is impossible to predict the exact number and sex of offspring. It is critical to approximate your animal numbers in Section 5 of the *Rodent Breeding and Weaning Supplement*.

- III. Appendix B of the *ILAR Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (2003) includes useful information for determining the total number of animals produced in order to obtain the number of animals needed to perform the research. For additional assistance, you may contact DLAR resources. Note that protocol approval does not guarantee that the desired space for housing animals will be available. For more information about space availability contact the Division of Laboratory Animal Resources (DLAR) at 412-648-8950 or dlar@pitt.edu.

Weaning Procedures

All weaning must conform to DLAR Specific Operating Procedure (SOP) 401, entitled "Breeding Weaning and Separating Mice." This document is appended below.

A. Pain and Suffering Concerns for Genetically Modified Strains

Any debility that genetically modified animals may experience is a cause of concern. It is important to provide as much support and comfort for mutant animals as possible. Some strains may require specific husbandry interventions to enable or promote well-being. See

Section 3, Part C of the Rodent *Breeding and Weaning Supplement*.

The general health of novel genetically modified animals should be assessed soon after their availability. In case a severe debilitating phenotype develops, the PI must provide the IACUC with this information in writing when the new mutant has been developed or at the next annual review of the animal-use protocol. Examples of severe phenotypes include abnormal behavior (circling, flipping), adverse anatomical changes (malocclusion, missing limb), adverse physiologic or organ dysfunction (progeria, lacking essential liver enzymes), etc. that impacts on the animals' health and well being. If health concerns are identified, please describe how the animals will be assessed and managed. Next, identify humane endpoints for removal from the study. For proper and efficient colony management, it is essential that breeding records be maintained. These records should be available for review during regular business hours. There are many commercial software packages available for record keeping. There are commercial vendor sources available to produce, manage and provide contract breeding and weaning services. Contact DLAR at 412-648-8950 or dlar@pitt.edu for additional information.

Minimal record keeping should include:

1. The species and strain designation (e.g., C57BL/6J, B6D2F1, etc.)
2. All phenotypes and genotypes
3. Breeder identification numbers (each breeding animal should have a unique identifier that is not repeated in subsequent generations)
4. Set-up date (mating date)
5. Breeder date of birth (DOB)
6. An accounting of all colony offspring including:
 - a. Animals weaned and retained for colony maintenance
 - b. Animals used for research
 - c. Culled littermates (e.g., undesired genotype)

IV. Additional Campus Resources

- Researchers are encouraged to investigate the services offered by the University of Pittsburgh Transgenic and Chimeric Mouse Facility, by emailing its director at: khillan@pitt.edu. The TCMF can create and maintain special rodent lines for University of Pittsburgh researchers.
 - More information about rodent breeding is available in DLAR SOP 401 entitled "Breeding, Weaning, and Separating Mice," which is appended below.
 - DLAR Veterinary Services are also available to provide support and advice in project
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planning. Contact DLAR Veterinary Services by email (DLAR@pitt.edu) or phone (412-648-8950).

V. APPROVAL

The University of Pittsburgh's Institutional Animal Care and Use Committee has reviewed and approved this SOP as attested by the signature of the Committee Chairperson.

Frank Jenkins, Ph.D.
Committee Chairperson

May 18, 2009
Date

Rodent Breeding and Weaning Supplement
<http://www.iacuc.pitt.edu/sop/RodentBreeding.pdf>

A. INDIVIDUAL(S) RESPONSIBLE FOR COLONY MAINTENANCE

P.I. NAME:		DEPARTMENT:	
WORK PHONE #:		EMERGENCY PHONE (after hours) #:	
E-Mail:			
Other Individuals Involved in Colony Maintenance (Must Also be Listed in Item 33 in Main Application)			

Describe training and experience of these individuals as it relates colony maintenance & breeding of animals:

SECTION 1: BREEDING COLONY JUSTIFICATION

What strains will be bred?
Provide a justification for establishing and maintaining a breeding colony of animals at the University of Pittsburgh. Refer to the Introduction of the University of Pittsburgh IACUC on Breeding Colonies found on the IACUC web site in the Policies section under Implementing and Designing a Small Animal Study

SECTION 2: HOUSING AND HUSBANDRY ISSUES:

List the location(s) where animals will be housed and bred.

BUILDING AND ROOM NUMBER

Are there any special husbandry requirements needed for the maintenance of the colony?	<input type="checkbox"/> Yes <input type="checkbox"/> No
If you answered yes, please describe the needs (e.g., special diets, special housing, immunocompromized strains, etc.).	

Will you be breeding genetically modified animals?	<input type="checkbox"/> Yes <input type="checkbox"/> No
Breeding of animals with a unique genotype requires the submission of a registration to the Institutional Biosafety (RDNA) Committee. The RDNA Registration Form can be downloaded from: http://www.rcco.pitt.edu/rdna	

SECTION 3: COLONY MANAGEMENT INFORMATION

Please provide the following information below. Refer to the University of Pittsburgh's IACUC Policy on Breeding Colonies for additional instruction, found on the IACUC web site in the Policies section under [Implementing and Designing a Small Animal Study](#)

a. Breeding Scheme

<input type="checkbox"/>	Harem Mating
<input type="checkbox"/>	Pair Breeding If this method is selected, what techniques will you use to properly manage litters within one cage? Describe how you will ensure that multiple litters do not occur within one cage?
<input type="checkbox"/>	Timed (hand) Mating
<input type="checkbox"/>	Other – Describe and provide a justification:
<input type="checkbox"/>	Post-Partum Breeding – Within 1-3 days after delivery

b. **Weaning Plan** – As per IACUC policy, no greater than one litter is to be kept in a cage. The Principal Investigator is responsible for weaning unless otherwise contracted.

<input type="checkbox"/>	Animals will be weaned at 21-28 days
<input type="checkbox"/>	The breeding requires additional time for weaning (beyond 28 days). Please describe and justify below:

c. Phenotype Information

Are there any health concerns associated with the development of the phenotypes for the strains described?	<input type="checkbox"/> Yes <input type="checkbox"/> No
If yes, please describe the health concern (e.g., behavioral, anatomical and/or physiological) and describe how the health of these animals will be managed.	

SECTION 4: GENOTYPING AND OTHER COLONY MANAGEMENT PROCEDURES – The goal of this section is to identify techniques and procedures used to manage your colony.

a. **Genetic Identification** - Refer to the University of Pittsburgh IACUC Guidelines for Genotyping of Rodents found at http://www.iacuc.pitt.edu/sop/policies_DesignSmAnimalStudy.asp.

Indicate how animals in the breeding colony will be identified genetically and the age of the animals for genotyping (if applicable).

Sample type:

<input type="checkbox"/>	Tail Clip. If clipping is done on animals over 28 days of age, local or general anesthesia is required. If this is the case what anesthetic method(s) will be used? (Specify to the right) Reference: "Drug List (PDF)" found in the Policies section of the IACUC website under Designing & Implementing an Animal Study.	
<input type="checkbox"/>	Blood sample --Describe the collection procedure:	
<input type="checkbox"/>	Anesthesia method (if applicable):	
<input type="checkbox"/>	Other , (e.g., ear notch, buccal swab). Specify:	

a. Age of animals for genotyping:

<input type="checkbox"/>	0-21 days (anesthesia is recommended)
<input type="checkbox"/>	21-23 days (anesthesia is highly recommended)
<input type="checkbox"/>	21-28 days (anesthesia is strongly recommended)
<input type="checkbox"/>	> 28 days and older (adult post-weaning - anesthesia is mandatory)

b. What method of animal identification will be used?

<input type="checkbox"/>	Ear notch
<input type="checkbox"/>	Ear tag
<input type="checkbox"/>	Tattoo
<input type="checkbox"/>	Microchip implant
<input type="checkbox"/>	Cage card identification only

Euthanasia:

Will animals that cannot be utilized be euthanized in the same manner as described above (items 61-63) in the main part of the IACUC Protocol Application?	<input type="checkbox"/> Yes <input type="checkbox"/> No
If no, please describe the alternate euthanasia method planned:	

c. Breeder Manipulations

Manipulations of breeder animals

Ovulation agents used?	<input type="checkbox"/> Yes <input type="checkbox"/> No
Experimental compounds?	<input type="checkbox"/> Yes <input type="checkbox"/> No
In-utero therapies / therapeutics?	<input type="checkbox"/> Yes <input type="checkbox"/> No

NOTE: All of these agents should be listed in the tables in Section K of the main protocol application

SECTION 5: ESTIMATED NUMBER OF ANIMALS TO ESTABLISH AND MAINTAIN THE COLONY.

A mechanism for tracking colony management should be employed to allow review during semiannual IACUC inspections. If you need assistance in estimating numbers, please refer to ILAR *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003)*:

<http://www.nap.edu/books/0309089034/html>.

Enter Estimates:

Estimated number of weaned and adult animals to be subject to experimental manipulations.	
Estimated number of suckling animals to be subject to experimental manipulations.	
Estimated number of breeders held but not subject to experimental manipulations.	
Estimated number of suckling animals to be euthanized at or prior to weaning, and <u>not</u> subject to experimental manipulation.	
TOTAL ESTIMATE:	

ISSUE DATE: 7/25/2001

REVISION DATE: 3/08/2004, 10/20/2005, 6/2006, 8/21/2006, 5/18/2009

DLAR 401

**Breeding, Weaning, and Separating Mice
Standard Operating Procedure**

I. Purpose

A. To provide guidance on breeding practices for mice in order to prevent overcrowding and related health issues, maintain one (1) litter of pups per box, assure accurate, up-to-date record keeping, and prompt reporting of non-compliance.

II. Scope

A. Personnel affected

1. DLAR Veterinary staff
2. DLAR Husbandry staff
3. DLAR Regulatory Coordinator
4. Principal Investigators(PIs) and their lab members

B. Training required

1. EH&S training on Chemical Hygiene and Blood Borne Pathogens
2. IACUC training modules on Research Integrity, Use of Laboratory Animals, and Small Animal Research
3. Participation in Animal Facility Orientation and knowledge of IACUC Policy: Rodent Breeding Colonies (<http://www.iacuc.pitt.edu/sop/RodentBreeding.pdf>)

III. Procedures

A. Counting days from birth

1. The day of parturition is day zero (0). The counting of days begins the day following birth, e.g. Tuesday is day one (1) if pups are born on Monday.

B. Breeding schemes

1. Pair mating: one male may be left with one female and one (1) litter of pups, provided the pups are weaned between 21 and 23 days to make room for the next litter. IACUC protocol approval must be granted to wean beyond day 23. Protocol approval requires an explanation as to how the PI will avoid overcrowding and multiple litters, and avoid exceeding cage density requirements.
2. Harem mating: one male may be placed with a breeding group of up to three females. The PI or their staff must remove the female(s) when she is recognized to be pregnant (14-17 days) and placed in a separate cage to avoid overcrowding and maintain one (1) litter of pups per cage. Pregnancy can be determined by weighing the female(s), visual inspection and by palpation. Meeting all requirements of harem mating, pups may be weaned at anytime through day 28, although it is suggested that weaning occur between 21 – 24 days. IACUC protocol approval must be granted to

wean beyond day 28. Protocol approval requires an explanation as to how the PI will avoid overcrowding and multiple litters, and avoid exceeding cage density requirements.

- a) No more than one litter of pups may be present in a cage at a given time.
- b) When females are noted to be pregnant, PI or lab member must house them individually and notify DLAR animal husbandry staff so that cage change can be scheduled to allow new litters to remain undisturbed for 3 to 5 days following delivery.
- c) If a litter is born to a harem breeding cage before the adults are separated, PI or lab member must remove all mice except the mother and pups to a separate cage.

C. Weaning

1. Based on the breeding scheme used (as outlined above) the PI or lab member must wean litters by either 23 or 28 days of age.
 - a) Exemptions are allowed if outlined in an IACUC-approved protocol.
 - b) Special health circumstances may require exceptions approved by DLAR veterinary staff on a case-by-case basis. (e.g. health situations)
 - (1) The DLAR Veterinarian or Veterinary Technical Staff is to mark Veterinarian approval on the animal's health report, the date, and any conditions associated with the Veterinary exception.
2. PI or lab member must separate and house pups at a cage density of no greater than four adult males or five adult females per cage.
3. PI or lab member may obtain supplies for weaning such as cages, water bottles, and cage cards during business hours.
 - a) Contact DLAR Husbandry Supervisor up to 24 hours in advance via phone, email or in person to make arrangements.
 - b) Request appropriate amounts and plan to use the supplies on the date requested. Unused items are returned to circulation.
4. PI or lab member is responsible for weaning and separating the pups by the IACUC-approved protocol or Veterinarian deadline, unless prior fee-for-service contractual arrangements have been made with the DLAR to wean and separate the pups. No reminder notices to wean and separate the pups will be issued.

D. Recordkeeping

1. Breeding

- a) PI or lab member must record new litters on the breeding cage card with date of birth (DOB) and number of pups born.
 - (1) If this is not done, DLAR staff determines the litter's age by size; and weaning and compliance dates are calculated by this estimation of the litter's age.

2. Weaning

a) PI or lab member must record complete information (PI name, protocol number, species, strain, source: in-house bred, date of weaning, and gender) on new cage cards when weaning pups.

(1) If information is not correctly recorded,

(a) DLAR staff first notifies the PI, requesting correct completion of the cage cards.

(b) If compliance is not provided, DLAR staff reports to the RCCO that the investigator has not provided mandatory cage card information as required by the IACUC-approved protocol.

b) PI, lab member, or whoever weans the litter must record weaned litters on the Weaning Logs located on a clipboard inside the animal room.

(1) DLAR Husbandry staff posts Weaning Logs in animal rooms and submits them to their supervisor at the end of each month.

(2) The DLAR Supervisor submits the completed Weaning Logs to the Regulatory Coordinator to track annual usage per federal guidelines.

c) PI, lab member, or whoever weans the litter must update the inventory sheet located on a clipboard inside the animal room.

(1) DLAR Husbandry staff posts inventory sheets in animal rooms and submits them to their supervisor at the end of each month.

3. Billing

a) DLAR staff must report to their supervisor their separating overcrowded cages in correcting the non-compliance issue.

(1) The DLAR Supervisor indicates the cage separation activity on their monthly billing statement as, "Separation Charge" and applies the fiscal year's service fee.

4. Tracking

a) DLAR Regulatory Coordinator tracks animal numbers through weaning logs and deducts the number used from the number permitted by the respective protocols.

(1) PIs are notified via email when they reach 80% of their approved animal numbers.

(2) PIs are notified via email when they reach 100% their approved animal numbers. The DLAR Executive Director, Attending Veterinarian, Husbandry Supervisor, and the IACUC Office are copied on this notice.

(3) Pups used in research prior to weaning are counted towards the number permitted on the protocol. All other pups are counted towards protocol limits at weaning age.

E. Non-compliance

1. Multiple litters in one cage

- a) DLAR staff record on the health report form any cages where more than one litter is present, regardless of litter ages.
- b) DLAR Veterinary Technical staff verifies protocol parameters to confirm whether the cage is non-compliant or has an IACUC-approved exception.

(1) If no exception exists, DLAR Veterinary Technical staff then notifies PI via email that a health case has been generated for the cage due to multiple litters, copying the assigned veterinarian and the Husbandry Supervisor.

- c) If PI or lab member has not separated the litters within 24 hours, DLAR staff separates the litters on the next business day and charges service fees.

(1) DLAR notifies PI via email that the litters were separated, copying the assigned veterinarian, the Husbandry Supervisor, and the IACUC office.

(a) IACUC office email: iacuc@pitt.edu

(2) DLAR assumes no responsibility for errors in recording of strains or genetic information on new cards.

2. Litters beyond the approved weaning date

- a) On the next business day following the maximum weaning age on the IACUC-approved protocol, DLAR staff record on the health report form any cages where a litter is older than the allowed weaning age.

- b) DLAR Veterinary Technical staff verifies protocol parameters to confirm whether the cage is non-compliant, or has a Veterinarian or an IACUC-approved exception.

(1) If no exception exists, the next business day, DLAR Veterinary Technical staff notifies PI via email that a health case has been generated due to presence of a litter whose age is beyond the approved weaning age, and that the litter was weaned, copying the assigned veterinarian, the Husbandry Supervisor, and the IACUC office.

(a) IACUC office email: iacuc@pitt.edu

(2) DLAR assumes no responsibility for errors in recording of strains or genetic information on new cards.

Approval:

Charles P. Bennett, Jr.
Executive Director, DLAR

[Signature]
Clinical Director, Attending Veterinarian, DLAR

[Signature]
Associate Director of Husbandry, DLAR

[Signature]
Associate Director of Administration, DLAR

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