	UCSD INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE POLICY MANUAL	POLICY #6.06
	Rodent Breeding Colonies	Approval 7/13/01 Revision 6/20/03 Revision 11/17/04 Revision 6/18/14 Revision 5/16/18 Revision 7/17/19

I. Background and Purpose

The UCSD policy is adopted from the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Federal Regulations and The Guide for the Care and Use of Laboratory Animals require appropriate management and housing for breeding.

II. Who Should Read This Policy

All investigators and ACP personnel who run breeding colonies or participate in the activities of a breeding colony.

III. Definitions

N/A

IV. Policy

1. Breeding of laboratory animals may not be performed except as approved in an animal use protocol.

2. Individuals participating in breeding programs must attend the ACP Breeding Colony training class.

3. If the breeding colony is located outside of the ACP-managed vivarium, the PI must obtain IACUC approval for a Satellite Facility and fulfill all obligations as detailed in IACUC Policy on Satellite Facilities.

4. The PI is responsible for weaning on time and preventing overcrowding and multiple litters.

5. The PI is responsible for recording date of birth on each cage card within 3 days of birth.

6. Labs must keep records of all the animals born in the colony and the number of animals born must be reported monthly at https://iacuc.ucsd.edu/. Reports must be submitted even if the number of animals born is "0".

7. Toe clipping (removal of the first bone of certain toes corresponding to a predetermined numbering code) as a method of identification may only be used in an approved protocol with justification of why no other methods are feasible. Toe-clipping is limited to rodents 7 days old or less and should be limited to one digit per extremity. Aseptic practices should be followed (The Guide, page 75).

8. For mouse breeding colonies, the following applies:
With an Animal Use Protocol that includes approval for a Breeding Colony, the following husbandry practices have been determined to fall within the density recommendations of the Guide and may be selected by investigators housing animals in a standard mouse cage (~75 square inches).

Breeding Configuration		Maximum Weaning or Separation Age	Comments
A	One female with litter (any number of pups)	28 days	Regardless of initial housing/breeding configuration, action must be taken (e.g., remove adult, wean litter) to ensure the combination of adults and pups conforms to one of the Breeding Configurations (A-D) within 48 h of the birth of a new litter.
B	Two adults and one litter (any number of pups)	28 days	
C	Two dams and two litters, ≤5 pups total	21 days (≤5 pups total)	
D	Multiple females, one male	Reduce number of adults to 2 within 7 days of birth of 1st litter	

Notes:

- Regardless of weaning date, tail clipping after 21 days of age requires anesthesia.
- Flexibility to wean past 21 days in configuration B is contingent upon active management of the breeding colony. Undermanaged colonies (including: 2nd litters frequently born before previous litter is weaned, undocumented new litters, litters not weaned by 28 days) may be restricted to a maximum weaning age of 21 days at IACUC discretion.
- If pups are still too small to wean at the maximum weaning age, the cage must be flagged with a pink Veterinary Observation card and an Animal Health Report must be submitted online (https://animalcare.ucsd.edu/pages/animal-health/health-reporting.html) to request an extension in the weaning date of the litter, unless a breeding colony exception for that strain has been approved on the protocol.
- A 2nd litter born in a cage with an older litter is at high risk for trampling. This situation should be avoided by planning to separate or wean before the second litter is born. If a second litter is born, the older litter should be separated or weaned as soon as possible, but no later than 48 hours following the birth of a new litter.

9. For rat breeding using standard rat cages (136-142 square inches) the following applies:
    a. Prior to the birth of a litter, the number of adult rats permitted in the cage must comply with the recommendations in The Guide (see V. Related Documents below). For example, one male weighing up to 400 grams may be housed with two females weighing up to 400 grams, or one male weighing up to 500 grams may be housed with one female weighing up to 400 grams.

b. Regardless of the rats' weight, within 24 hours after a litter is born, the number of adults must be reduced to two.
c. After 7 days of the birth of a litter, only the female and her litter are permitted to remain in the cage.

d. Rat pups must be weaned by 21 days of age.

10. Exceptions to the husbandry practices in 8. and 9. above (e.g., use of non-standard size caging, altered wean dates, etc.) must be described in the Animal Use Protocol and will be considered for approval by the IACUC on a case-by-case basis. A scientific justification for the exception must be provided.

11. For species other than mice and rats, the breeding scheme must comply with the UCSD Housing Requirements for Laboratory Animals: http://iacuc.ucsd.edu/policies/CageSizes.pdf

V. Related Documents

The Guide for the Care and Use of Laboratory Animals	http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-laboratory-animals.pdf
IACUC Policy 36 Identification	http://blink.ucsd.edu/_files/sponsor-tab/iacuc/Policy 36 Identification.pdf
Housing Requirements for Laboratory Animals	http://iacuc.ucsd.edu/policies/CageSizes.pdf

VI. Additional information

Genotyping of Rodents: The generation of transgenic rodents commonly requires collection of tissue samples for genetic analysis. Tail tip excision, ear punch, peripheral blood collection or analyses of saliva are acceptable methods of tissue collection provided the guidelines below are followed. The IACUC requires that anesthesia must be administered to rodents when the sampling method is associated with more than momentary pain or distress.

Ear Punching: Ear punching is commonly used as an identification method in rodents. It is performed using an instrument that removes a small (0.5-1 mm) circular section of tissue from the ear pinna. Multiple samples can be collected from one or both ears. Collection of the small tissue samples produced during ear punching may generate enough tissue (DNA) to allow analysis by PCR. Anesthesia is not required when performing ear punches on rodents.

Peripheral Blood Collection: Peripheral blood can provide tissue for genetic analysis. For more information or training in blood collection methods from mice, please contact ACP Veterinary Services (acp-vetservices@ucsd.edu).

Saliva Analysis: Genetic analysis of oral epithelial cells collected in saliva from mice has been described and offers an alternative and noninvasive method of genetic analysis in the mouse. This method involves collecting a saliva sample from weanling rodents by oral wash using a plastic pipette tip followed by nested PCR analysis.

Tail Tip Excision

In pre-weanling rodents: Anesthesia is not required for rodents ≤ 21 days of age if less than 5 mm of the tail is excised. Anesthesia is required for rodents < 21 days for excision of more than 5 mm or if repeated excisions of tail are required.

In adult rodents: Anesthesia is required for rodents that undergo tail tip excision at > 21 days of age.

Hemostasis: It is important that complete hemostasis be achieved when performing tail tip excisions, for example by the use of cautery agents such as silver nitrate. The use of a heated scalpel blade is a recommended technique because it accomplishes both amputation and cautery. The scalpel blade can be heated easily in a glass bead sterilizer.

Acceptable Methods of Identification of Rodents:

Ear Punching: Ear punching is commonly used as an identification method in rodents. It is performed using an instrument that removes a small (0.5-1 mm) circular section of tissue from the ear pinna. Multiple samples can be collected from one or both ears. Collection of the small tissue samples produced during ear punching may generate enough tissue (DNA) to allow analysis by PCR. Anesthesia is not required when performing ear punches on rodents.

Micro-Tattooing: A permanent mark, which is easily readable, can now be achieved with the use of micro-tattooing forceps on rodents. It is humane, rapid, easy to apply, and allows for an almost infinite amount of ID numbers. Requires anesthesia and training.

Microchip: Injection of small microchip subcutaneously between the scapulae and read with a transponder. This method is easy, safe, reliable, and the microchips are sometimes reusable after sterilization.

Ear-tagging: With ear tagging, a metal tag with an ID # is attached to one ear of the rodent. Due to the size of the tag and the rate at which the ear develops, this is routinely performed on weaned animals. However, disadvantages are that tags are burdensome to the animal and can fall out.

Acceptable <u>only with scientific justification</u>:

Toe clipping:

Toe clipping (removal of the first bone of certain toes corresponding to a predetermined numbering code) as a method of identification should be used only when no other individual identification method is feasible.

Because toe-clipping can alter the gait or weight-bearing ability of a rodent's rear limbs, *The Guide* limits its use to justified instances. According to the 8th edition (NRC 2011), toe-clipping "should be used only when no other individual identification method is feasible." The IACUC has adopted the following policy in accordance with these guidelines:

Investigators considering using toe-clipping as a means of rodent identification must first show that they have considered alternative methods of identification.

The investigator must provide the IACUC with a justification of why toe-clipping is necessary for identification of rodents, including a discussion of why alternative methods are unsatisfactory.

Toe-clipping is limited to rodents 7 days old or less and should be limited to one digit per extremity.

Improving Breeding Efficiency

Please contact the ACP veterinarians (acp-veterinarians-l@ad.ucsd.edu) for guidance on improving breeding performance and for determining the issues that may be affecting your particular environment and mouse strains. ACP Veterinary Staff offer this consultation free of charge.

Adding enrichment or providing nutritional supplements can improve breeding success, but may need to be added to the protocol.

The following changes do NOT require a protocol amendment and have been shown to improve breeding in some strains:

- paper towel for nesting
- cotton nesting material (e.g. Nestlets)
- paper towel tube/paper tubes
- cardboard or plastic huts or igloos
- cardboard tunnels
- other paper products (e.g., Enviro-dri, Crink-l' Nest)
- ACP-supplied breeder chows (10-12% fat)
- ACP-supplied commercially available bedding (e.g., wood chip, spiral wood shavings, corn cob, paper bedding)

The following changes DO require a protocol amendment:

- All other plastic or wood enrichments
- All other dietary enrichments (LoveMash, sunflower seeds)

Please Note:

- Amendments to add enrichment devices or commonly used dietary supplements may be eligible for a quicker review. Please refer to IACUC Policy 39 on the Protocol Review Process for more information.
- Always speak with an ACP veterinarian or ACP facility supervisor prior to placing materials into animal housing as there may be special requirements (e.g. sterilization) or restrictions. Please read IACUC Policy 12 on Housing and Environmental Enrichment for more information on enrichment devices.

- Principal Investigators are responsible for making arrangements with ACP to provide enrichment and for ACP to supply specialty bedding or breeder chow on a recharge basis. Materials and labor costs not associated with standard housing will be the responsibility of the principal investigator.

Listed below are other factors to consider that could affect the success of your breeding colony.

Age of Breeders: Females <9 months, ideally <6 months; Males <12 months and have produced a litter in the last 3 months.

Age of Colony: Long-inbred, small colonies accumulate mutations; replacement with fresh stock from a vendor may help.

Cage Placement: Lowest shelf of rack, in low light and away from areas of high traffic.

Lighting: Ensure light cycle is optimal and lights are not turned on during dark cycle.

Environmental Factors: Assess and minimize vibration, noise, ultrasonic emissions, fluctuations in humidity.

Cage Disturbances: Reduce unnecessary handling and disturbance of cages.

Cage Changing Frequency: Reducing the number of animals in the cage, or cage density, reduces the frequency of cage changes needed.

Personnel Exposure: Minimize number and turnover of personnel handling the animals.

Seasonal Changes: Lower productivity may occur with fluctuations in weather or air pressure.

Post-Partum Breeding: Some strains will fare better if the dams are not gestating while weaning.

Aunting Female: Adding a non-pregnant, experienced female mouse to the cage may provide support to the pregnant dam.

If you have questions or concerns regarding your breeding colony, please contact the ACP veterinarians (acp-veterinarians-l@ad.ucsd.edu).