Title: Rodent Breeding  
SOP Number: 075  
Purpose: To establish general guidelines for rodent breeding and colony management in order to ensure the highest quality care for the mice while promoting physical and psychological well-being.

REPRODUCTION  
A. Sexual maturity  
a. Mice generally become sexually mature between five and eight weeks of age.  
b. Mice are usually mated between six and eight weeks old.  
c. Rats reach sexual maturity around four to five weeks of age.  
B. Reproductive life span  
a. Typically, laboratory mice can breed about 7-8 months,  
b. Produce four or more litters  
c. Strain specific characteristics may affect fertility and maternal characteristics and reduce total number of litters produced  
C. Gestation  
a. The gestation period for laboratory mice is generally 18.5 to 21 days depending on mouse strain.  
b. Rat gestation time is generally 21 to 23 days.  
D. Litter size  
a. Litter size varies among strains ranging from 2-3 pups/litter in some transgenic strains to 12 pups/litter in other inbred or outbred strains of mice.  
b. The usual litter size for rats is 6 to 12 pups

HOUSING  
A. Females  
a. Non-pregnant females may be housed together if they are compatible  
b. Pregnant females are generally housed individually  
c. Females may be re-grouped with compatible females after weaning  
B. Males  
a. Males may be housed together until they have bred  
b. After their first mating, males are then housed individually to reduce aggression  
C. Caging  
a. Pregnant females and those raising litters may be placed on the bottom of the rack to reduce lighting, vibration, and noise in breeding rooms.  
b. Enrichment may be provided to breeders.  
   i. Males and females may be provided with huts, wooden sticks/block, tunnels, and wheels on a rotational schedule.
ii. Pregnant females are provided with additional nesting materials such as alpha-dri and cellulose bedding.

iii. Shepherd shacks are provided to the female mice to help with the reduction of noise and light.

c. To reduce stress and prevent cannibalism, cage changes are postponed until after the female has given birth between days 5 and 7.

D. Lighting
a. Breeding rooms may be on a 14 - 10 light - dark cycle.

E. Diet
a. Males and females are fed a standard rodent diet.
b. Pregnant females are fed a protein enriched breeder diet.
c. Pups are offered softened rodent chow at day 14.

BREEDING
A. Breeding will be performed by the ACS Breeding Manager unless otherwise approved by the Veterinarians. Technician time may be applied to the Investigator’s ACS invoice each month. If space becomes limited in the barrier suite, preference will be given to ACS managed colonies and PI managed colonies will be relocated.
   a. If issues (i.e., overcrowding, separating, weaning etc.) are found in PI managed colonies that ACS has to perform, then ACS time may be charged at the investigator’s expense.

B. Breeding schemes will be determined through consultation with commercial vendors and the PI to maximize desired genotype or numbers. Sometimes multiple generations are needed before getting the desired genotype.

C. Pair, Trio or Harem breeding may be used.
   a. The females will be placed into the male cage for breeding.
   b. It is recommended to wait approximately 1 week in between breedings
      i. Post-partum breeding is not recommended in order reduce overcrowding and trampling of pups.

D. Pregnancy check
   a. Females will be checked for copulation plugs 12-24 hours after mating.
   b. The female is weighed on day 14. If gaining weight and the abdomen is visibly distended, then she is presumed to be pregnant and may be housed individually.

E. Replacement breeders
   a. Male breeders are replaced when they have not produced a positive pregnancy in a receptive female after 4 to 6 weeks.
   b. Females are replaced when they have cannibalized two consecutive litters or when the quality of the pups is not adequate.

F. Breeding of common, commercially available inbred and outbred strains for experimental purposes is highly discouraged due to genetic drift, formation of substrains and wastage of animals. If approved by the veterinarian, then only F1 generations can be bred.
RAISING A LITTER

A. Care is taken to handle the litter as little as possible and only when necessary. It is preferred that one person be designated to managing the colony, as rodents respond best to calm and consistent handling.
   a. Pups are counted and sexed during the first cage change between days 5 and 7.
   b. Clean gloves are worn and changed before handling the next litter.

B. Litter Fostering
   a. A foster mother that has had at least 2 or 3 successful litters will be chosen. To help in differentiating between both litters, a mother that has a different coat color than the foster pups is ideal but not required.
   b. The natural litter will be removed and reduced in size if needed, to accommodate the foster pups. The foster pups will be removed and cleaned of debris.
   c. Clean gloves will be used during the transfer. The foster mother will be restrained to encourage her to urinate on the gloves. The urine will be rubbed on the foster pups. Scent can also be transferred by rubbing bedding from the foster cage onto the pups. The foster pups are then placed in the cage among the mother’s own pups and mixed together, waiting 15 minutes before returning the mother.
   d. The cage will be placed on a quiet place on the rack and observed for signs of acceptance. If the foster mother does not accept the pups after 2 or 3 hours, another mother will be chosen.

C. Weaning
   a. Pups will be weaned between the age of 21-28 days depending on the size of the pups. Most outbreds and inbreds are weaned at 21 days, while some transgenics are too small and therefore weaning is extended to 28 days.
   b. Pups are sexed and transferred to same-sex cages with littermates.
   c. If pups are small in size even after reaching weaning age, soft chow and hydrogel may be provided on the cage floor for food and water.

D. Genotyping
   a. At weaning, pups may be genotyped using DNA isolated from:
      i. Ear punch
         1. The ear is cleaned with 70% ethanol and allowed to dry.
         2. 2-3 mm of the ear, from the middle of the pinna will be taken.
         3. Ear punch is also used as an identification method and the university ear punch system will be used.
         4. Anesthesia is not required.
         5. Ear punching may also be used as a means of identification.
      ii. Tail snip
         1. Genotyping may be performed or confirmed by taking a snip of the tail following SOP054.
ANIMAL USE
A. Colonies will be established based on need and managed according to principles of animal reduction.
B. Pups will be weaned and transferred to appropriate PI's experimental IACUC approved protocols.
C. Extra or unused pups may be euthanized or transferred to other PIs' protocols (i.e., training) to encourage reduction.

ANIMAL HEALTH MONITORING
a. Animals are observed daily by animal care staff for any evidence or illness or change in behavior.
   a. Everyone with access to the animal facility is responsible for immediately informing the facility manager or university veterinarians when an animal becomes ill or a change in behavior is observed.
   b. In the event of suspected illness:
      a. Complete the “Animal Treatment Record” in the Notebook. Indicate the date, room number/ pen number/animal number/cage or animal ID, the problem observed, and ensure that the name (or initials) of the person making the report is recorded.
      b. Contact the University Veterinarian or the ACS Facility Manager at:

         Dr. Tiffanie Brooks, ACS Attending Veterinarian
         806-834-8588 Office
         806-239-2120 Cell

         Dr. Paul Stonum, ACS Clinical Veterinarian
         806-834-7373 Office
         660-562-4425 Cell

         Sydnee Woodman, ACS Facilities Manager
         806-834-2872 Office
         602-758-0670 Cell

      c. Provide all the above information to the individual contacted above, who will give advice and authorization for the action(s) that should be taken.