Rodent Colony Management

This packet contains the following information:

- DLAM Rodent Breeding Information Sheet
- Weaning log
- Breeding log
- Factors That May Affect Breeding
- Estimating Rodent Fetal Age and Delivery by Palpation
- Characteristics of Mice from Birth to Four Weeks of Age
- Mouse Cage Density Policy
- Rat Cage Density Policy
- Rodent Weaning SOP
- Sexing Mice and Rats
- Mouse and Rat Identification Methods
- Rodent Ear Punch Pattern
- Rodent Tail Biopsies SOP

DLAM RODENT BREEDING INFORMATION SHEET

Breeding Parameters

Mice: breeding age: 6 – 8 weeks old
estrous cycle: 4 days
gestation: 19 – 21 days
retirement: 6 – 9 months or 5 – 6 litters

Rats: breeding age: females 8 – 9 weeks
males 10 – 12 weeks
estrous cycle: 4 – 5 days
gestation: 21 – 23 days
retirement: 6 – 9 months or 5 – 6 litters

Breeding Systems

In general, the male should be housed alone for 48 hours prior to placing the female(s) into his cage.

Monogamous:
Recommended
1 male: 1 female

Harem:
1 male: 2 females
Separate out females when pregnancy is confirmed to prevent overcrowding and pup mortality.

Polygamous: 1 male rotated between several females

Timed breeding:
Determination of exact gestation period based on date of conception.

Technique: Place female in a male's cage in the late afternoon.
The next morning, check for a vaginal plug and separate breeders.
The morning that the plug is detected is considered day 0.5.

Retirement Schedule

Replacement breeders should be selected from offspring of the 2nd (preferred) through 6th litter. The litter should contain at least four healthy pups.

Data Management

We recommend the following types of records:

1. Pedigree of colony.
2. Inventory log of all animals within colony – individual ID numbers, DOB, date weaned, parent ID numbers, strain, genotype, etc… (e.g. weaning log)
3. Breeding logs of all breeder females.
    a. Pairing info – date paired and with whom, date separated/retired
    b. Litter info – DOB, number of pups born, sex of pups, number and date weaned.

Weaning

Mouse pups are generally weaned between 21 and 28 days of age, and must be weaned by 28 days of age. Rats are generally weaned between 21 and 23 days of age, and must be weaned by 23 days of age.

INVESTIGATOR: _____ DATE: _____ ROOM #: _____

#	ID#	CAGE#	STRAIN	SEX	DOB	DAM	SIRE	DATE WEANED	COMMENTS
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									

BREEDING LOG

FEMALE ID_____ DOB_____ PARENTS _____

Paired with male ID# _____

Male DOB _____ Male Parents _____

Date Paired _____ Date Separated _____

Pregnant _____ Pups and #_____

Weaned _____ # of Pups _____

Paired with male ID# _____

Male DOB _____ Male Parents _____

Date Paired _____ Date Separated _____

Pregnant _____ Pups and #_____

Weaned _____ # of Pups _____

Paired with male ID# _____

Male DOB _____ Male Parents _____

Date Paired _____ Date Separated _____

Pregnant _____ Pups and #_____

Weaned _____ # of Pups _____

FACTORS THAT MAY AFFECT BREEDING

Bruce Effect - A pregnant female may abort her pups if introduced to a new male within 24 hours of conception.

Whitten Effect - Estrus can be induced by placing soiled bedding from the male's cage in the female's cage. Most females will come into estrus within 72 hours of exposure to the male's scent. You can use the Whitten effect to enhance the breeding efficiency of your rodents. It is more reliable in mice than in rats.

Lee-Boot Effect - The increased incidence of false pregnancies seen in females that are housed together. This is callused by a pheromone in the animal's urine that causes the estrous cycle to slow down and eventually stop. The Lee-Boot effect may prolong the time for a female to become pregnant following pairing with the male.

Estimating Rodent Fetal Age and Delivery by Palpation

Fetuses can initially be palpated between the 10^{th} and 12^{th} day of gestation.

Gently scruff the female and place your forefinger behind the ribs over the lumbar area. Next, gently roll the abdomen between your thumb and forefinger. This is what the fetuses will feel like:

Day 10-12: they will feel like knots on a string
Day 14: they will feel like firm pea-sized masses
Day 15-16: they will feel mushy
Day 17-18: they will feel bean-shaped

Near delivery time, you can feel the head, body and hips.

Signs to help determine when delivery is about to happen:

- A gap in the pelvic symphysis; this can be detected by placing your finger just above the vaginal opening and feeling for increased spacing of the pubic bones.

- Other signs may include vaginal discharge, observation of pup movement in the female's abdomen and increased activity or excitability.

Characteristics of Mice from Birth to Four Weeks of Age

Age	Characteristics
Birth	Blood red skin color
1 day	Lighter red skin color; milk visible in stomach
2 days	Lighter skin color; ears flat against head
3 days	Ear elevated about 45° away from head
4 days	Ears elevated 90° away from head
5 days	Skin thicker; milk no longer visible in stomach
6 days	Fur starts as a fine stubble over back
7 days	Complete coat of fine, fuzzy fur is visible
8 days	Lower incisors visible, but not erupted
9 days	Inguinal nipples visible in female
10 days	Lower incisors erupted
11 days	Upper incisors erupted
13-14 days	Eyelids open; slit-like palpebral opening
3 weeks	Oval palpebral opening; fine soft fur; triangular shape to head
4 weeks	Round palpebral opening; smooth fur; trapezoidal shape to head

MOUSE CAGE DENSITY POLICY

Overcrowded mouse cages represent a significant animal welfare concern. Such cages are noncompliant with Public Health Service (PHS) Policy and our Assurance to PHS. *The Guide for the Care and Use of Laboratory Animals* states the PHS recommendations for housing densities. In order to standardize housing densities and prevent or eliminate the possibility of overcrowding within cages, the University's Animal Resource has adopted the following UCAR-approved policy. **Ventilated cages accommodate up to five adult mice. Static cages accommodate four adult mice in standard isolation (SI) and three adult mice using microIsolator technology (MIT) husbandry.** Cage densities exceeding these numbers represent clear policy violation.

Breeding

- The two breeding schemes permitted are:
    1. Monogamous pairing (1 male: 1 female) - this method is preferred to prevent overcrowding.
    2. Trio grouping (1 male: 2 females) - females must be placed in individual cages prior to parturition.

- Male and female mice should be separated after pregnancy confirmation to avoid post-partum insemination. A post-partum estrus occurs within 14 to 28 hours after parturition in mice.

- No more than two adult females and one litter of pups may be housed in a standard mouse cage without UCAR approval.

- The breeding strategy must be described in the UCAR protocol. This includes the breeding scheme, whether continuous or non-continuous breeding will occur and the weaning age of pups. Justification is required for any scheme other than monogamous and trio, for continuous breeding and/or for cage densities which exceed those described above.

Weaning

Investigators who choose to manage their own breeding colonies are responsible for timely weaning. Conventional mice are typically weaned at 21 days of age. The Animal Resource staff reports the date new pups are found in a log maintained in a binder in each mouse room. There is a separate log sheet for each investigator. The date when pups reach 29 days of age is calculated in the last column of the log. Litters not weaned before their 29th day of age will be reported to the investigator on day 29. DLAM will separate unweaned litters the following morning for a charge of $50.00 per cage. Delayed weaning protocols must be approved by UCAR with specification of actual weaning ages. Additionally, a special request must be submitted to the DLAM/Vivarium office identifying the group of mice approved for delayed weaning.

Where continuous breeding is approved, weaning of older litters between 17 and 20 days may be necessary. Should the presence of an older litter constitute a threat to a newborn litter, DLAM will notify the PI to separate immediately. In the absence of an immediate response by the investigator, DLAM will wean the older litter for a fee. The investigator will be informed.

The DLAM veterinary staff provides training in the management of rodent colonies for investigators and their staff. DLAM also offers colony management services to those PIs who choose this option.

Overcrowded Cages

Overcrowded cages (> five adult mice in ventilated cages, > four adult mice in static SI cages and > three adult mice in static MIT cages) will be reported to investigator. DLAM will remove mice from overcrowded cages if the investigator has not done so by the day following notification. There is a fee for this service.

Identification

A completed cage card must be present on all mouse cages. Please refer to the Animal Resource website (http://www.urmc.rochester.edu/vivarium/Barcoding.cfm) for information on cage card activation. The information on the card should include: the investigator's name, the approved UCAR protocol number, an animal identification number (if applicable), the mouse strain/stock and the account number. Individual animal identification such as ear punches, ear tags, toe clips, tattoos and implantable transponders is encouraged, especially in cases where animals are group housed and/or appear identical. All methods of identification must be described in the animal protocol and approved by UCAR.

The DLAM and Vivarium staff is available to discuss any questions you may have regarding this policy. Please do not hesitate to contact the Animal Resource Office at X5-2651.

RAT CAGE DENSITY POLICY

Overcrowded rat cages represent a significant animal welfare concern. Such cages are noncompliant with Public Health Service (PHS) Policy and our Assurance to PHS. The *Guide for the Care and Use of Laboratory Animals* states the PHS recommendations for housing densities. In order to standardize housing densities and prevent or eliminate the possibility of overcrowding within cages, the University's Animal Resource has adopted the following UCAR-approved policy: **No more than two adult males or three adult female rats may be housed per standard rat plastic cage (10.5 in. X 19 in. X 8 in.). Pregnant females may only be housed in solid bottom plastic cages.**

The use of wire bottom cages is not permitted unless scientifically justified and approved by UCAR. A small wire bottom cage (11.5 in. X 7 in. X 8 in.) may accommodate one rat of any weight or two rats weighing less than 400 grams each. A large wire bottom cage (11.5 in. X 16.5 in. X 8 in.) may accommodate four rats each weighing less than 400 grams, or three rats each weighing between 400 and 500 grams.

Breeding

- The two breeding schemes permitted are:

    1. Monogamous pairing (1 male: 1 female) – this method is preferred to prevent overcrowding.

    2. Trio grouping (1 male: 2 females) – females must be placed in individual cages prior to parturition.

- Male and female rats should be separated after pregnancy confirmation to avoid post-partum insemination. A post-partum estrus occurs within 48 hours of parturition.

- No more than two adult females and one litter of pups may be housed in a standard rat cage.

- The breeding strategy must be described in the UCAR protocol. This includes the breeding scheme, whether continuous or non-continuous breeding will occur and the weaning age of pups. Justification is required for any scheme other than monogamous and trio, for continuous breeding and/or for cage densities which exceed those described above.

Weaning

Investigators who choose to manage their own breeding colonies are responsible for timely weaning. Rats are generally weaned at 21 days of age. At this age, the pups are placed on inventory by the vivarium staff and the PI is notified. At 23 days of age, the PI will be notified if litters have not been weaned. The following day, these rats will be weaned by DLAM for a $50.00 fee. Delayed weaning protocols must be approved by UCAR with specification of actual weaning ages. Additionally, a special request must be submitted to the DLAM/Vivarium office identifying the group of rats approved for delayed weaning.

Where continuous breeding is approved, weaning of older litters between 17 and 20 days may be necessary. Should the presence of an older litter constitute a threat to a newborn litter, DLAM will notify the PI to remove the older litter immediately. In the absence of a prompt response by the investigator, DLAM will wean the older litter for a fee. The investigator will be informed.

The DLAM veterinary staff provides training in the management of rodent colonies for investigators and their staff. DLAM also offers colony management services to those PIs who choose this option.

Overcrowded Cages

Overcrowded cages (more than two adult males or three adult females) will be reported to investigators. DLAM will remove rats from overcrowded cages if the investigator has not done so by the day following notification. There is a fee for this service.

Identification

A completed cage card must be present on all rat cages. The information on the card should include: the investigator's name, the approved UCAR protocol number, an animal identification number (if applicable), the rat strain/stock and the account number. The use of individual animal identification such as ear punches, ear tags, tattoos or implantable transponders is encouraged, especially in cases in which animals are group housed and/or appear identical. All methods of identification must be described in the animal protocol and approved by UCAR.

The DLAM and vivarium staff are available to discuss any questions you may have regarding this policy. Please do not hesitate to contact the Animal Resource Office at X5-2651.

UR Animal Resource SOP# D-4 **Title:** RODENT WEANING

Original Date: November 1998 Original Composer: Diane Moorman-White, DVM
Wendy Bates, DVM

Date of Previous Revision: October 19, 2011

Date of Current Revision: November 29, 2011 Revised By: Wendy Bates, DVM
Departmental Approval:

PURPOSE

To provide a standardized method for weaning rodent cages and completing new cage cards.

MATERIALS

Rodent cages to be weaned, clean (+/- autoclaved) complete cage set-ups, blank cage cards and pen

PROCEDURE

1. At 21-28 days of age for mice or 21-23 days of age for rats, determine if pups are ready to be weaned. This decision should be based on pup size, activity level, ability to reach food and water, etc.

2. Prepare cage cards for cages which will hold weaned animals. The cage card should indicate the cage from which the rodents originated. The investigator's name, strain, number, sex, UCAR #, and account # should also be completed. Include the birth and weaning dates on the bottom portion of the cage card.

3. For mice and rats, if you have preprinted cage cards with bar codes, activate each card by filling out a barcode activation form located in the animal room. Return this form to the Animal Resource office or place it in a black barcode depository box. If there is no pre-printed card, fill out a temporary cage card, which is found in the animal room. Place the bottom copy on the cage and submit the top two copies to the Animal resource office or place in a barcode depository box. Refer to http://www.urmc.rochester.edu/vivarium/Barcoding.cfm for more information on cage card activation.

4. Separate offspring from the parent cage according to sex. Place same sex animals in each cage. Place 3-4 pieces of rodent chow on cage floor. If the cage will be placed on a rack with automatic watering, **place a water bottle on the cage as well**. A supply of water bottles is available in each room or suite. Make sure all cages have bedding, food, water, Nestlets (mice) or Nesting Sheets (rats) and cage cards before placing on rack.

5. Write the number of mice or rats weaned and the weaning date on the parent's cage card. You can use shorthand for # females: # males - always write the number of female pups first. For example, three female pups and two male pups would be designated as 3:2.

PARENT CAGE

ID # Cage #	P.I. Sharpe
STRAIN C57BL/6	CO P.I._____
SEX ♀/♂	UCAR # 99-6
ID of ♀+♂ and pair date	ACCT# 5-1234-5

1. 1-31-98 3:2 w. 2-21-98
2.
3.

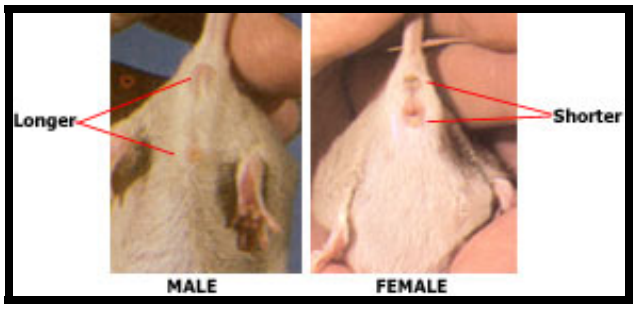
WEANED OFFSPRING CAGES

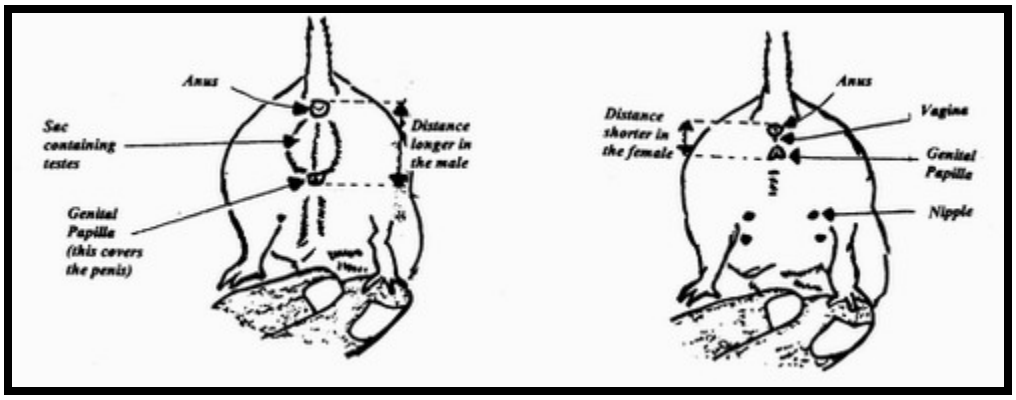
ID# Cage # STRAIN C57BL/6 SEX 2 males _____ Parent ID #s DOB W Pup ID #s	PI Sharpe CO PI_____ UCAR # 99-6 ACCT# 5-1234-5

ID # Cage # STRAIN C57BL/6 SEX 3 females _____ Parent ID #s DOB W Pup ID #s	PI Sharpe CO PI _____ UCAR# 99-6 ACCT # 5-1234-5

6. If there are separate cage cards for the male and female breeders, write the pup information (birth date, weaning date, number and sex) on the female's cage card.

SEXING MICE AND RATS



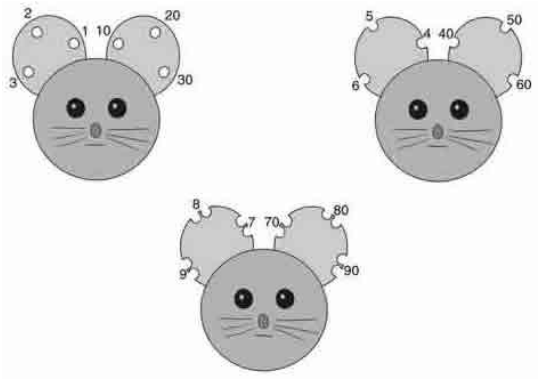


MOUSE AND RAT IDENTIFICATION METHODS

There are several acceptable methods to permanently identify laboratory rodents. A description of the identification method used must be included in the Procedures (Descriptive Information) section of your approved University Committee on Animal Resources (UCAR) protocol.

- **EAR PUNCH:** This is a commonly used procedure which employs a special metal punch instrument to place a hole in the ear of the rodent, following a code (below). **Advantages:** (1) quick and easy to perform (2) inexpensive (3) relatively atraumatic, (4) no anesthesia required (5) punched tissue can be used for DNA (PCR) screening. **Disadvantages:** (1) cannot be performed on pups under two weeks of age due to size and position of ears (2) potential exists for ear damage (3) may be difficult to read.

Ear punch numbering system



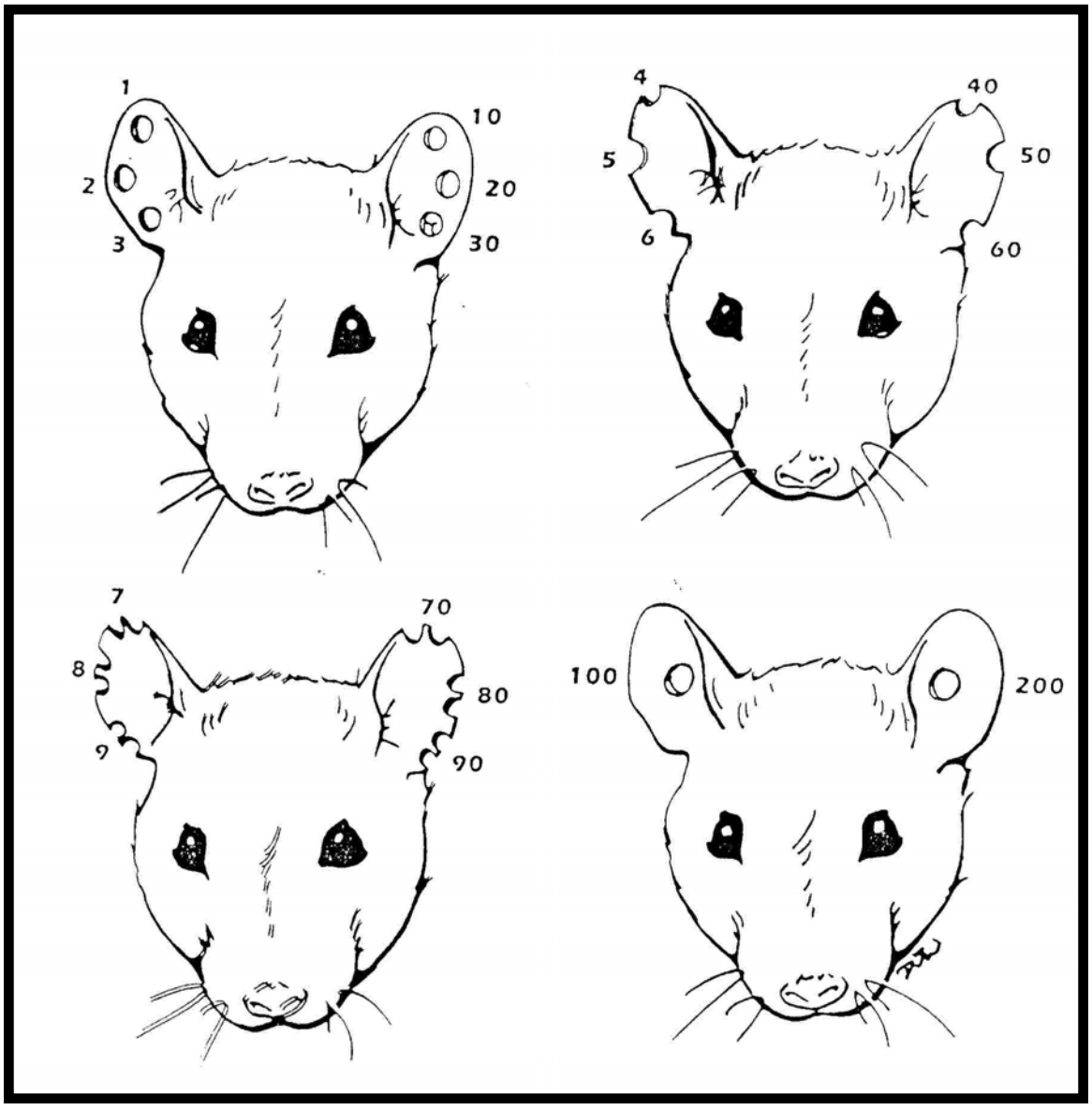
- **EAR TAGGING:** Numbered metal clips can be applied to the base of the pinna with special pliers. Various sized tags exist; the appropriate size must be selected for the species being identified. **Advantages:** (1) quick and easy to perform (2) relatively atraumatic (3) no anesthesia required (4) relatively inexpensive. **Disadvantages:** (1) cannot be performed on pups less than three weeks of age due to size and weight of tags (2) tags can fall out (3) tags may cause granulomas at site of application.

- **TOE CLIPPING:** This method involves the removal of the distal portion of one toe per foot on no more that two feet for individual animal identification purposes in addition to genotyping. The Guide for the Care and Use of Laboratory Animals states that this identification method is only appropriate for altricial neonates and is to be used when a less invasive method of identification is not practical. Because this method may cause more than momentary pain, its use must be scientifically justified and approved by UCAR. Toe clipping must be performed in accordance with the UCAR Toe Clipping Policy. **Advantages:** (1) easy to read (2) inexpensive (3) can be successfully employed in neonates (4) clipped tissue can be used for DNA (PCR) screening. **Disadvantages:** (1) may cause pain, (2) lameness, (3) infection and (4) decreased grasping ability.

- **TATTOOING:** Tattoo ink can be injected under the skin of all rodents, using either a tattoo needle or a hypodermic needle and syringe. Appropriate tattoo sites include: tail –all rodents, ears –guinea pigs. Neonatal rodents may be tattooed on the ear, tail, hock or toe. **Advantages:** (1) easy to read, (2) can be used on neonates. **Disadvantages:** (1) requires anesthesia (2) may require special equipment (3) potential for infection (4) tattoos can fade or spread as the animal ages (5) may be difficult to read in pigmented animals.

- **ELECTRONIC TRANSPONDERS:** microchip transponders are implanted via subcutaneous injection. A special recording instrument reads and displays the number on the scanner. **Advantages:** (1) no anesthesia required (2) easy to read (3) fast (4) some chips can be linked to computer system that records other data about the animal. **Disadvantages:** (1) initial cost of equipment (2) chips can fall out (3) requires special equipment to read identification (4) potential for infection.

Rodent Ear Punch Pattern



UR Animal Resource SOP# D-6 **Title:** RODENT TAIL BIOPSIES

Original Date: February 1999 Original Composer: Diane Moorman-White, DVM
Wendy Bates, DVM

Date of Previous Revision: March 7, 2003

Date of Current Revision: April 11, 2008 Revised by: Andrew Winterborn, DVM

Departmental Approval: Reviewed by: DLAM staff at weekly meeting 9-4-09

PURPOSE

To provide instruction for obtaining genetic material for DNA isolation via tail biopsy.

MATERIALS

*Mice or rats of weaning age (3-4 weeks old) ideally
Identification instrument
Straight edged razor blades – 1 blade per 2-3 animals
Autoclaved nestlet material
Specimen vials (e.g. eppendorf tubes)
Ketamine 60-90 mg/kg IP for older rodents
Styptic or antibiotic powder for older rodents
Tail biopsy log

PROCEDURE

1. At weaning, identify animals by ear punch, ear tag, etc.

2. Manually restrain the mouse/rat with distal portion of tail situated on surface of nestlet.

3. Using 1/3-1/2 of a straight edged blade, remove ~ 7 mm of distal tail. Some bleeding will occur, but no special treatment is required for weaning age animals.

4. Return rodent to cage.

5. Place tail tissue in specimen vial and label with animal ID number and sex. If you are maintaining a tail biopsy log, make sure you record this information on the log sheet.

6. The unused part(s) of the blade can be used to transect the tail tip on the next animal, making certain to avoid any blood contamination. Once each part of the blade has been used once (2-3 mice), discard the blade in a sharps container.

7. Change nestlets when they become soiled, or between investigator's animals.

8. Store specimens in a freezer designated by investigator.

*Tail biopsies may be performed on mice or rats of any age. Anesthesia (e.g. ketamine) is required for rodents beyond weaning age. Inject ketamine (diluted 1:10 with sterile saline or water for injection for mice) at a dosage of 60-90 mg/kg intraperitoneally. Once rodent is anesthetized, follow tail biopsy procedure above. Heavy bleeding is more likely in older mice and rats. For this reason, it is recommended to dip each tail in styptic powder or an antibiotic powder (e.g. Biozide).

If you have any questions about colony management or any other animal related issues such as handling, injections, specimen collection or surgery, do not hesitate to contact DLAM at X5-2651. We provide individualized or group training sessions for special procedures. Please remember animal research is a privilege, and animals should always be treated with care and respect.