Mouse Breeding Colony Management

Know where you want to go …or you might end up somewhere else!
Mouse Breeding Colony Management

Why Mice?

- Mice comprise the majority of all research animals
- They are genetically similar to humans
- They mature quickly and can advance in generations quickly
- They are inexpensive to maintain
- They have a short life span and can be studied throughout their entire life cycle
- They are small and easy to house
- They are easy to breed
Mouse Breeding Colony Management

Why do I Need A Breeding Colony

- To produce a new line, strain, or expand an existing colony?
- To maintain a line?
- To supply animals for research?
- To provide animals for experimental use
- To provide enough animals for replacement breeders
- To keep the colony viable
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General Breeding Information

**Sexually mature** - 6-8 weeks of age

**Average estrous cycle** - 4-5 days

**Estrous** - approximately 20 hours

**Gestation** - 19-21 days can be strain dependent

**Weaning age** - 20-21 days but strain dependent some transgenic strain require up to 28 days. Longer than normal weaning age (21 days) requires approval from the IACUC

**Post-partum estrus** - approximately 20 hours after parturition

**Reproductive life** - approximately 6 litters
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FVB

Albino coat color
Adults > 30 grams
Aggressive
Average breeders
Good mothers
Large litters
  9.5 pups/litter average
  (JAX)
C57BL/6

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Black coat color
Adults 25-30 grams
Developed by C.C. Little ~ 1921

Health:
  - Eye defects
  - Malocclusion
  - Dystocia
  - UD (ulcerative dermatitis)
  - Hydrocephalic (JAX)

Commonly used as an inbred background strain

Average breeders:
  - 6.6 pups/litter & 87% weaned (JAX)
Several sub-strains range in color from white to chinchilla or agouti (129SvEv Taconic).

Docile

Source of ES cells

Variable breeders (genotype dependent)

- 129/J: 4.6 pups/litter
- 129/SvJ: 6.0 pups/litter
# Mouse Breeding Colony Management

## Reproduction Performance for Common Strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Breeding Performance</th>
<th>Average Litter Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD-1 (outbred)</td>
<td>Excellent</td>
<td>7+</td>
</tr>
<tr>
<td>B6C3F1(hybrid)</td>
<td>Excellent</td>
<td>7+</td>
</tr>
<tr>
<td>DBA/2</td>
<td>Poor</td>
<td>3-4</td>
</tr>
<tr>
<td>Tg/KO</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
If you don’t have a goal, it’s hard to make a plan...
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Three “R”s of Research

**REDUCE** the number of animals in research (or excessive production)

**REFINE** experiments to minimize pain, distress, or unnecessary production

**REPLACE** animal use with computer models or in vitro research
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Colony Goals

**Maximize output**: per cage, per unit area, and per unit of labor by selecting breeders properly and by replacing unproductive breeders

**Maximize quality**: health, genetic authenticity, and uniformity of offspring

**Equate supply with demand**: low wastage and flexibility of production
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What is the Goal of the Project

- Number of mice needed per week or month to meet experimental needs
- Continuous vs Intermittent production
- Can you use Males and Females or only one sex
- How often can you expect a litter
- How many pups of the appropriate genotype can you expect per litter
- How often will you have to replace breeders
- Is a Genetic Profile needed
- How long will the breeding colony be maintained
- What will happen to the mice when the project is completed
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How to set Goals for Production
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Know Mouse Behavior

- Gnawing mammals (rodents)
- Omnivorous, semi-continuous feeders
- Nocturnal, secretive & nesting
- Thigmotactic
- Social & communal with male-dominated hierarchy
  - Males will fight: injuries & fatalities possible.
- Communicate via pheromones
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Know Mouse Life Span

- Genotype dependent
- Biological life span ~ 2 years

- **Optimal breeding life span is much shorter**
  - female ~ 9 months
  - male ~ 1 year

- Basic life stages:
  - Neonate: birth to wean (21-28 days)
  - Sexual maturity: ~6 weeks
  - Adult size: 8 - 10 weeks
  - Geriatric: 18 months
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Know Mouse Reproduction Data

- Most strains reach sexual maturation between 6 to 8 weeks of age. Start breeding at 7-8 weeks age.
  - Age of sexual maturity is strain dependent.
  - Breeding too young or too old can result in poor performance.
    - Too long between litters
    - Litters very small; unthrifty
    - Dystocia
- Breed at night, about 1 a.m.
- Gestation is roughly 19-21 days
- Wean at roughly 21-28 days
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Know Mouse Reproduction Data

The Estrous Cycle:

- 4-5 days long.
- Can last up to 10 hours.
- Signs of estrus include:
  - Swollen vulvar area
  - Redness at vulva
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Select the Proper Breeding System

Intensive pairs or trios
Non-intensive breeding system
Timed matings
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Select the Proper Breeding System

Intensive Breeding System

Mate one male with one or two females in the same cage for the entire useful breeding life.
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Select the Proper Breeding System

Disadvantage to Intensive Breeding System

- High demand for space, cages, and equipment
- Supports more males than are actually required for production
- Must wean older litter before second litter is born or risk death of newborns
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Select the Proper Breeding System

Intensive Breeding System

“Should I Use Pairs or Trios”

- When average litter size is small (<7) it is better to use two females in a cage
  - Both moms will care for any pups born and will tend to cycle together
  - This is especially true for transgenic and knockout mice

- When average litter size is large (>7) it is better to use only a single female to prevent overcrowding in the cage as pups mature
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Select the Proper Breeding System

Non-intensive breeding system

Females are placed in the male’s cage only for breeding and then separated.
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Select the Proper Breeding System

Non-intensive breeding system

Advantages

- Reduced risk of fighting between aggressive females
- Males cannot kill the newborns
- Increased flexibility of production

Disadvantages

- High Labor costs
- Lost access to postpartum estrus
- Requires more observation of moms to identify mice that are poor producers verses mice that are “forgotten” by technician
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Select the Proper Breeding System

Timed matings

Used when you need to know exact day of gestation to collect embryos or forecast delivery date

Females are added to males cage then checked each morning for “plugs” to confirm mating

When plug is found that is “DAY 1/2” (calculate that breeding actually took place at midnight / early morning that day)
What is a “Plug” in Mouse Breeding

- The ejaculate from the male's accessory sex glands forms a short-lived, white to yellowish plug in the vagina of the female.

- Presence of a vaginal plug is often used to determine if copulation occurred between mice.

- By checking female breeding mice each morning, the presence of a vaginal plug allows one to estimate the approximate time of mating as the middle of the preceding night.
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Checking for Plugs

Restraint
  on wire lid is easiest by scruff
Tools
  forceps
  toothpick
  probe
  fingers
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Checking for Plugs

- Vaginal plug
- No Vaginal Plug
Checking for Plugs

PRESENCE OF A PLUG DOES NOT GUARANTEE PREGNANCY

15% or more of plugged females are either not pregnant or never gestate due to reabsorption of fetuses.
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Checking for Plugs

ABSENCE OF A PLUG DOES NOT GUARANTEE FEMALE IS NOT PREGNANT

Plug may have dissolved before you saw it, or many have been too deep, too small, etc.
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Gestation & Detection of Pregnancy

Mouse gestation is 19-21 days.
In absence of plug check, detection of pregnancy is hit or miss.

- Observation
- Parturition
- Palpation

At about 14 days, may detect what feels like a string of pearls
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Parturition

- Giving birth at the natural end of gestation
- Best chance of litter survival when female is left undisturbed for 2-3 days before and 4-5 days after parturition
- Arrange for cage to be changed beforehand or left unchanged during this period if sensitive strain
- Reduce handling and observations to a minimum
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Postnatal Development & Weaning

- Recognizing developmental stages helps determine how old pups are
- Weaning on time prevents doubling up of litters and trampling to death of new litters
  - Timely weaning also prevents “Overcrowded Cage” charges
- Normal v. abnormal development:
  - runts
  - malocclusion
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Mouse Age Determination
Day 1: Blood red in color and hairless. Very small, no milk spot. Eyes and ears are closed
Day 2: Dark pink, Milk spot is present, Eyes and ears closed
Day 3: Pink. Slightly larger, milk spot is bigger and more opaque. Ear nubs are apparent
Day 4: Pink. Ears stand out from head
Day 5: ‘Stubble’ or ‘dander’ on back, early signs of pigment (on dark furred mice) Milk spot fading
Day 6: Fur and pigment starting to show. Ears more prominent and detailed
Day 7: Bigger. Fur and pigment filling in
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Mouse Age Determination

Days 8-10: Feet look more formed. Inner ears look fully developed. Eyes are still closed most of the time. Fur and pigment fully established.

Days 12-14: eyes are open. Pups are mobile and may seem hyper or jumpy due to auditory development.

Days 14-16: begins to eat solid food. Looks anatomically formed but slightly juvenile in posture and attitude.

Days 17-21: Fully active. Looks like a complete (but small) adult. Ready for weaning at 20-21 days old.
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Aging Mouse Pups

Day 0-1

FOR ADDITIONAL PHOTOS AND INFORMATION TURN CARD OVER

PROVIDED BY: St. Jude Children's Research Hospital, Memphis, TN Animal Resources Center
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When to Wean

Very Important to Wean on Time!

Note size of 21 day old pups and if mother is pregnant again

Timely Weaning prevents Trampling Deaths and overcrowded charges!
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Set Up New Weanling Cages

- Carry over some dirty bedding and nestlet from parent cage
- Put 5-6 pellets of food on cage floor
- Be sure food and bottle are correctly placed
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Separate Males & Females
Sexing 21 day old pups:
- Anogenital distance
- Presence of nipples

Maximum # Mice & Pups per Cage Refer to:
- DLAR Mouse Housing Density Policy
- DLAR Housing Information
- IACUC Policy 110
Mouse Breeding Colony Management

Weaning Index

- Calculate a weaning index for each breeding pair and for each strain, To estimate and track production

- A weaning index incorporates all unfavorable factors to give you the average numbers weaned per month per female

- This number can vary considerably depending on the strain that you are working with

- The lower the index, the more time and resources will be needed to reach your goal
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Weaning Index

To Calculate For Each Female Take:

Total number of pups **weaned**
Divided by the number of months she has been mated

**Example**

20 (pups weaned) ÷ 5 (months mated) = 4 pups per month
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Weaning Index

Factors That Influence A Weaning Index

- Some induced mutations cause low production or poor viability (failure to thrive) or genetic lethality
- Poor breeder productivity, pre-weaning deaths, cannibalism, still-born pups
- Delayed implantation or environmental incidents
- Long gaps between litters
- Harem breeding requires separation from males eliminating post-partum breeding opportunity
- Age of males and females: The older they are, the less productive
Retire & Replace

- Retire mice over 6-8 months old or after 5-6 litters
- Replace breeders that have produced 2 consecutive poor litters (quality or quantity)
- Replace males that have not produced a positive pregnancy after 3-6 weeks with a receptive, fertile female with a different male
Mouse Breeding Colony Management

Organize your Colony & Projects

- Use different and **consistent** color of cage card for each strain
- Physically separate similar strains (names, coat colors, etc.) as much as possible
- Use correct nomenclature and identification on every card
- Keep older cards in the cage card holder until cage is eliminated from the colony
- Identify mice by physical ID (ear punch, tag, etc.)
Mouse Breeding Colony Management

Organize your Colony & Projects

Identify Strains or Projects
# Mouse Breeding Colony Management

## Cage Cards for Breeding Cages

<table>
<thead>
<tr>
<th>PI:</th>
<th>Protocol#</th>
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<tbody>
<tr>
<td>Strain:</td>
<td>Mating Date</td>
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<tr>
<td></td>
<td>Mating #</td>
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<table>
<thead>
<tr>
<th>Female ID#</th>
<th>DOB</th>
<th>Genotype</th>
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<th>Male ID#</th>
<th>DOB</th>
<th>Genotype</th>
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<table>
<thead>
<tr>
<th>Date Born</th>
<th># Born</th>
<th>Date Weaned</th>
<th>Females Weaned</th>
<th>Males Weaned</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
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</table>

breeding card
### Mouse Breeding Colony Management

**Information for Breeding Cage Cards**

- **Breed and strain**
- **Ancestry**
- **Animal ID #s**
- **Sex**
- **Date mated**
- **Date of birth with number of pups**
- **Date weaned with sex and number of weanlings**
- **Veterinary information**

![Breeding Cage Card Example](image.png)
Mouse Breeding Colony Management

Record-Keeping

Accurate and current records are one of the most important factors in an efficient breeding program:

- Aids troubleshooting
- Saves time
- Improves organization

Facilitates compliance with divisional, university, local, state, and federal guidelines and laws
A well-maintained record keeping system would allow one to monitor:

- Individual animals, their ancestors, siblings and descendants
- Matings between animals
- Litters born from such matings, and the individuals within litters that are used experimentally or for the next generation of breeding
- Experimental material (tissues and DNA samples) obtained from individual animals
- Test results from the samples
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Record-Keeping

Types of Records:

- Colony, strain or project
- Physical identification
  - Cage cards
  - Individual animal
- Electronic database
  - SCION from Topaz
  - MS Excel spreadsheet
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Animal Identification

Ear Punch or Notch

Tattoo

Ear Tag
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Animal Identification

- Microchips are durable ID
- They require the use of scanners to read
- They can be used to measure other bodily functions such as heart rate, temperature, etc.
- They can be expensive compared to tattoo or ear punching
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Troubleshooting

My mice aren't breeding...
My mice keep eating their pups...
My female died and I need to foster a litter...
How can I increase litter size....
Help! My pups aren't what I expected...
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My Mice are not Breeding…

- Check sex of mice in the cage (should be 1 male and 1 or 2 females)

- Check age of mice (retire at 6-9 months)

- Check environmental factors in the room (light cycle, noises, traffic flows, etc.)

- Try using a different male

- Are you sure they aren’t breeding? Try doing plug checks to see if the loss of pups is occurring after conception

- Replace the breeders with a younger pair
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My Mice are Eating Their Pups…

- Mice may cannibalize litters when disturbed during the first few days after delivery
- Some mice will cannibalize only their first litter
- Make sure the room is quiet and the cage location avoids unnecessary traffic..

- Move mice to a more secluded location in the mouse room and do not handle or disturb nest for several days after delivery
- Try removing the male before delivery (non-intensive system)
- Foster pups if needed and replace that breeder
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In a Perfect World

- Breeding mice would involve nothing more than putting a male and a female together...

- wait a few weeks and.........
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In a Perfect World

TAA-- DAAH!

- Babies are born
- Are cared for by mom
- Mature to weaning
- Life is good!
Who Lives In a Perfect World

Sometimes things happen…

- Moms die

- Genetic or physical abnormalities prevent moms from caring for litters

- Some mice are just bad moms!
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Time Will Come to Foster a Litter
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When Do I Foster?

- Foster when keeping the pups alive is crucial to the strain

- Foster when there is no hope the mom can or will care for them

- Foster early! Each hour spent without proper care is critical; especially to newborns
Select an Experienced Mom

- Select a mom which already had a normal size litter about the same age as the one to be fostered
- For best results, select a litter that is no more than 48 hours older than the litter to be fostered
- Set litter aside and add in 2 new pups with 3 of foster mom’s own pups
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Some Moms are Better than Others

- Ideal foster mom and litter will be of a different coat color as the foster pups for easy identification.
- Select moms that have had 2-3 successful litters weaned already.
- B6CBAF1 strain make excellent foster moms.
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Keep Total Litter Size the Same

• Remove as many of her own pups as you wish to replace with foster pups

• Milk production will suffer if total litter size varies by more than 2 from the original number

• Humanely Euthanize extra pups from mom’s original litter
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Mix the Litters

- Set litter / pups to be fostered aside
- Keep pups to be fostered warm and ensure pups viable and active
- With clean gloved hand mix foster pups with remaining pups in the nest from her birth litter
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Leave Them Alone

- Leave mom and litter in a secluded, quiet place and observe from a distance her acceptance of the new pups

- Moms will usually clean and nurse the new arrivals within the hour, if they are going to accept them

- Successful foster should be noted in the first 6-12 hours or try another mom
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You can Never Have too Many Reminders

- Make reminder notes of the ID on the cage card so you will know which are which at weaning.
- Make similar notes on the birth mom’s cage as well.
Successful Fosters

- Best results come from the least amount of stress put on the fostering mom. Keep handling to a minimum.

- BE SURE to correctly identify strain and pedigree of the fosters when time to wean!

- Note the success of the foster mom for use in the future
How to Increase Litter Size

- Litter size is often a trait specific to the strain
- Selecting breeders for good performance may insure maximum production
- Feed higher fat breeder chow (2918 = 6% fat; 2919 = 9% fat)
- Intensive breeding system with 2 females will often increase overall success of the colony
- Retire and replace lower than average breeders promptly to maximize colony production
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Handling Unexpected Results

If your weanlings are not what you expected… wrong phenotype, wrong genotype, abnormally large litter size, or yield unexpected experimental results then you should

❑ Suspect your Breeders Have a Problem

 Or

❑ Check your Breeding Colony Records
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Handling Unexpected Results

- Check the weanling card for the wrong ID or wrong parent information (match up parent and DOB info).
- Check the breeder card for the wrong ID (a homozygous cage? Back-crossed cage? Wrong strain name? Wrong selected color of cage card? Mixed up cage cards?).
- Re-check the breeders genotype (strain impurity can result in greater than average litter size).
- Re-check your records for data on previous litters and any genotype testing on pups.
- Eliminate that cage and start over.
Dystocia

Female is dilated and clearly stressed:
   Remove pups and foster surviving pups
Female has delivered a few pups (dead or alive) and stops for several hours, but is active and alert:
   Observe to make sure delivers rest of pups (usually dead), wait 7-10 days, then re-mate

Heroics if needed:
   Immediately resuscitate any viable pups
**If valuable female euthanized or dies, immediately remove ovaries for ovary transplants to maintain gene pool**
Mouse Breeding Colony Management

Dietary Management

Harlan(ENVIGO) Global Rodent diets

2918 Irradiated

Key Features
* 18% Protein
* 6% Fat
* + Moderate phytoestrogen

2919 Irradiated

Key Features
* 19% Protein
* 9% Fat

The irradiated version is identical to the standard version, with the exception of packaging.

Teklad Global Diets® at a glance
Dietary Management

Weanlings may need a softened diet

- Dough Diet on Cage Floor
- Moistened Food Pellets on Cage Floor
- H₂O DietGel 76A
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Breeding Tips

- Keep rooms quiet and avoid handling cages as much as possible—especially avoid loud or sudden impact noises such as loud conversation, equipment collision, or radios

- Minimize traffic in and out of the room

- Minimize any source of vibration to cage location

- Do not wear perfume, cologne, or other strong scents in the room
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Breeding Tips

☐ Use clean gloved hand to handle mice and avoid scent transfer between cages. Keep gloves moistened with disinfectant

☐ Work gently, slowly, and quietly with problem breeders and use the same technique and technician for breeding work

☐ Do not change cage for 2 days before or 3 days after delivery

☐ If moms are scattering or killing the newborns, try removing the male before delivery

☐ **PAY ATTENTION to the mice and keep good records**
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Cost of Poor Breeding Colony Management

- Lost experiment time due to lack of mice
- Lost per diem and materials for unproductive cages
- Increased expenses if average litter size is not optimized
- Failure to notice….
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Most Common Mistake

**FAILURE TO NOTICE**

- individual mouse deaths, problems, performance
- individual cages-- breeding problems, production drops, age to retire
- entire colony trends of problems, performance (or lack of performance)
# Mouse Breeding Colony Management

## Need Assistance

<table>
<thead>
<tr>
<th>Name</th>
<th>Office</th>
<th>E-mail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glenn Florence</td>
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</tr>
</tbody>
</table>

* Indicates a Pager Number
QUESTIONS

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Mouse Breeding Colony Management

Credits

“Mouse Breeding Basics” Presentation Dr. Haughton, Cheryl

“Mouse Breeding Colony Management” Presentation
University of Kentucky DLAR

Multiple websites for pictures