# Mouse Handling Training (2021)

## Laboratory Animal facility, HKUST (CWB)

**Contents:**



Note: This material serves as part of the training program for animal users of Laboratory Animal Facility, HKUST (CWB). The rest of the training program includes

- General Animal Use Training
- LAF User Manual
- Hands-on training and orientation in the facility, and
- Biosafety course (provided by HSEO)

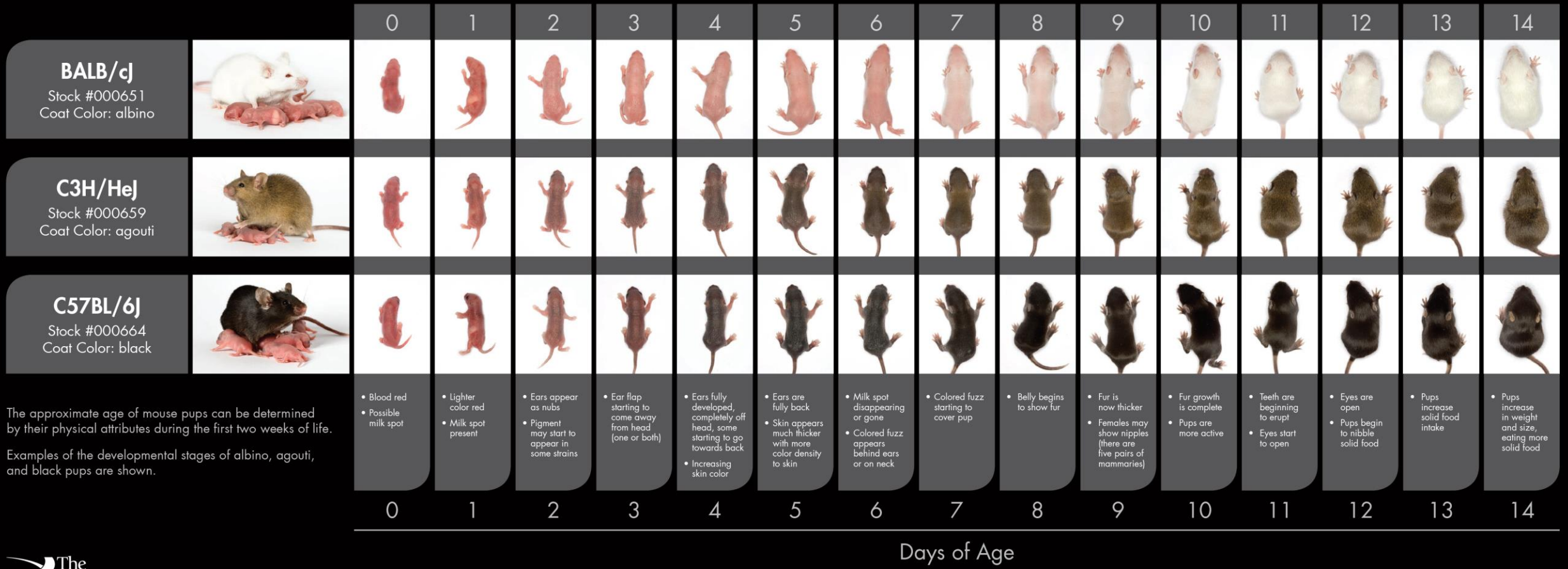
# Part 1

# Basic Manual Skills in Mouse Handling

# Estimating the Age of Pups

香港科技大學
THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY
實驗動物中心 (清水灣)
LABORATORY ANIMAL FACILITY (CWB)

## JAX® Mice Pup Appearance by Age




The approximate age of mouse pups can be determined by their physical attributes during the first two weeks of life.

Examples of the developmental stages of albino, agouti, and black pups are shown.

The Jackson Laboratory
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**To order JAX® Mice:** 1-800-422-6423 orderquest@jax.org www.jax.org

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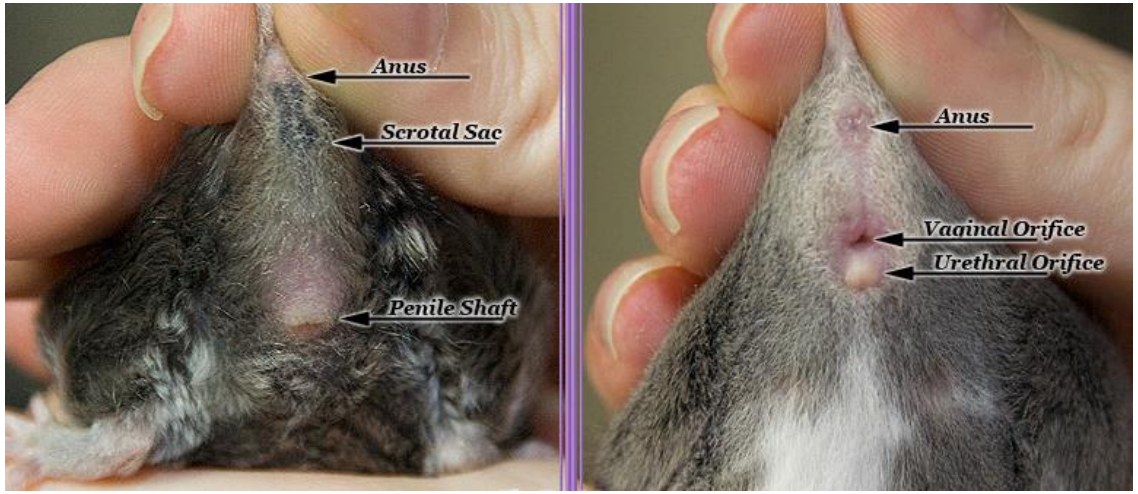
# Sexing Mice

- Both male and female have protruding external genitalia
- Male: Longer distance between the external genitalia and the anus
- Female adult: presence of vaginal opening (orifice)
- Female adult: present of a furless line between the vaginal opening and anus
- Female pups: pale nipples in pigmented pups

Male Female

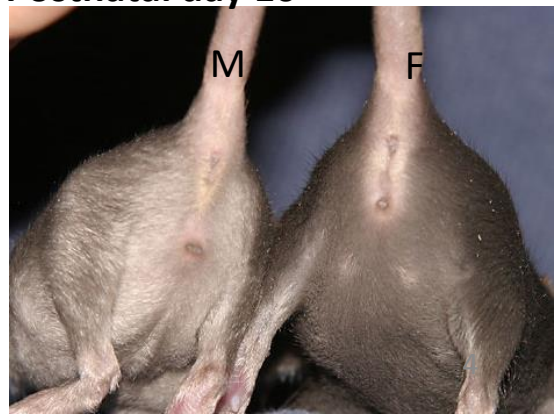



**Adults**

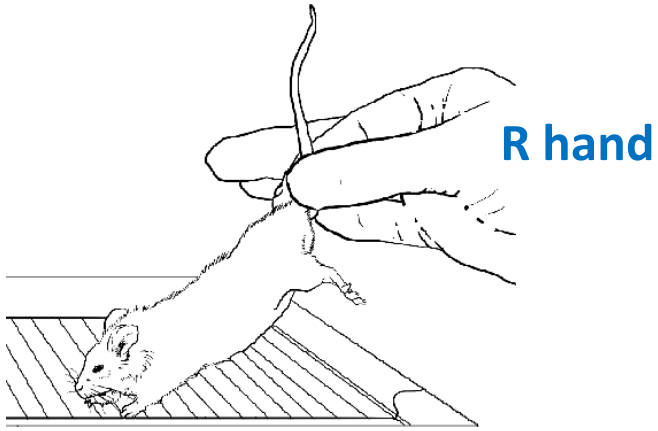
**Postnatal day 2**


**Postnatal day 6**

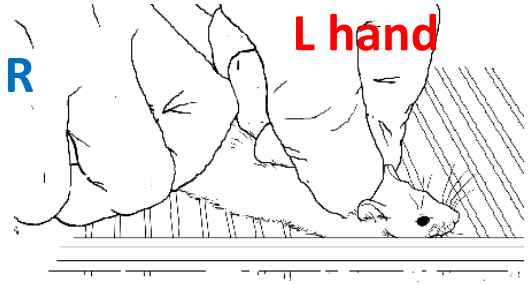

**Postnatal day 16**



# Restraining a Mouse




Hold the base of the tail
And place the mouse on a surface for grasping




Grasp loose skin on the back of the neck


Secure the tail with the fourth and fifth fingers

**Recommended training videos:**
**Mouse restrain: https://www.youtube.com/watch?v=ZBPVvRZvIEw (shared by NIH OACO)**
**Rat restrain: https://www.youtube.com/watch?v=SqGN7TCKYZ8 (shared by NIH OACO)**

# Rodent Identifications

## Ear punching

- Applicable to any mice older than 2wks
- Causes minimal discomfort (no bones affected)
- Ear disc as biopsy for genotyping
- Consult LAF staff for recommendations on ear marking system

## Ear Tagging

- Applicable to any mice older than 3wks
- No biopsy harvested for genotyping
- Special equipment required

# Rodent Identifications

## Toe Clipping

- Applicable to the mice at 7 days of age or younger
- Applicable only when scientifically justified with no other alternatives
- Minimize the number of digits clipped
- Prefer not to remove toes from fore paws
- If forepaw must be used, avoid clipping the hallus (“thumb”) which will affect the grasping ability of the animal
- Small sharp scissors must be used and personnel should be trained in performing the procedure
- Tissue as DNA source for genotyping

Ref:
Animal Research Advisory Committee Guidelines, Office of Animal Care and Use, NIH
Guide for the Care and Use of Laboratory Animals (NCR 2011)

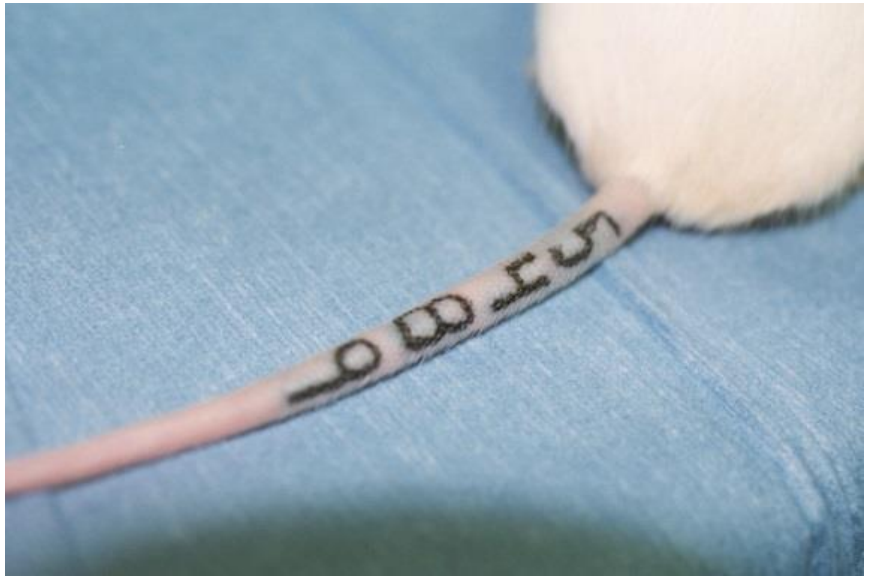
# Rodent Identifications

**Tattoo**

- Specialized equipment and ink are required
- No tissue available for genotyping

**Marker pen**

- Non-toxic, “permanent” marker is required
- May last for a couple of days only, remarking may be required



# Compound Administration

## Intraperitoneal Injection

- Body cavity **outside** internal organs
- At the left lower quadrant of the abdomen,
- The needle is pushed at an approximately 10° angle
- Insert about 0.5 -1 cm of the needle – MUST ASPIRATE
- *Hold in head lower than body position*

## Subcutaneous Injection

- Top of the shoulder blades is the most usual site
- Grab loose skin
- Insert about 0.5 -1 cm of the needle – MUST ASPIRATE
- Parallel to muscle layer

# Compound Administration

## Intramuscular Injection in Rats

- Rat: front or back of the thigh; injection vol. < 0.05ml
- Causes pain to the animals due to the distension of the injected muscle

## Intravenous Injection

- Injection site: lateral tail vein
- Warming of tail/animals is required to dilate the tail vein (key of success)
- Extensive training is required (Key of success)

# Compound Administration

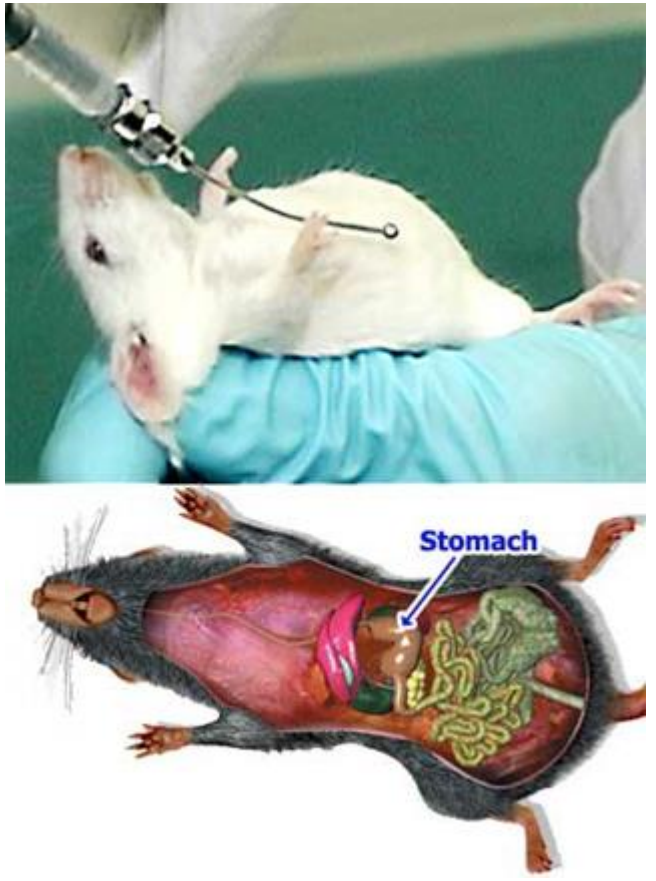
## General note for material injection

- Use a new needle for each animal to reduce risk of infection
- Shaving and site disinfection is usually not necessary on rodents
- Materials with high and low pH can cause pain on the injection site
- Further induction of discomfort can be reduced by injecting material close to body temperature
- Please refer to the *Introductory to Lab Animal Handling and Procedures* lecture handout for a more in-depth review

# Compound Administration

## Gastric Gavage

- Stomach tube or dosing cannula is used
- Estimate length of insertion externally before inserting the cannula
- Extensive training is required.
- If unintentionally administered into the lung (indicated by respiratory distress), animal should be euthanized




**Recommended readings and training videos:**
http://www.procedureswithcare.org.uk/oral-gavage-in-the-mouse/
http://www.procedureswithcare.org.uk/oral-gavage-in-the-rat/

# Survival Surgery

**General Conditions** *(Guide for the Care and Use of Laboratory Animals, 8th edition, NRC)*

- Appropriate pre-operative and post-operative care of animals in accordance with established veterinary medical and nursing practices are required
- At the time of use, the aseptic surgery should be conducted in an area which is dedicated for surgeries and related activities
- At all times, the surgery should be exposed to minimal contamination
- All survival surgeries will be performed by using **aseptic procedures**, including masks, sterile gloves, sterile instruments, and aseptic techniques
- Please refer to the *Introductory to Lab Animal Handling and Procedures* lecture handout for a more in-depth review

# Survival Surgery

## Important notes for survival surgery

- **BE FAMILIAR WITH THE SURGERY BY RECEIVING THE APPROPRIATE TRAINING** (from LAF staff, your seniors or experts from another laboratory)
- Avoid using too much disinfectant which can cause hypothermic in the animals
- Lubricant should be applied to the eyes of rodents to prevent drying under anesthesia
- Handling tissues gently with the appropriate dissecting instruments
- Apply surgical aseptic techniques

**Recommended readings and training videos:**
http://www.procedureswithcare.org.uk/aseptic-technique-in-rodent-surgery-tutorial/

# Survival Surgery

**Aseptic techniques**

- Procedures to eliminate contamination of the surgical site and wound infection
- Wound infection causes
  - pain and distress (animal welfare issues) and
  - physiological changes (affect scientific outcome)

# Survival Surgery

## Important notes to aseptic techniques

- The appropriate personal protective equipment must be worn
- Wash hand thoroughly to eliminate potential contaminants
- Use only sterilized dissecting instrument or tools in contact with the wound
- Autoclaved items or purchased pre-sterilized materials may be used
- Alcohol alone is NOT considered as an effective disinfectant
- Ideally an independent set of sterilized dissecting instrument should be used for each animal

**Recommended readings and training videos:**

https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent_surgery.pdf

http://www.procedureswithcare.org.uk/aseptic-technique-in-rodent-surgery-tutorial/

# Euthanasia

## What is euthanasia?

- In Greeks *eu* means good and *thanatos* means death
- The term is usually used to describe ending the life of an individual animal in a way that minimizes or eliminates pain and distress. A good death is tantamount to the humane termination of an animal's life

## Important guideline

- AVMA Guidelines for the Euthanasia of Animals: 2020 Edition
- Euthanasia not accepted by AVMA must be justified by scientific needs

**Recommended reading:**
AVMA Guidelines for the Euthanasia of Animals: 2013 Edition (see page 48-51 for laboratory animals)

# Euthanasia of Mice and Rats

## General considerations

- Methods of euthanasia likely to elicit distress vocalizations or pheromones that other animals in the room could hear or smell should be performed in another location
- Before euthanasia, minimize activities that contribute to distress, including
  - transport
  - handling
  - disruption of compatible groups, and
  - elimination of established scent marks

**Recommended reading and videos:**

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition (see page 48-51 for laboratory animals)

# Acceptable Method (Mice and Rats)

## Non-inhalant agent injection

***Injection of dissociative agent combinations***

- Ketamine at lethal does
- in combination with xylazine or benzodiazpines ($\alpha_2$-adrenergic receptor agonist)
- via intraperitoneal route

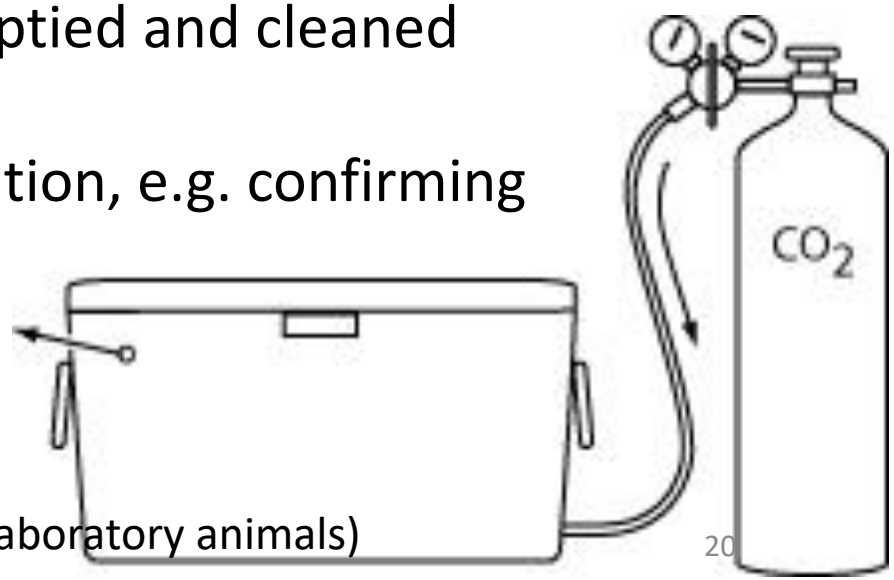
***Injection of Barbiturates and barbituric acid derivatives (Pentobarbital)***

- at three times the anesthetic dose
- via intraperitoneal route

# Acceptable with Condition Method (Mice and Rats)

## Carbon dioxide inhalation

- Use home cage and must not have more than 5 mice in one cage
- Compress $CO_2$ gas in cylinder as the source, dry ice is NOT ACCEPTABLE
- Optimal filling rate to the euthanasia chamber should displace 30-70% of the chamber volume per minute (strictly follow the specified flow rate post at the euthanasia station in LAF)
- Prefilled chamber is NOT ACCEPTABLE
- Chamber ( if not the home cage) should be emptied and cleaned between uses
- Death MUST BE confirmed by physical examination, e.g. confirming breathing and heart beat arrest
- Death may be ensured by a physical method (e.g. cervical dislocation and decapitation)




**Ref:**

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition (see page 48-51 for laboratory animals)
NIH Guidelines for the Euthanasia of Rodent Fetuses and Neonates

# Euthanasia of Rodent fetuses

## Fetuses in utero

- Fetuses in utero are unconscious
- Hypoxia does not evoke a response
- Unnecessary to remove fetuses for euthanasia after the dam is euthanized by acceptable method

## Fetuses removed from uterus

- Fetuses removed from dams may be aroused
- Mouse and rat fetuses of >E15 may be euthanized by decapitation
- Fetuses >E15 should be euthanized or anesthetized before chemical fixation
- Anesthesia may be induced by hypothermia in cold saline (with the amniotic sac intact) until the fetuses become immobile

**Ref:**

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition (see page 48-51 for laboratory animals)
NIH Guidelines for the Euthanasia of Rodent Fetuses and Neonates

# Euthanasia of Rodent neonates

**Acceptable methods for neonates up to 10 days of age**

- Injection of chemical anesthetics, e.g. pentobarbital, and then decapitation
- Anesthetic inhalation, e.g. $CO_2$ or isoflurane, and then decapitation
- $CO_2$ inhalation cannot be used alone as the animals are resistant to hypoxia, decapitation may be use as a secondary physical procedures for euthanasia

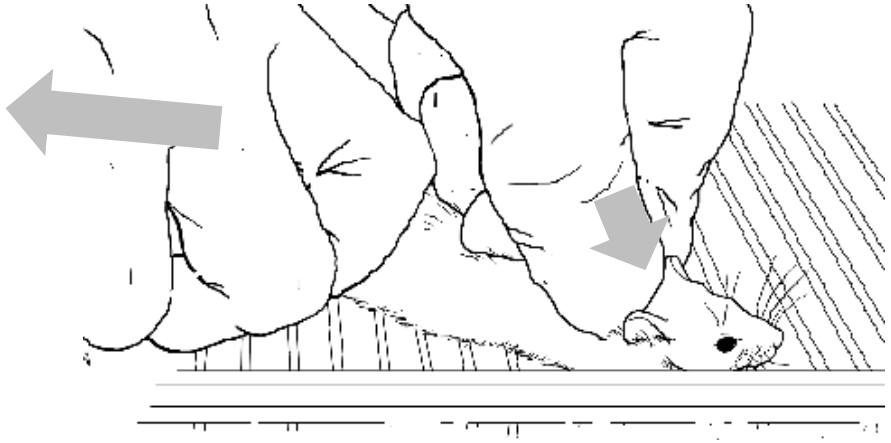
**Acceptable methods for neonates over 10 days of age**

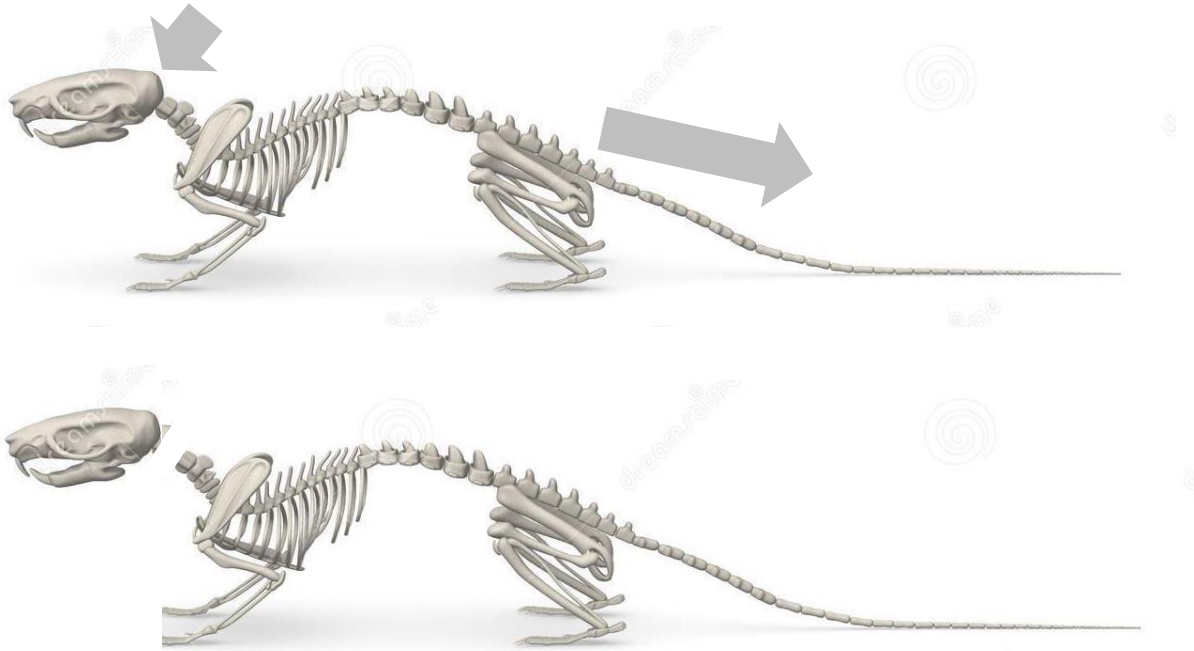
- *AVMA Guidelines for the Euthanasia of Animals* is applied

**Ref:**

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition (see page 48-51 for laboratory animals)
NIH Guidelines for the Euthanasia of Rodent Fetuses and Neonates

# Cervical dislocation





- Let the mouse grasp the grid
- Restrain the rodent by grasping the base of the tail
- Place fingers against the back of the neck at the base of the skull
- Quickly pull backwardS with the hand holding the tail base
- Verify dislocation by feeling for a separation of cervical vertebrae
- Confirm respiratory arrest, no heart beat

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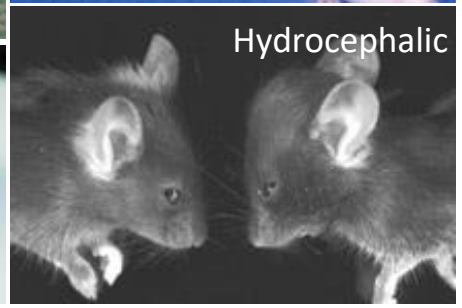
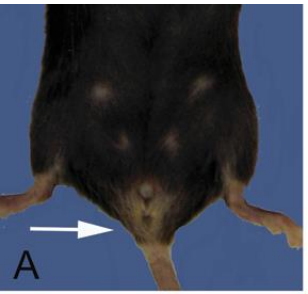
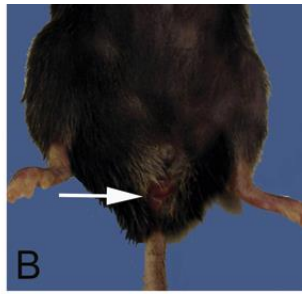
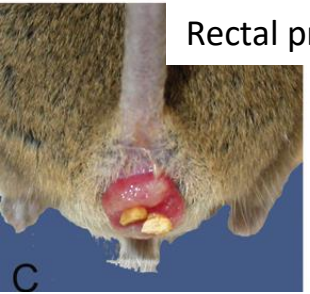
# Common problems in your mouse: What is abnormal?

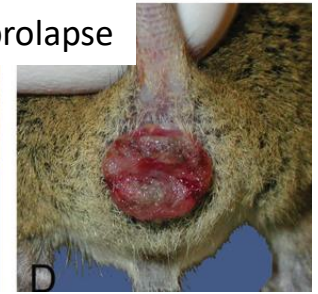



| | Observation | Possible reason (apart from genetic problem) |
|---|---|---|
| Shape | Thin | Low food consumption, infection/problem with ingestion |
| | Undersize, weak pup | Occasional too much competition in uterus/getting milk |
| | Hunched | Severe pain/illness due to different reasons |
| | Outgrown teeth | Misalign of upper and lower jaw, failed to wear each other |
| | Rectal prolapse | Bacterial infection, heavy burden of parasites in guts |
| | Hydrocephalic | Cerebrospinal fluid accumulated in the brain, inherited/infection |
| Movement | Spinning/flipping | Problem in balance, infection of ears |
| | Paralysis | Spinal cord damage, illness |
| | Labored breath | Problem with nose/lung, infection, presence of mass/fluid in lungs |
| Fur/skin | Hair loss | Behavioral abnormal barbering (one in the cage looks normal), scratching due to parasite/skin infection |
| | Coat color change | Genetic contamination |
| | Staining (urine, feces etc) | Illness, diarrhea |
| | Wound (tails, hind feet) | Fighting, cocaging of non-compatible mice |
| | Scaly skin | Infection by Corynebacterium bovis |
| | Ulceration | Infection of (fight) wound/tumor outgrowth |

# Part 2

# Mouse Nomenclature

# Mouse Nomenclature

**Standardization of nomenclature:**

- Aids in scientific communication
- Link gene to specific alleles and genetic background
- Avoid confusion of similar strains or genetic modifications

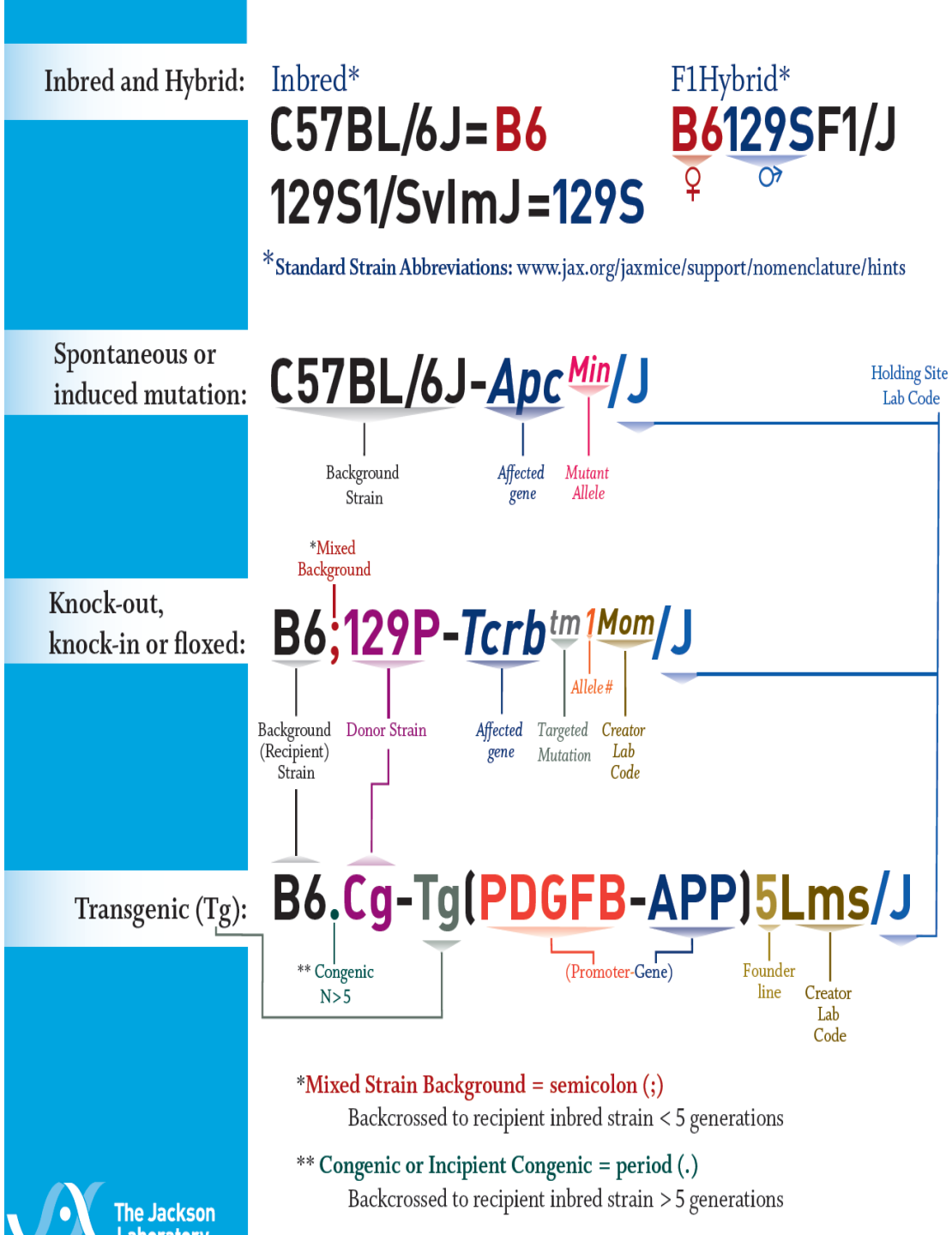
**Creator Lab code:**

- Identifying a unique allele
- Lab code maintained by the international Laboratory Code Registry
- You may apply “Hkust” as the Lab code to those transgenic strains generated in HKUST

  (pls see http://ilarlabcode.nas.edu/search_codes_nodep.php?cat=h)

# Mouse Nomenclature






**Reference and recommended reading:**

***JAX Full Guidelines:***
http://www.informatics.jax.org/mgihome/nomen/gene.shtml

***Nomenclature for mutant alleles generated in ES cell lines by the International Knockout Mouse Consortium (IKMC):***
http://www.informatics.jax.org/mgihome/nomen/IKMCnomen.shtml

# Part 3

# Calculating Colony Size

# Sizing Mouse Colonies

## Sizing Mouse Colonies

### Example:

How many breeding females are needed to produce 10 female & 10 male homozygotes per week using a homozygous female x homozygous male breeding scheme?

### Strain characteristics

| | |
|---|---|
| Breeding scheme | Homozygotes x Homozygote |
| Breeding lifespan | 32 weeks |
| Number of litters produced | 4 litters |
| Litter frequency | 1 litter/8 weeks (4 litters/32 weeks) |
| Litter size | 6 pups (3 females, 3 males) |
| Offspring genotypes | Homozygotes only |
| Percent useful offspring | 100% |

### *Number of experimental mice needed*

| | | |
|---|---|---|
| 1. | Number of mice needed | 20 |
| 2. | Age requirements<br>If must be same age, enter 1<br>If can have a two-week age range (*e.g.*, five to six weeks old), enter 2<br>If can have a four-week age range (*e.g.*, five to eight weeks old), enter 4 | 1 |
| 3. | Frequency with which mice are needed<br>If weekly, enter 1<br>If every other week, enter 2<br>If once a month, enter 4 | 1 |
| 4. | Divide Line 1 by the smaller of Line 2 or Line 3 (round ***up*** to nearest whole number) | 20 |
| 5. | Sexes needed<br>If both sexes needed, enter 1<br>If one sex needed, enter 2 | 1 |
| 6. | Breeding scheme<br>If homozygote x homozygote, enter 1<br>If heterozygote x homozygote, enter 2<br>If heterozygote x heterozygote, enter 4 | 1 |
| 7. | Some surplus (insurance) mice desired<br>If no, enter 1<br>If yes, enter a "fudge factor" to ensure overproduction *e.g.*, if 10% more mice are desired, enter 1.1) | 1.1 |
| 8. | Number of mice to be produced weekly<br>Multiply Lines 4 x 5 x 6 x 7 (round ***up*** to nearest whole number) | 22 |

### *Colony productivity*

| | | |
|---|---|---|
| 9. | Average number of pups weaned per litter | 6 |
| 10. | Average number of litters produced per breeder female | 4 |
| 11. | Average productive female's breeding lifespan (weeks) | 32 |
| 12. | Calculate colony productivity (number of weaned pups/female/week<br>Divide Line 10 by Line 11, multiply by Line 9 (round to nearest hundredth) | 0.75 |
| 13. | Calculate number of breeding females needed<br>Divide Line 8 by Line 12 (round up to nearest whole number) | 30 |

### *Number of breeding females needed to keep colony productive*

| | | |
|---|---|---|
| 14. | Calculate number of replacement breeders needed per week<br>Divide Line 13 by Line 11 (round up to nearest whole number) | 1 |
| 15. | Calculate the number of additional breeders needed to provide replacement breeders<br>Divide Line 14 by (2 x Line 12), then multiply by Line 5 (round up to nearest whole number) | 1 |

### *Total number of breeders needed*

| | | |
|---|---|---|
| 16. | Add Line 13 and Line 15 | 31 |

### *Number of cages needed per week*

| | |
|---|---|
| 17. | Breeding cages<br>For pair breeding (one breeding female per cage): **31 cages needed (16 boxes)**<br>or trio breeding (two breeding females per cage): **16 cages needed (8 boxes)** |
| 18. | Weaning cages<br>~11 females & ~11 males weaned per week will require ~ **6 cages** (5 animals per cage separated by sex), ~ 3 boxes |

# 試算表 (The Jackson Laboratory)

- **實驗小鼠需求量**

| | | |
|---|---|---|
| 1 | 需要小鼠數目 | 20 |
| 2 | 需要小鼠年齡<br>a. 需要同年齡小鼠, 填1<br>b. 需要年齡差距在2週以內, 填2<br>c. 需要年齡差距在4週以內, 填4 | 1 |
| 3 | 需要小鼠頻率<br>a. 每週, 填1<br>b. 每2週, 填2<br>c. 每4週, 填4 | 1 |
| 4 | 題3的結果除以題2的結果 (計算到整數) | 1 |
| 5 | 需要小鼠性別<br>a. 性別不拘, 填1<br>b. 單一性別, 填2 | 1 |
| 6 | 繁殖策略<br>a. Homozygote x homozygote, 填1<br>b. Heterozygote x homozygote, 填2<br>c. Heterozygote x heterozygote, 填4 | 4 |
| 7 | 需要保留備用小鼠<br>a. 不需保留, 填1<br>b. 依保留比例填寫, 例如保留10%, 填寫1.1 | 1.1 |
| 8 | 每週需產出小鼠數量 (結果1 x4 x5 x6 x7, 計算到整數) | 88 |

- **繁殖母鼠需求量**

| | | |
|---|---|---|
| 9 | 平均每胎離乳小鼠數量 | 6 |
| 10 | 平均每隻母鼠產出胎數 | 4 |
| 11 | 平均每隻母鼠繁殖期 (週數) | 32 |
| 12 | 計算繁殖指數 (題目 10 x 9 /11, 計算到小數點後二位) | 0.75 |
| 13 | 計算需要的繁殖母鼠 (題目8/ 12, 計算到整數) | 118 |

- 種源維持-換種需求

| | | |
|---|---|---|
| 14 | 平均每週需換種數量 (題目13/ 11, 計算到整數) | 4 |
| 15 | 母鼠需求量 (題目14/ 12, 計算到整數) | 6 |

- 種母鼠需求量

| | | |
|---|---|---|
| 16 | 題目13+15 | 124 |
| | 每週產出heterozgytes公鼠及母鼠, 因此不需額外計算留種量 | |

- 飼育籠數

| | | |
|---|---|---|
| 17 | 繁殖籠 (1公1母)<br>繁殖籠 (1公2母) | 118<br>59 |
| 18 | 離乳籠<br>每週約離乳11公11母 (最多5隻1籠, 公母分籠) | 6 |

# Factors to consider when calculating the Number of Breeders Needed

- Cohort size required for experiments and controls (age and sex matched)
- How often cohorts are required
- Which gender to use
- What genotypes to use
- Universal breeding index
- Strain specific characteristics
- Age range to use
- Buffer to accommodate variation in productivity (e.g. 10%)

# Steady-State Colonies



To determine number of breeders required, consider the following:

- Average litter size (6/litter for a good breeders)
- Average number of litters (4 for good breeders)
- Rotation length – time from mating of animal to retirement (e.g. 32 weeks for good breeders but usually shorter)
- Frequency of productive mattings , e.g. 0.85 for a 15% non-productive(NP) rate
- Frequency of rotation

Colony productivity =

**# of Litters ÷rotation Length × litter size × NP**

Number of breeders needed =

**mice needed per week ÷colony productivity**

# Part 4

# Breeding Schemes

# Types of Breeding Schemes

1. Pair Mating
2. Trio Mating
3. Harem Mating
4. Timed Mating

# Pair mating

**When to use this pattern?**

- Permanent mating
- Pedigreed stocks where lineage tracing is required
- Where litters are large and having two litters in a cage could result in overcrowding, leading to increased mortality/prewean discard
- When can safely wean before 19 days of age – day of weaning will vary between strains
- To observe breeding performance
- To take advantage of post-partum estrus

**Remarks:**

- For known DOBs – Must wean prior to next delivery with visibly pregnant females
- For estimated DOBs- wean according to size and observe cage daily, TWICE a day or early morning once a day
- If new litter noted, user must wean immediately

*refer to ***LAF Policies on Overcrowding***

# Trio Mating

**When to use this pattern?**

- Pups have faster growth rates as raised by more than one female
- To maximize breeding efficiency as both females help care for young
- Best for strains that generate small litters or are difficult to breed (e.g. Genetically-modified mutants, special phenotypes, weak pups, weak moms)
- Multiple moms in the cage will help each other raise pups

**Remarks:**

- Separate pregnant females and housed individually if the females are far apart in pregnancy statuses
- Separate, preferably, the male, before the pregnant female delivers if the two females are synchronised or close to each other in pregnancy statuses
- Must accept responsibility and take action immediately for all overcrowding issues if used*

*refer to ***LAF Policies on Overcrowding***

# Harem Mating

**When to use this pattern?**

- Necessary for embryology studies
- Good for maximising breeding potential of each male
- Maximizes the number of offspring from each male
- Intensive breeding program
- Accurate aging of litters – helpful in planning age dependent projects

**Remarks:**

- Visibly pregnant females must be removed from the breeder unit to avoid postpartum estrus
- "Bruce effect" – if females not separated immediately after copulatory plug is noticed, male pheromones can prevent implantation of blastocysts
- Pregnant female should be removed before delivering pups, otherwise identification of the lactating female will be difficult after delivery
- Must have large enough cage space - Discouraged in standard shoebox cages due to increased cage population densities
- Must accept responsibility and take action immediately for all overcrowding issues if used*

*refer to ***LAF Policies on Overcrowding***

# Timed Mating

- Using the existence of copulatory plug to determine if mating has occurred
- Best to examine early in the morning
- Presence of plug does not guarantee pregnancy
- Difficulties of noticing plugs include: too deep in canal , or falls out easily
- If applied to Harem Mating, group females away from males prior to mating to synchronous cycle
- Success of pregnancy will depend on strain and stage of estrus cycle of the female when mated

# Points to consider when choosing a breeding pattern

- Estimate the survival and due date based on performance/experience.
- For common deaths due to premature weaning, harem mating is best.
- The selected breeding strategy must be closely followed and clearly stated on the cage label from the start.
- If the previous litter can be weaned in time to avoid being trampled to death, postpartum estrus mating is acceptable. Otherwise, the pregnant or timed-mated females should be removed from the male, especially if they are small and/or weak physically.
- Postpartum estrus mating must be avoided in strains that show slower maturing weanlings. This can be achieved by separating the male prior to delivery. Strains with slower maturing weanlings may also be more suitable for Harem or Timed mating

- Weaning age for mice is typically 19 days. Small, runted pups will occasionally be required to stay with mother until 28 days. Ideally, postpartum estrus should be avoided to allow for a longer nurturing time from mom. Runted or small-sized pups that experience a lower survival rate should be allowed to stay in the same cage with the next litter as long as the litter sizes are small. If the litter sizes are large the two litters should be separated to avoid competition of milk supply.
- Recommended not to touch or manipulate the mother or pups until the pups are at least 3 days of age. Always handle pups gently with minimal noise and shortest duration possible even after 3 days of age

# General information about mice

- Number of chromosomes 20
- Gestational length 19.5 days (19 days for B6 Mice)
- Birth weight 0.75–1.5 g
- Litter size 2–12 pups
- Weaning age 18–28 days
- Weaning weight ≥ 10g
- Sexual maturity: usually 6 weeks of age **+** good health status **+** adequate body weight
- Adult weight 20–40 g
- Estrous cycle 4–5 days
- Retirement age (female) ≤ 10 months
  - Last litter around 8 months of age
- Retirement age (male) ≤ 11 months

# Optimizing Breeding Performance

- Maintaining a line: Keep at least 2 active males and 4 active females at anytime
- Replacement:
  - When last generation reaches 6 months old, set up at least 2 males and 4 females for breeding
  - Terminate F(n) only when F(n+2) are proven healthy
  - Keep no more than 2 best males and 4 best females from each cage for breeding purposes
- Mixed-age breeders in a colony will produce more consistent number of pups.
- NP breeders: replace breeder when:
  - > 60 days old since first litter
  - > 60 days since last litter
  - >2 consecutive litters that do no wean any pups
  - > 60 days of female and male housed together with no pregnancy
- Use young mice: ideally start at 8 weeks old, for genetically modified mice, > 8 weeks is best
- pair young females with older and bigger males

# Breeding difficult strains

- Fostering
  - For strains with poor mothers or mothers with an undesirable phenotype
  - Foster mother should have a healthy litter already within 1-2 days of age of fostered pups
  - Best to have coat colour difference between natural and fostered pups (e.g. albino strains such as CD1 and FVB are usually good foster mothers)
- Ovarian transplants
- In Vitro Fertilization
- Allele-Specific Genotyping and unwanted Alleles
- Cryopreservation
  - Back-up in the event of disaster,  health problems and human errors
  - No immediate need for colony
  - Stabilize colony to prevent genetic drift or degradation

# Common problems in colony management and breeding strategies

- Colony no longer breeding
- Yielding small litter size
- Yielding runty pups
- Dystocia/pup mortality
- Overcrowding
- Pups in former litter not ready to be weaned (e.g. age, size, condition) and new litter has arrived in the same cage from a permanent mating pair
- Cannibalism
- Inaccurate estimated DOB due to infrequent inspection by the user leading to overdue weaning
- Vague signs of pregnancy in harem mating cage (e.g. only pregnant with 1-2 fetuses)

# Overcrowding

## Important note

- It is users' responsibility to maintain the animal colonies in good conditions by
  - keeping good animal record and
  - monitor the animals closely and manage them timely

# What is overcrowding?

- More than 5 adult (6 weeks of age or older) mice per green IVC
- For Trio and Harem mating, coexisting of two litters is only acceptable if
  - the total number of pups is not more than 15, and
  - The litters born is less than 1 week apart
- Coexistence of 2 litters must be weaned no later than 21 days old
- Diet gel, wet feed, long sipper sack can be given to juvenile to help them to survive. These supplementation can also be used during when being fed by mom but beware of competition of these supplementation between weaker and stronger pups if they're mixed in the litter
- When a litter of mice is born, there can be no more than 2 adult mice in the cage
- Prior progeny must be separated from the mother before she gives birth to a new litter
- Pending approval by LAF, a litter can be partially weaned, e.g. wean the bigger and stronger pups if they are of adequate age and weight, leaving the small and weak pups with mom a bit longer, as long as the other overcrowding violations are not met

# Summary

- Always apply the 3Rs
  - REPLACEMENT – replace the use of live animals whenever possible
  - REDUCTION – minimize number of animals user in each experiment
  - REFINEMENT – minimize pain, suffering, distress in research animals
- Choose a breeding strategy that best fits your needs while maintaining optimal welfare standards
- Once a breeding strategy is chosen, the conditions must be closely followed and the strategy clearly stated on the cage card
- Frequent visits by you on your colony managements to avoid overcrowding and/or violating welfare standards
- Contact a LAF staff member immediately upon any issues on your colony management
- Always practice an optimal level of biosecurity and occupational health and safety
- Utilize LAF facilities with respect and always be considerate of other users

# END
# of
# Mouse Handling Training