MOUSE CAGE DENSITY STANDARD

Revised 2019

UNC Research

Office of Animal Care and Use – IACUC Office
University of North Carolina - Chapel Hill
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UNC Standard on Mouse Cage Density

***Previously referred to as Mouse Breeding Policy.**

Link to Standard: University Standard on Mouse Cage Density

OR

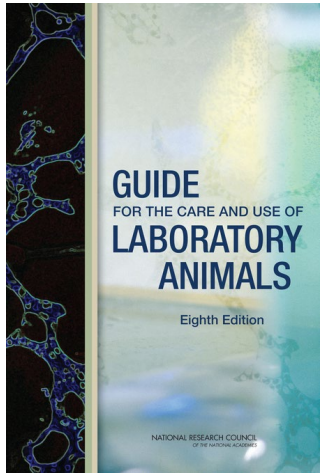
Start here: https://research.unc.edu/iacuc/

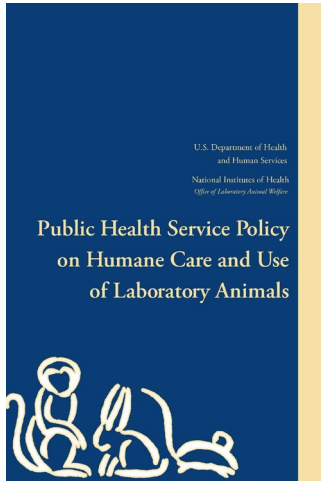
Click: Policy, Standards, Guidance, and Templates

Scroll to: University Standard for Mouse Cage Density

Why is Cage Density Important?

- Animal well-being
- Adherence to Federal Policies and Guidelines
- Scientific validity and reproducibility amongst institutions





Adult Mice in Groups

Animals	Weight of mice (grams)	Floor Area of cage needed per animal, (inches^2)	# Adult mice per static micro-isolator 70 (inches^2)	# Adult mice per green/blue line 78- 82 (Inches^2)
Mice in Groups*	<10	6	11	13
	Up to 15	8	8	9
	Up to 25	12	5	6
	>25	≥ 15	4	5
Female + litter		51 recommended space for the housing group	See previous chart	See previous chart

*Non-breeding cages allow a maximum of 5 adults of the same sex.

Breeding Cage Density Basics

	1 Adult	2 Adults	3 Adults**
Single litter	no limit on pups	no limit on pups	no limit on pups
Multiple litters*	14 pups	11 pups	9 pups

* *Pup numbers are not limited until the oldest litter reaches 14 days of age.*

** *Cages with more than 3 adult mice, plus pups, will be flagged for correction with an 'Action Required' (yellow) card.*

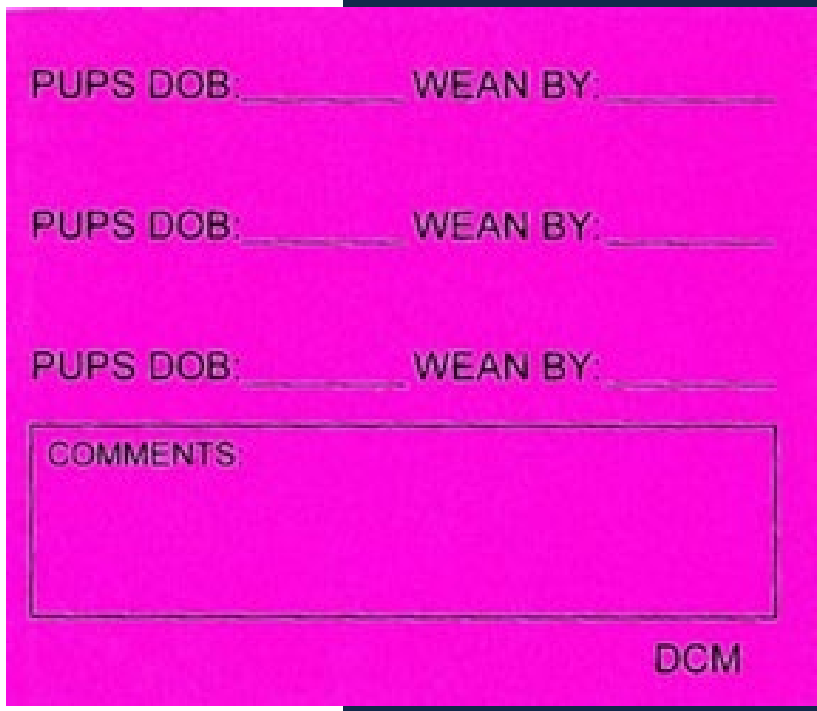
New to the Standard in 2019

- **Research staff are required** to record date of birth on the DCM Weaning Notice (pink) card <u>within three (3) days of birth</u>
  - If DCM notices the litter before the research staff, they will mark DOB and wean date of 23 days later

- If research staff do not plan to wean the mice on Day 23:
  - They must indicate ‘E’ if an IACUC approved Exception is in place

OR

  - They must indicate a reason in the ‘Comments’ section, prior to day 23



DCM “Pink” Card

Pink Card Instructions

This pink card should be used by DCM and the labs to record the litter's DOB and wean date

1. When a new litter is noted in the cage, document the pups date of birth (DOB) on the pink card using your best conservative estimate of age and the wean by date 23 days later
   - Use a china marker/wax pencil to record the information
   - As each litter is born, record DOB and wean by date on a separate line, if applicable
2. If there are no litters, remove the pink card and wipe clean, saving for further use (cards are laminated)

China Marker

New ‘Action Required’ Card (Yellow Card)

Front

Back

WHAT DOES THE YELLOW "Action Required" CARD MEAN?

- The cage exceeds UNC's Standard on Mouse Cage Density
- The lab has 48 hours (two consecutive calendar days) to address the density issue
- DCM will take action to bring the cage into compliance the morning of the 3rd day if not remedied by the lab
- Once you have brought the cage back into compliance, flip the action required card over and place it behind other cards in the cage card holder, so DCM knows you have taken care of the cage.
- Colonies with greater than 10% of total breeding cages flagged with the cards at any given time will be reported to the IACUC for further action.

Action Required/Yellow Card Practices

- **ON Day 1 (24):** DCM to mark cages exceeding cage density with a yellow "Action Required" card
- **ON Day 2 (25)**: Lab is charged **$5.15** if card is still present on the AM on Day 2
- **ON Day 4 (27):** DCM separates for lab (AM), charges **$53.56**

- Corrective action must be taken by the lab within *2* ***calendar*** days to avoid separation charges!
  - A cage flagged by DCM on a Wednesday must be separated by the lab by the end of the day Friday. DCM Separates on Saturday.
  - A cage flagged by DCM on a Friday, must be separated by Sunday to avoid separation by DCM on Monday

"Action Required" CARD IS PLACED ON THE CAGE……

Cage density must be addressed by research staff within two calendar days

On Monday: Comply by Wednesday
On Tuesday: Comply by Thursday
On Wednesday: Comply by Friday
On Thursday: Comply by Saturday
On Friday: Comply by Sunday
On Saturday: Comply by Monday
On Sunday: Comply by Tuesday

Updated DCM 'Action Required' Log

ACTION REQUIRED LOG

FOR DCM USE ONLY

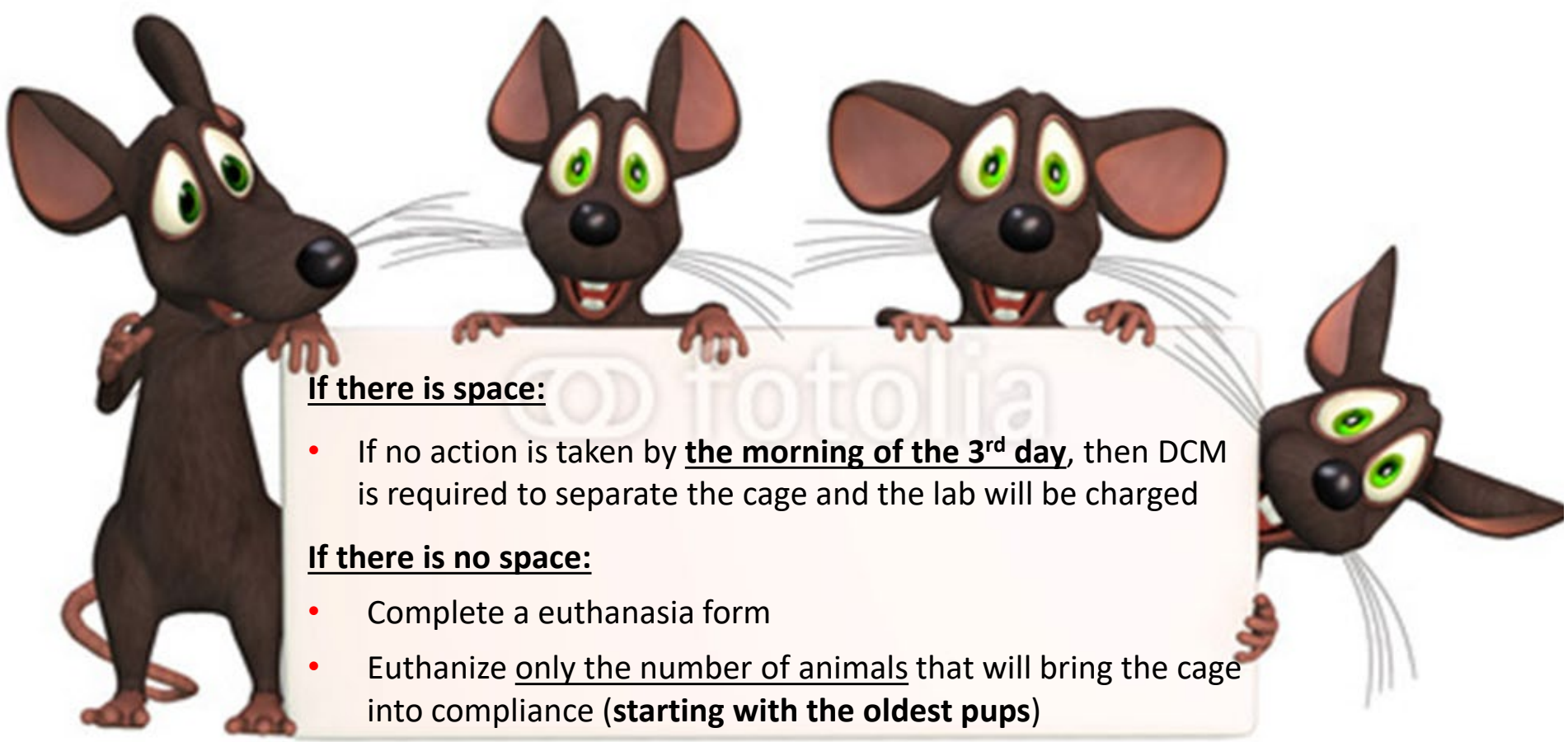
BUILDING:_____ **ROOM #:** _____

DATE	PI & PROTOCOL	CAGE CARD #	RACK #	DCM INITIALS (CORRECTED BY EOD?)*		TIME OF CHECK	ADDITIONAL COMMENTS (PRINT ON BACK IF NEEDED)
				YES	NO		

Day 1

Technically Day 1 PM/ Actually early AM Day 2

DCM Options



<u>If there is space:</u>

- If no action is taken by **<u>the morning of the 3rd day</u>**, then DCM is required to separate the cage and the lab will be charged

<u>If there is no space:</u>

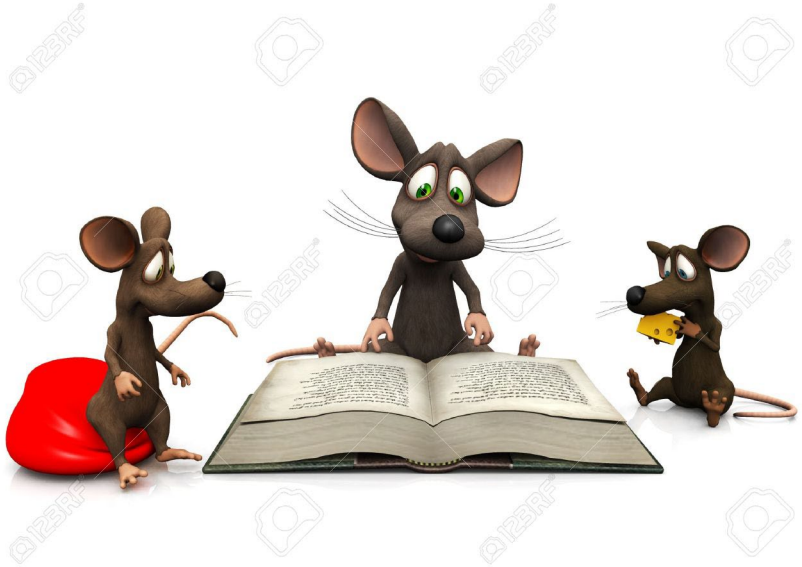
- Complete a euthanasia form
- Euthanize <u>only the number of animals</u> that will bring the cage into compliance (**starting with the oldest pups**)

Research Staff Should Know:

- Repeated or ongoing problems w/ Standard will be reported to the IACUC
- Contact IACUC if you cannot comply with Standard
- As a mouse breeder your lab is responsible for the management of your colony
- DCM is **not** required to contact you prior to marking or separating your cages so you should check at least every 3rd day!
- The lab will incur a **$53.56 separation fee** for every cage separated by DCM

Your Lab Didn't Wean…. Now What?

(This is after the 2-day period and there's no space to separate animals)



DCM finds *7 adult* mice in a cage that are over 25 grams, how many should be euthanized?

Maximum # of animals allowed in a Techniplast cage= 5

Weight	Floor area per animal	Tecniplast # per cage 78-82 sq in	Conventional # per cage 70 sq in
< 10 g	6 sq. in	13	11
10 – 15 g	8 sq. in	9	8
15 – 25 g	12 sq. in	6	5
> 25 g	15 sq. in	5	4

- 1 Adult Mouse
- 2 Adult Mice
- 3 Adult Mice

DCM finds 3 adult mice (30g each) and 12 pups from 2 litters in a cage, one litter is 17 days old and the other is 13 days old, how many mice should be euthanized?

	1 Adult	2 Adults	3 Adults
Single litter	no limit on pups	no limit on pups	no limit on pups
Multiple litters*	14 pups	11 pups	9 pups

* no need to limit the number of pups until pups reach 14 days of age

- **1 Adult Mouse**
- **3 (17) day old pups**
- **3 (13) day old pups**

DCM finds 9 weanling animals that are 14 grams in a cage, how many should be euthanized?

Weight	Floor area per animal	Tecniplast # per cage 78-82 sq in	Conventional # per cage 70 sq in
< 10 g	6 sq. in	13	11
10 – 15 g	8 sq. in	9	8
15 – 25 g	12 sq. in	6	5
> 25 g	15 sq. in	5	4

- 3 mice
- 2 mice
- None

Recap of Compliance Standards

- If the density is corrected on the same day yellow card is placed: No charge
- If the density is NOT corrected the same day the card is placed: **$5.15 charge**
- If on the third day, the density is still not corrected: DCM will separate and assess a **$53.56 charge**
- To avoid charges: Use Comments section of Pink Card & IACUC Exceptions!
  - Details on the next slide

“E”xceptions

- Necessary deviations will be considered by the IACUC on a case-by-case basis with scientific justification and documentation provided by the PI
  - Submit a protocol amendment (including Section 8) requesting approval for a specific situation, strain, etc. with scientific justification
  - The exception will not be in effect until the PI has received written notification from the IACUC

“E”xception Examples

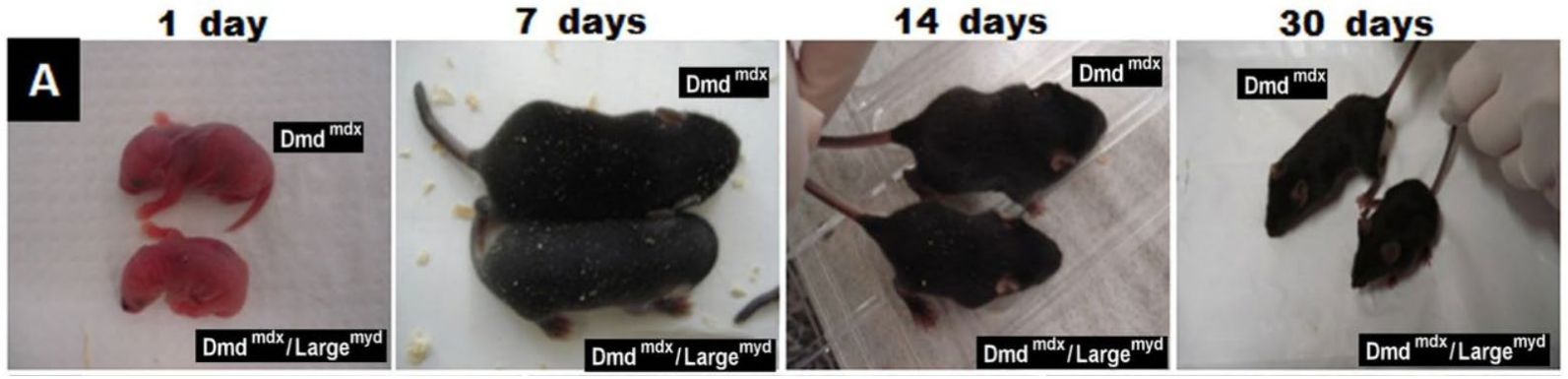
Example #1: Lines with larger than average litters

“E”xception Examples

Example #2: Small mouse pup phenotypes

“E”xception Examples

Example #3: Pups with phenotype that makes them appear more mature than they are



Breeding Cage Density Basics

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* *Pup numbers are not limited until the oldest litter reaches 14 days of age.*

** *Cages with more than 3 adult mice, plus pups, will be flagged for correction with an 'Action Required' (yellow) card.*

Cage Density Basics

For cages with **single litters**:

- No limit on pups with 1 adult
- No limit on pups with 2 adults
- No limit on pups with 3 adults

For cages with **multiple litters**: no limit on the number of pups, until one litter reaches 14 days old, then:

- 14 pups with 1 adult
- 11 pups with 2 adults
- 9 pups with 3 adults

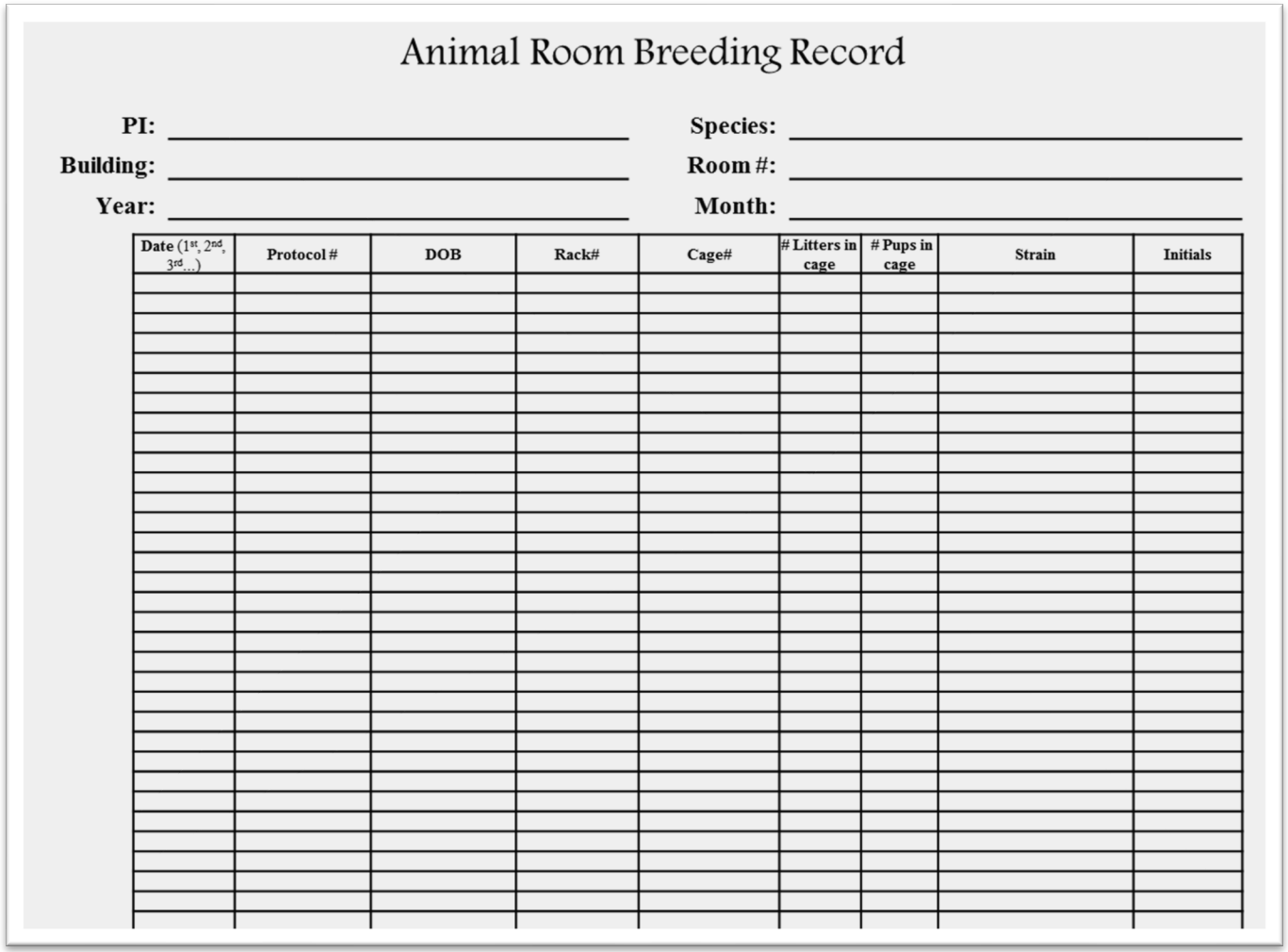
Breeding Cage Requirements

- Nesting material **MUST** be placed in all breeding cages at the time of setup and until pups are weaned

Breeding Etiquette

- **DO NOT** place more than one male in with females, this leads to potential fighting unless you have an Exception to do so
- Place food on floor prior to female giving birth
- Remove dead pups from cages once identified

Breeding Records



Animal Room Breeding Record

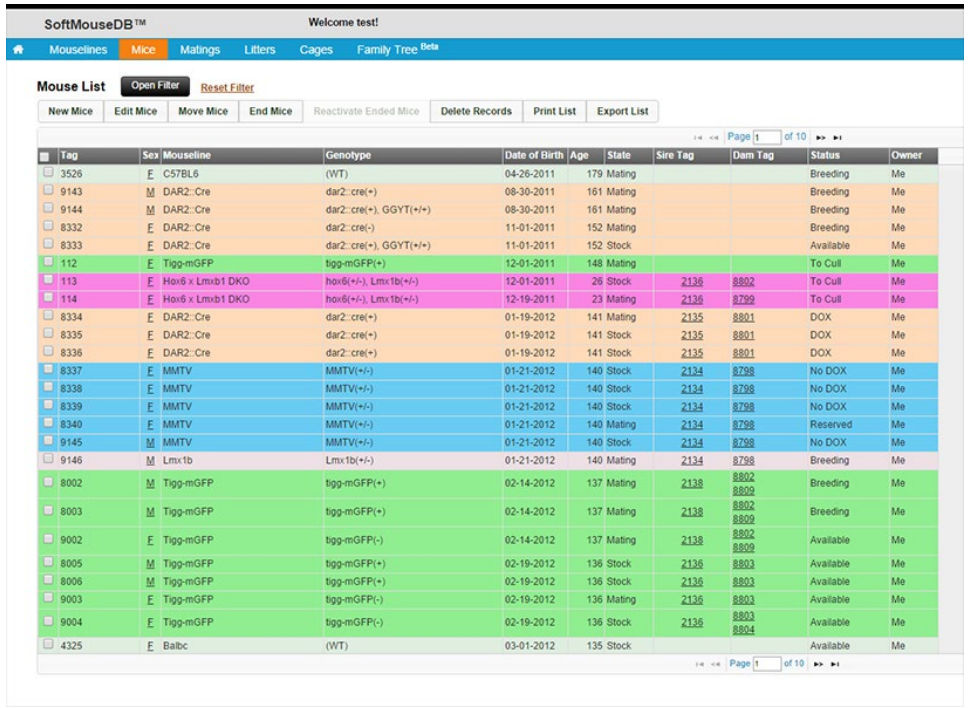
PI: _____ Species: _____

Building: _____ Room #: _____

Year: _____ Month: _____

Date (1st, 2nd, 3rd…)	Protocol #	DOB	Rack#	Cage#	# Litters in cage	# Pups in cage	Strain	Initials

Breeding Records



Genotyping

- Complete genotyping early (so results are ready prior to wean date) to prevent overcrowded cages
  - No need to wait until weaning – Genotype at D14-20, so genotypes are known at wean date!

Weaning Essentials

Wean mice by 23 days

- Most strains will reach 10 g by this age
  - If not, you need an Exception AND you should NOT use continuous breeding scheme
- Do not wean pups prior to reaching 10 g
- Consult DCM Veterinary Services on how to handle strains that do not reach 10 g by 23 days of age

Weaning Tips & Supportive Care

1. **Add feed on floor in new cage at weaning**
   If weaker strain or small pups, add feed on floor prior to weaning

2. **Toggle Lixit card is <u>REQUIRED</u> for all weaned cages**
   Researcher adds dates at placement (one week) and DCM toggles during daily health check

3. **Wean cohorts/groups rather than single mice whenever possible**
   Genotype early to avoid having to re-sort after weaning

4. **When weighing animals, ensure that a clean container is used between cages**
   Ask DCM for cups or disposables if needed, use hood disinfectant between cages

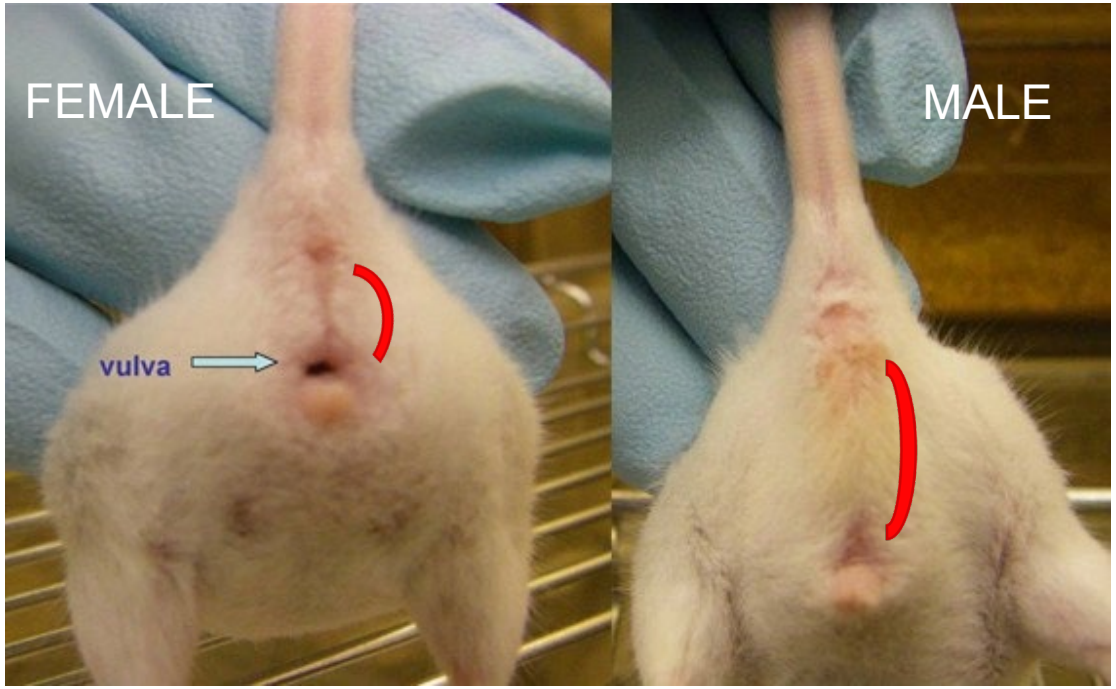
Weaning Tips & Supportive Care

TIPS FOR USING SUPPLEMENTAL FEED TO AID IN WEANING PUPS

- At 14-17days (or after the eyes are open), place moistened food and/or hydrogel into the supplemental feeder
  - This may help increase pups' nutrition prior to weaning

- Keep a supplemental feed in the cage for 5-7 days after weaning

Do I Have A Male Or Female



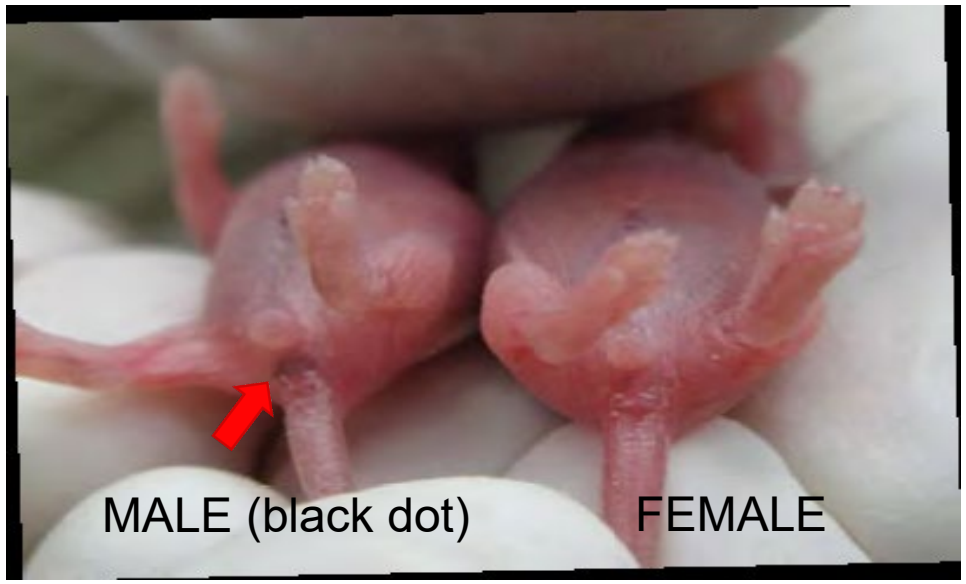
Males

- Greater anogenital distance
- Reach sexual maturity at 8 weeks
- Breed up to one year

Females

- Smaller anogenital distance
- Reach sexual maturity at 7-8 weeks
- Breed for approximately 6-8 months

Do I Have A Male Or Female



Neonatal Mice

- Differentiated by the presence of a black dot in male as pinkies

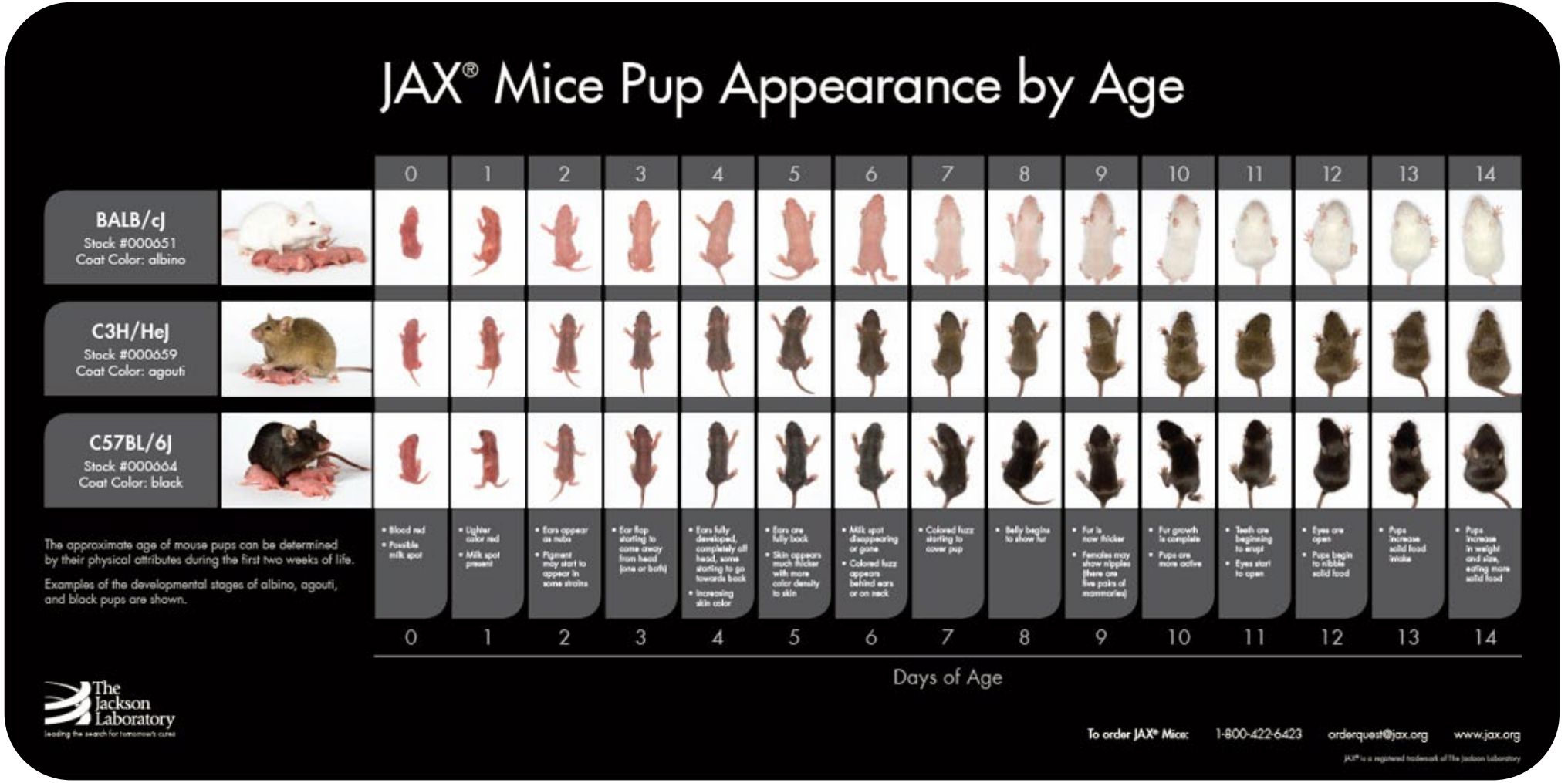
- Once furred, females will have teats visible

How to age young mice

Be As Accurate As Possible

DAY	EVENT	DAY	EVENT
1	Skin real red; short curly whisk; umbilical cord	8	Fuzz on belly
2	Straight, fine whisks; skin lighter red	9	Fur back; last day belly translucent, milk bands
3	Ear “nub” prominent	10	Top teeth erupted; belly slight fuzz
4	Pink smoother skin; ear flaps ½ up, down	11	Fully furred; fur short & smooth
5	Ears up; white fuzz across shoulders	12	Nipples visible
6	Bottom teeth erupted	13	Eyes open
7	Entire back slightly furred	14	Ears open

Jackson Labs Pup Appearance by Age



Pup Development Day 1 through Day 25

https://www.youtube.com/watch?v=NtlHP0-ltF4

Investigator Responsibilities

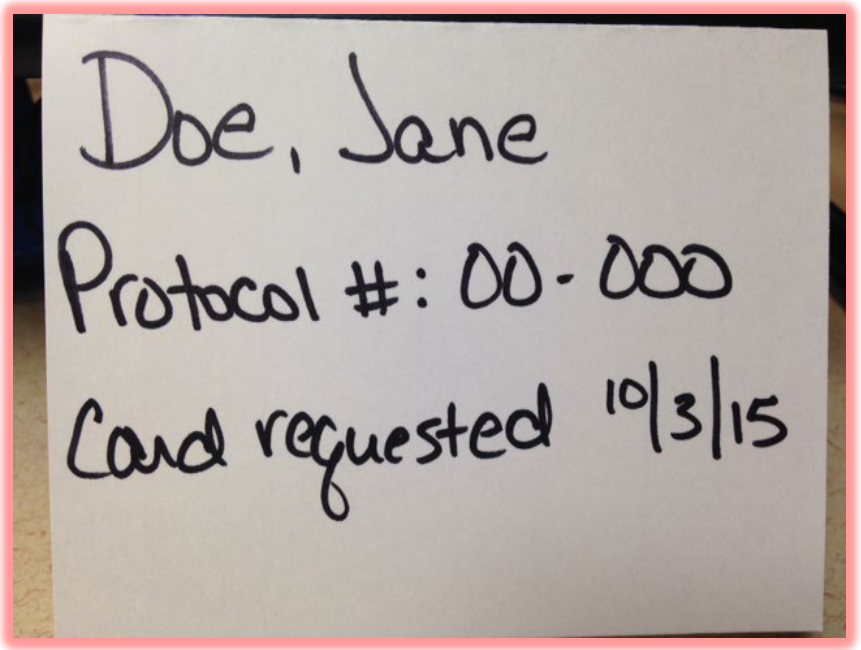
- Record Date of Birth for each litter on all breeding cages. This **must** be recorded within 3 days after birth of the litter on the PINK CARD!
- Write legibly so all information is understandable
- If you have an approved Exception for weaning, the ‘E’ must be written on the Pink Card!

Cage Identification – RFID cards

- All cages must be identified by the PI's name and protocol number. This is accomplished by the use of a DCM RFID scan card

No RFID Card?

- If a DCM RFID card is **NOT** available, the cage still must be labeled with the PI name, Protocol Number and Date Cage Cards Requested (can use a plain white card)



WHAT IS WRONG WITH THESE “CAGES”?

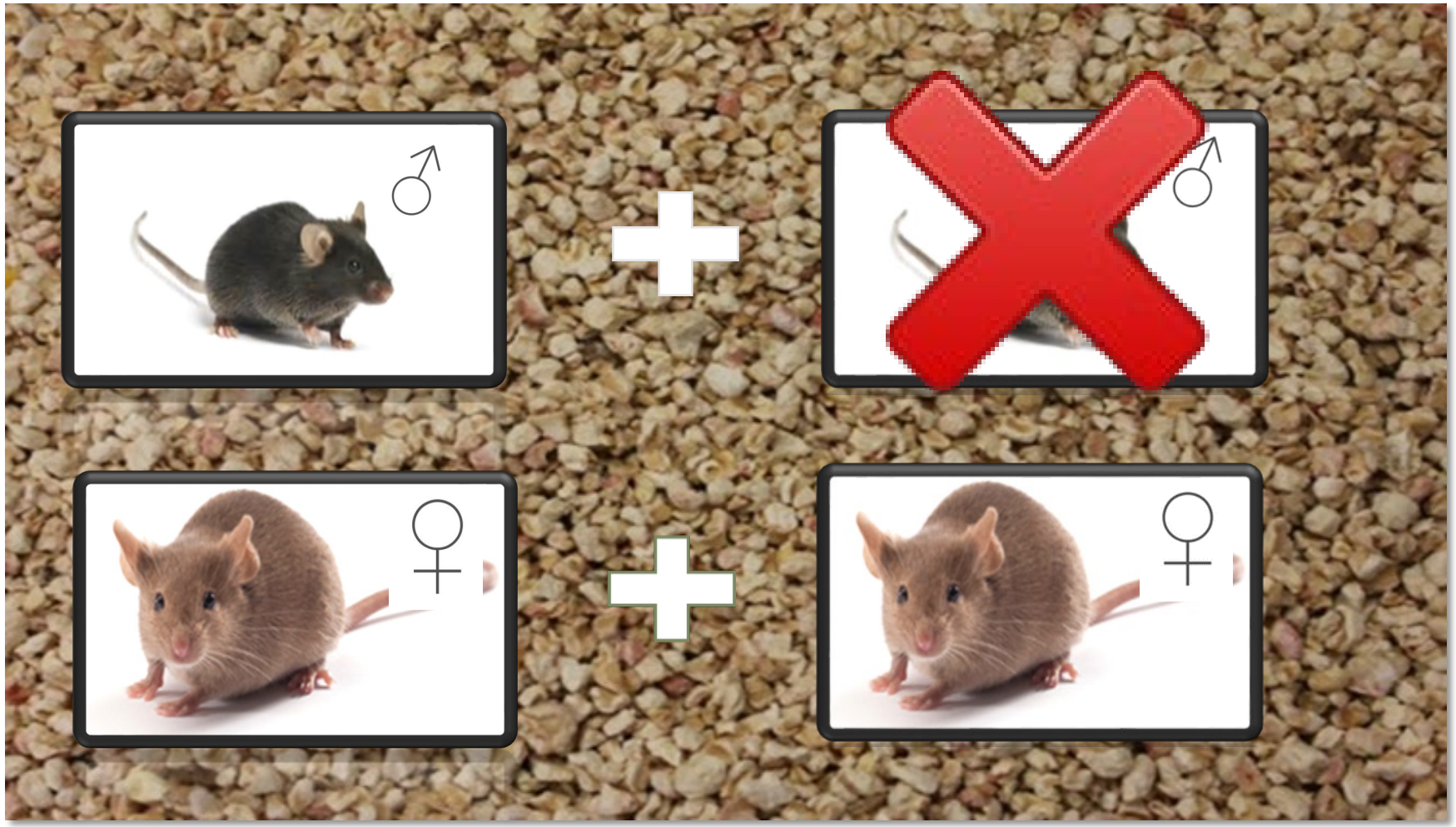
A few hypothetical examples…

Cage 1

Cage 1

Nestlet and food

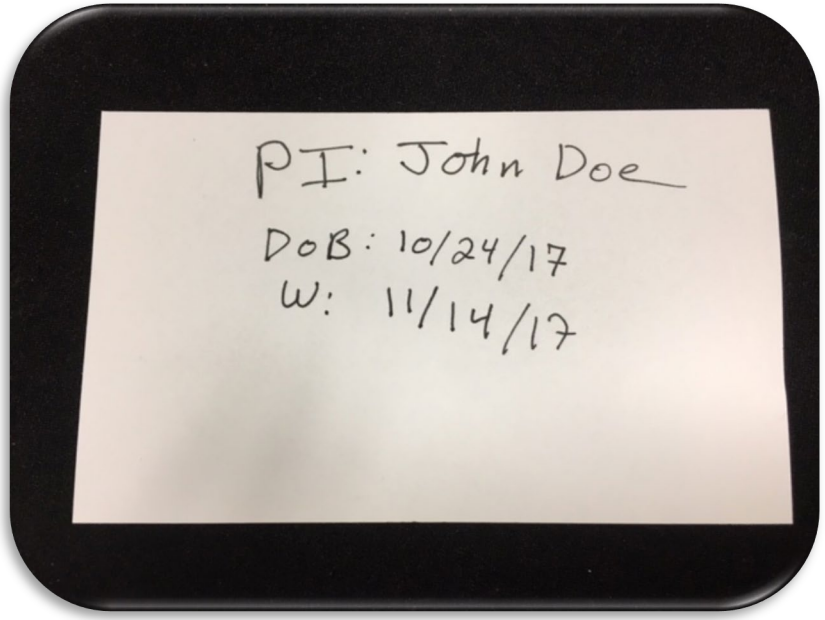
Cage 2



Fight wounds

- Male mice are territorial
- Severe fight wounds may require euthanasia

What information is missing?



- Protocol number
- Date card was requested

What's The Issue?

While checking your colony you come across a cage with 10 adult mice over 25 grams in it.

The cage has too many adults

What's The Issue?

While checking your cages you notice 12 (13 gram) weanlings in a cage with an Action Required Card on the cage.

They all weigh 13 grams – only 9 should be in the cage

Weaning Essentials

Weaned pup cage density is based on body weight

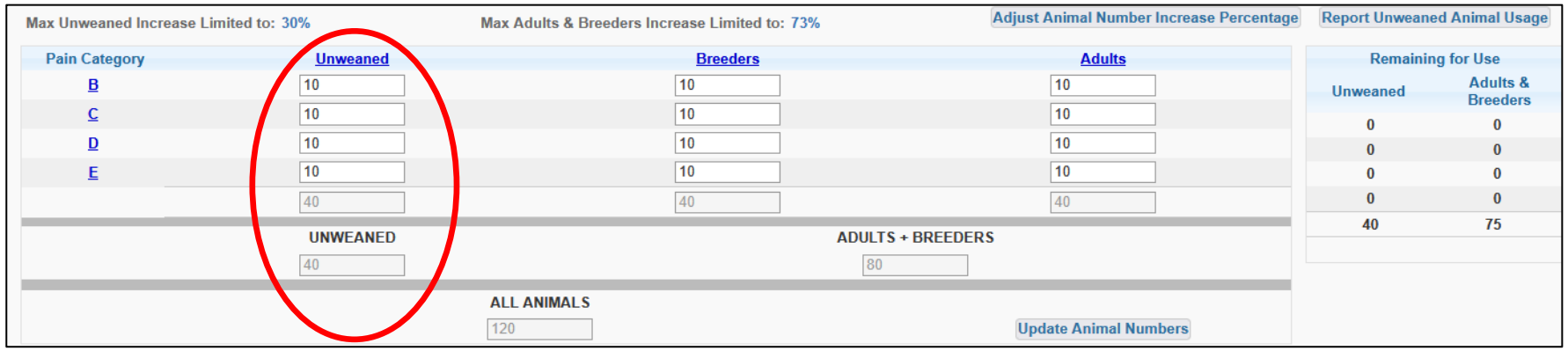
Weight	Floor area per animal	Tecniplast # per cage 78-82 sq in	Conventional # per cage 70 sq in
< 10 g	6 sq. in	13	11
10 – 15 g	8 sq. in	9	8
15 – 25 g	12 sq. in	6	5
> 25 g	15 sq. in	5	4

After Correcting the Cage Density:

- Flip over the Action Required card
- Throw the Action Required card away
- Add the Action Required card to your private collection

Euthanasia: Rodent Pups and Adults

- The method of euthanasia of pups and adults must be described and approved in the application.
- Ensure lab members only utilize methods approved in the application and that they are trained and certified to use.
- Unweaned pups are vertebrate animals and must be treated humanely and in compliance with federal and institutional policies
- The number of pups euthanized prior to weaning should be estimated in the ‘unweaned’ column of ACAP table 4.0.3 (Animal Numbers Table). The PI/Lab is responsible for accounting and tracking the number of pups euthanized prior to weaning.



Donated Animals

- If you have extra animals you cannot use, please contact the DCM Training team (DCMTraining@unc.edu). They may be able to use the animals for their training classes.
- Requirements: Animals must *not* be transgenic or experimentally manipulated.

Network of Laboratory Animal Coordinators (NLAC)

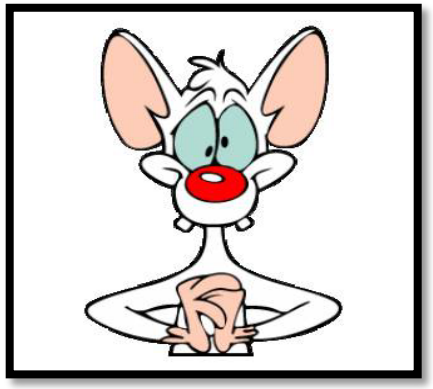
- Listserv: A useful collection of people at UNC that exchange ideas and experiences.
- Serves as the liaison that improves communication between PIs and the IACUC.

https://research.unc.edu/nlac/

Colony Management Lecture

- The Division of Comparative Medicine Colony Management Core conducts voluntary Mouse Colony Management lectures on an *as needed basis*.
- If interested, please contact:

  DCMTraining@unc.edu



QUESTIONS???

Email: iacuc@med.unc.edu

Need help with all those breeding cages?

DCM Colony Management
Natallia Riddick, Ph.D.: natallia_riddick@med.unc.edu
Colony Management Supervisor, DCM
Office: 919-843-6565

Preclinical Research Unit for Experimental and Colony Support
Charlene Santos (Director): cmross@email.unc.edu
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Alicia Chandler (Manager): chandlea@email.unc.edu

UNC Research