

MOUSE CAGE DENSITY STANDARD Revised 2019

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UNC Standard on Mouse Cage Density

*Previously referred to as Mouse Breeding Policy.



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- Link to Standard: [University Standard on Mouse Cage Density](#)
OR
- Start here: <https://research.unc.edu/iacuc/> Click: [Policy, Standards and Guidance](#) Scroll to: University on Standard on Mouse Cage Density

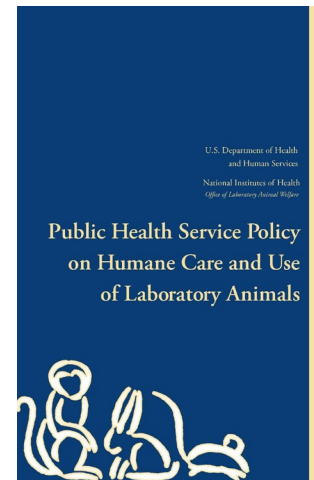
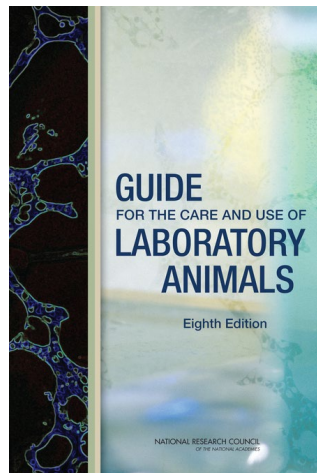


Why is Cage Density Important?



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



Adult Mice in Groups



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Animals	Weight of mice (grams)	Floor Area of cage needed per animal, (inches²)	# Adult mice per static micro-isolator 70 (inches²)	# Adult mice per green/blue line 78- 82 (Inches²)
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 including no more than 5 weaned animals per cage.

Breeding Cage Density Basics



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	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.

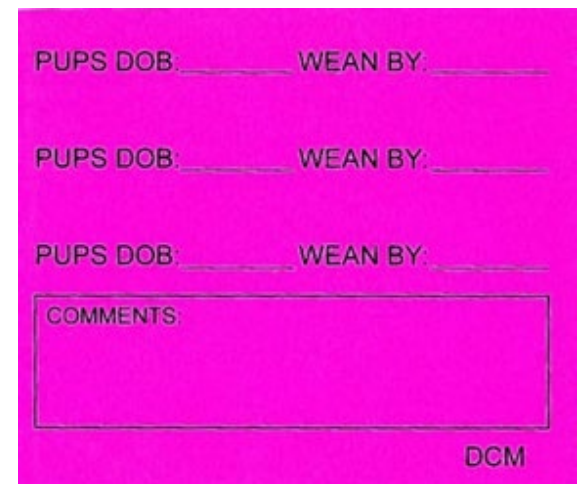
New to the Standard in 2019



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



PUPS DOB: _____ WEAN BY: _____

PUPS DOB: _____ WEAN BY: _____

PUPS DOB: _____ WEAN BY: _____

COMMENTS:

DCM

Pink Card Instructions



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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
2. Record the wean by date for day 23 using a china marker/wax pencil
3. This pink card should be used by DCM and the labs to record the litter's DOB
4. As each litter is born, record DOB and wean by date on a separate line, if applicable
5. If there are no litters, remove the pink card and wipe clean, saving for further use



China
Marker

New 'Action Required' Card (Yellow Card)



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DO NOT REMOVE
FOR DCM USE ONLY
ACTION REQUIRED BY:

____ _
____ _

A few common resolutions for separating cages to maintain compliance:

1. Separate weanlings at 23 days of age
2. 1 adult: remove oldest litter if second litter drops
3. 2 adults: Remove pups >14 days if there are 2 litters in cage or if there are more than 11 pups. Keep the pups with an adult female if <23 days old.
4. 3 adults: Remove pups >14 days old if there are more than 9 pups in the cage. Keep the pups with an adult female if <23 days old.
5. Separate pregnant dam

PI Name:

Protocol:

Building:

Room:

Date:

Cage card:

#new cages:

Your initials:

WHAT DOES THE YELLOW “**Action Required**” CARD MEAN?



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- 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
- 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.
- Once you have brought the cage back into compliance flip the action required card over on the front of the cage, so DCM knows you have taken care of the cage.

Action Required/Yellow Card Practices



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- **ON Day 24:** DCM to mark cages exceeding cage density with a yellow “Action Required” card
- **ON Day 25:** Lab is assessed \$5 charge if card is still present on the AM on Day 25
- **ON Day 27:** DCM separates for lab (AM), charges \$52
- 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 (24), must be separated by Sunday (26) to avoid separation by DCM on Monday (27)

Action Required” CARD IS PLACED ON THE CAGE.....



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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

Updated DCM 'Action Required' Log



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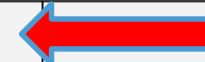
ACTION REQUIRED LOG FOR DCM USE ONLY

BUILDING: _____

ROOM #: _____

Day 24 

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



Technically
Day 24 PM /
Actually early
AM Day 25

Breeding Cage Density Basics



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	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.*

Cage Density Basics



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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



Cage Density Basics



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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

Weaning Essentials



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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



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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 REQUIRED 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



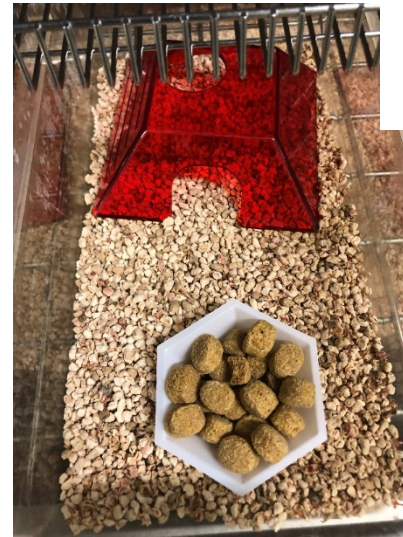
Weaning Tips & Supportive Care



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TIPS FOR USING SUPPLEMENTAL FEEDER 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 feeder in the cage for 5-7 days after weaning



Recap of Compliance Standards



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- 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 charge
- If on the third day, the density is still not corrected: DCM will separate and assess a \$52 charge
- To avoid charges: Use Comments section of Pink Card & IACUC Exceptions!
 - See next slide for details



“E”xceptions



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- 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



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Example #1: Lines with larger than average litters



“E”xception



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Example #2: Small mouse pup phenotypes

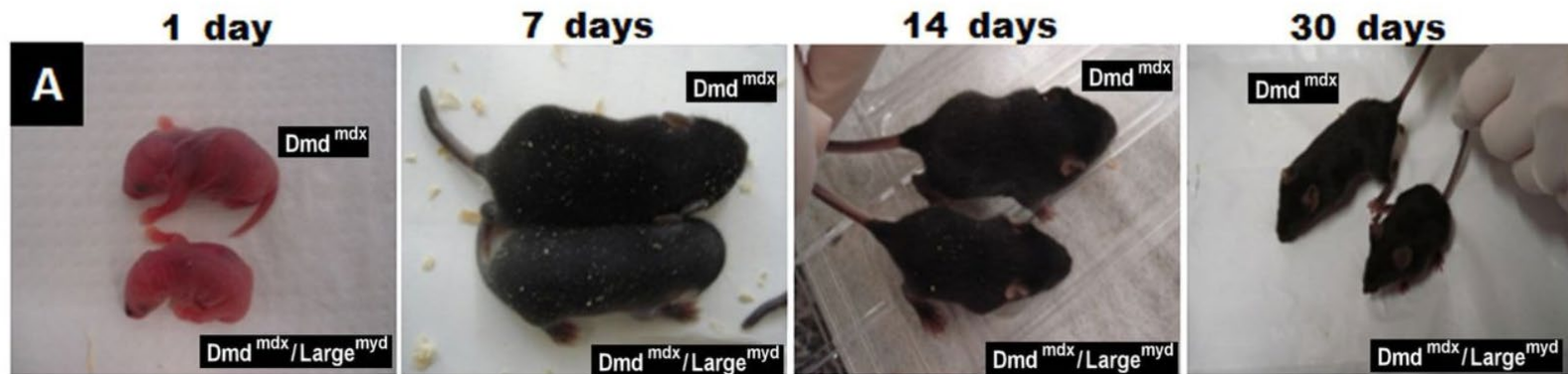


“E”xception



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



Investigator Responsibilities



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





- 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



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- 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

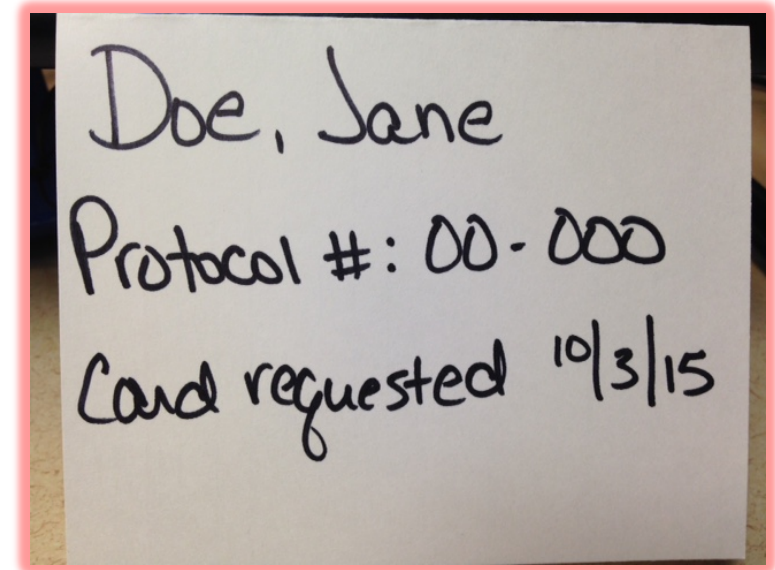


No RFID Card?



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- 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)



Breeding Records



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Animal Room Breeding Record

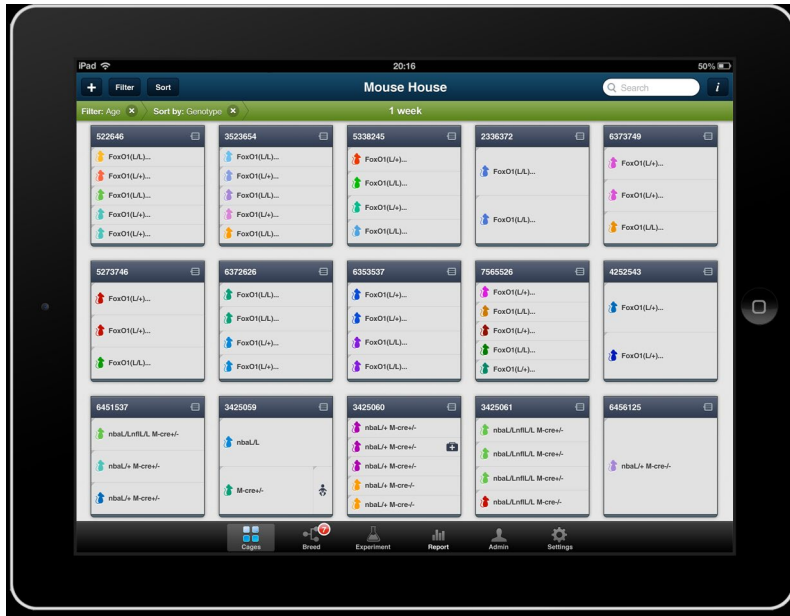
PI: _____ **Species:** _____
Building: _____ **Room #:** _____
Year: _____ **Month:** _____

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

Breeding Records



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SoftMouseDB™ Welcome test!

Mouselines Mice Matings Litters Cages Family Tree

Mouse List [Open Filter](#) [Reset Filter](#)

New Mice Edit Mice Move Mice End Mice Reactivate Ended Mice Delete Records Print List Export List

Tag	Sex	Mouseline	Genotype	Date of Birth	Age	State	Sire Tag	Dam Tag	Status	Owner
3526	E	CS7BL6	(WT)	04-26-2011	179	Mating			Breeding	Me
9143	M	DAR2:Cre	dar2:cre(+)	08-30-2011	161	Mating			Breeding	Me
9144	M	DAR2:Cre	dar2:cre(+), GGYT(+)	08-30-2011	161	Mating			Breeding	Me
8332	E	DAR2:Cre	dar2:cre(-)	11-01-2011	152	Mating			Breeding	Me
8333	E	DAR2:Cre	dar2:cre(-), GGYT(+)	11-01-2011	152	Stock			Available	Me
112	E	Tigg-mGFP	tigg-mGFP(+)	12-01-2011	148	Mating			To Cull	Me
113	E	Hox6 x Lmx1b DKO	hox6(-/-), Lmx1b(+/-)	12-01-2011	26	Stock	2136	8802	To Cull	Me
114	E	Hox6 x Lmx1b DKO	hox6(-/-), Lmx1b(+/-)	12-19-2011	23	Mating	2136	8799	To Cull	Me
8334	E	DAR2:Cre	dar2:cre(-)	01-19-2012	141	Mating	2135	8801	DOX	Me
8335	E	DAR2:Cre	dar2:cre(-)	01-19-2012	141	Stock	2135	8801	DOX	Me
8336	E	DAR2:Cre	dar2:cre(-)	01-19-2012	141	Stock	2135	8801	DOX	Me
8337	E	MNTV	MNTV(+)	01-21-2012	140	Stock	2134	8798	No DOX	Me
8338	E	MNTV	MNTV(+)	01-21-2012	140	Stock	2134	8798	No DOX	Me
8339	E	MNTV	MNTV(+)	01-21-2012	140	Stock	2134	8798	No DOX	Me
8340	E	MNTV	MNTV(+)	01-21-2012	140	Mating	2134	8798	Reserved	Me
9145	M	MNTV	MNTV(+)	01-21-2012	140	Stock	2134	8798	No DOX	Me
9146	M	Lmx1b	Lmx1b(+/-)	01-21-2012	140	Mating	2134	8798	Breeding	Me
8002	M	Tigg-mGFP	tigg-mGFP(+)	02-14-2012	137	Mating	2138	8802	Breeding	Me
8003	M	Tigg-mGFP	tigg-mGFP(+)	02-14-2012	137	Mating	2138	8802	Breeding	Me
9002	E	Tigg-mGFP	tigg-mGFP(-)	02-14-2012	137	Mating	2138	8802	Available	Me
8005	M	Tigg-mGFP	tigg-mGFP(+)	02-19-2012	136	Stock	2136	8803	Available	Me
8006	M	Tigg-mGFP	tigg-mGFP(+)	02-19-2012	136	Stock	2136	8803	Available	Me
9003	E	Tigg-mGFP	tigg-mGFP(-)	02-19-2012	136	Mating	2136	8803	Available	Me
9004	E	Tigg-mGFP	tigg-mGFP(-)	02-19-2012	136	Stock	2136	8803	Available	Me
4325	E	Balb/c	(WT)	03-01-2012	135	Stock			Available	Me

Breeding Etiquette



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- **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 Etiquette



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- 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!





How to age young mice

Be As Accurate As Possible



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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

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	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
BALB/cj Stock #000651 Coat Color: albino															
C3H/Hej Stock #000659 Coat Color: agouti															
C57BL/6j Stock #000664 Coat Color: black															
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.	<ul style="list-style-type: none"> Blood red Possible milk spot 	<ul style="list-style-type: none"> Lighter color red Milk spot present 	<ul style="list-style-type: none"> Ears appear as nubs Pinnae may start to appear in some strains 	<ul style="list-style-type: none"> Ear flap starting to come away from head (one or both) 	<ul style="list-style-type: none"> Ears fully developed, completely off head, some starting to go towards back Increasing skin color 	<ul style="list-style-type: none"> Ears are fully back Skin appears much thicker with more color density to skin 	<ul style="list-style-type: none"> Milk spot disappearing or gone Colored fuzz appears behind ears or on neck 	<ul style="list-style-type: none"> Colored fuzz starting to cover pup 	<ul style="list-style-type: none"> Belly begins to show fur 	<ul style="list-style-type: none"> Fur is now thicker Females may show nipples (have one five pairs of mammae) 	<ul style="list-style-type: none"> Fur growth is complete Pups are more active 	<ul style="list-style-type: none"> Teeth are beginning to erupt Eyes start to open 	<ul style="list-style-type: none"> Eyes are open Pups begin to nibble solid food 	<ul style="list-style-type: none"> Pups increase solid food intake 	<ul style="list-style-type: none"> Pups increase in weight and size, eating more solid food
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14

Pup Development Day 1 through Day 25



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Do I Have A Male Or Female?



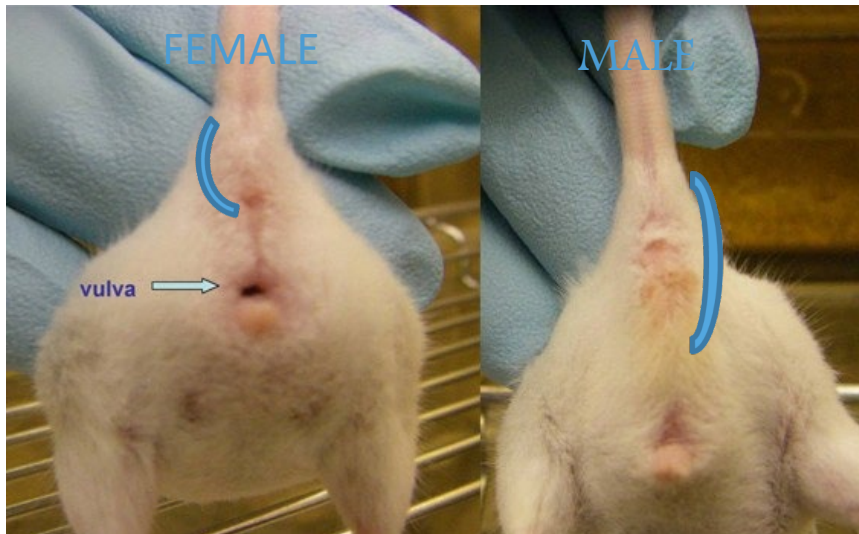
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Males

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

Females

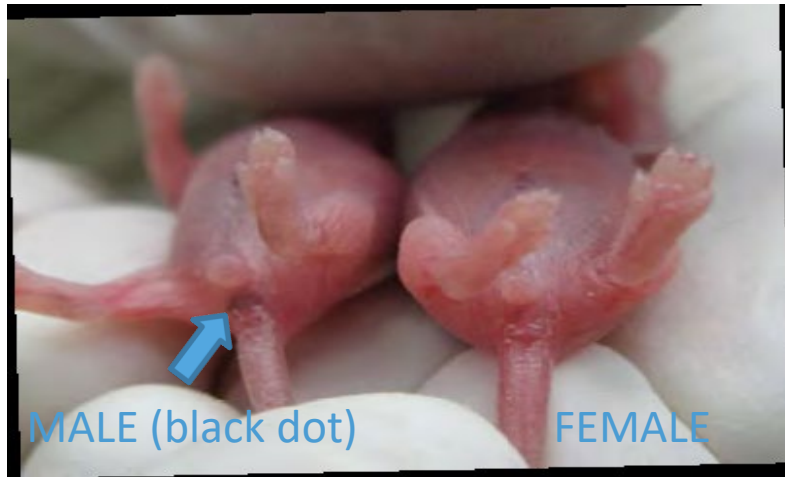
- Reach maturity at 7-8 weeks
- Breed for approximately 6-8 months



Do I Have A Male Or Female?



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Neonatal Mice

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



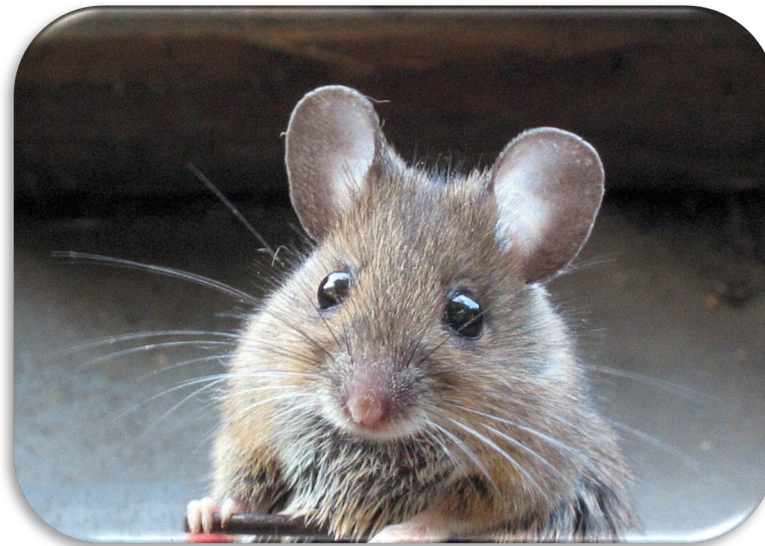
- Once furred females will have teats present

A few hypothetical examples...



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- What is wrong with these “cages”?



Cage 1



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Cage 1



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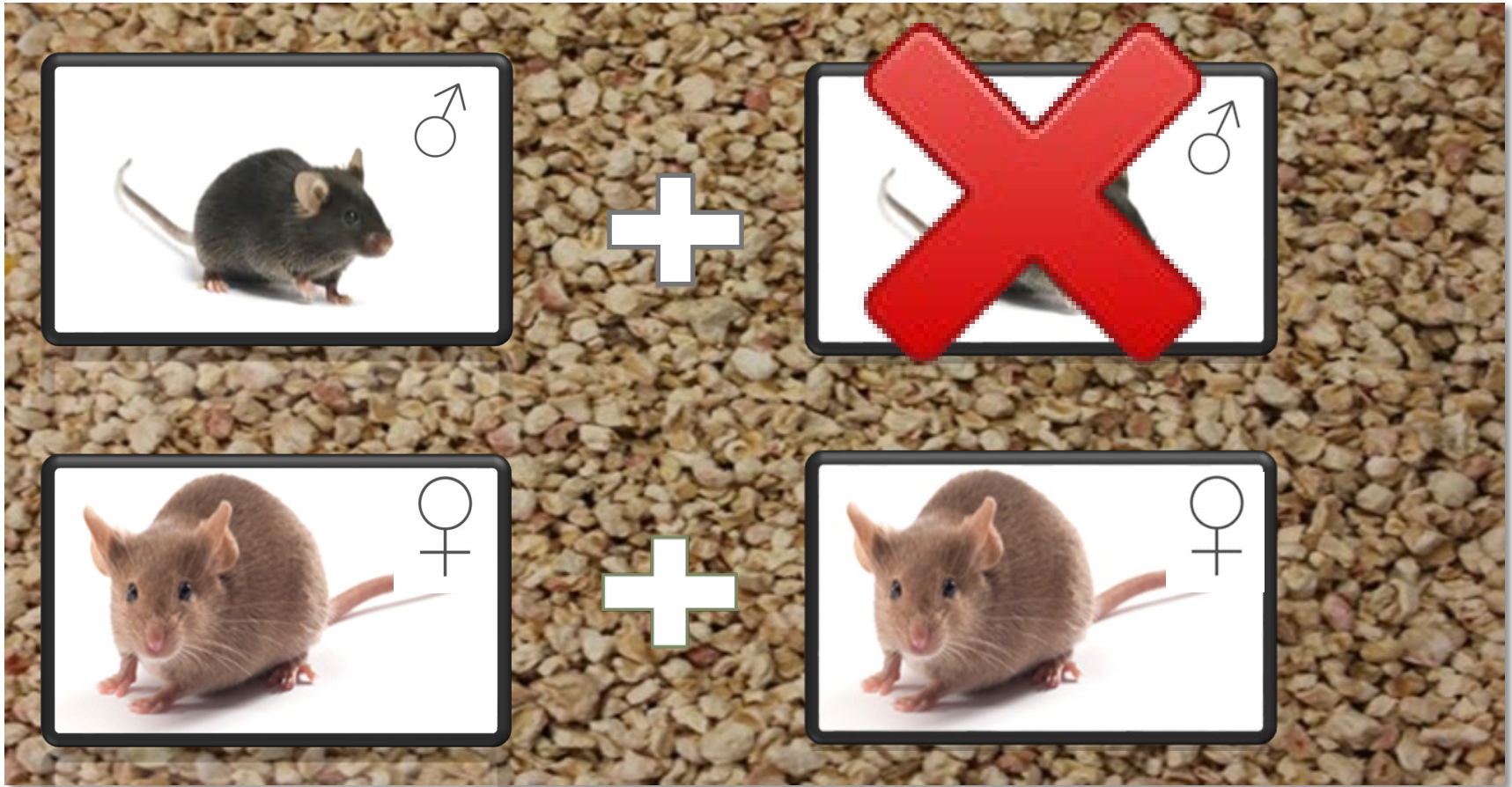
*Nestlet and
food*



Cage 2



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Fight wounds



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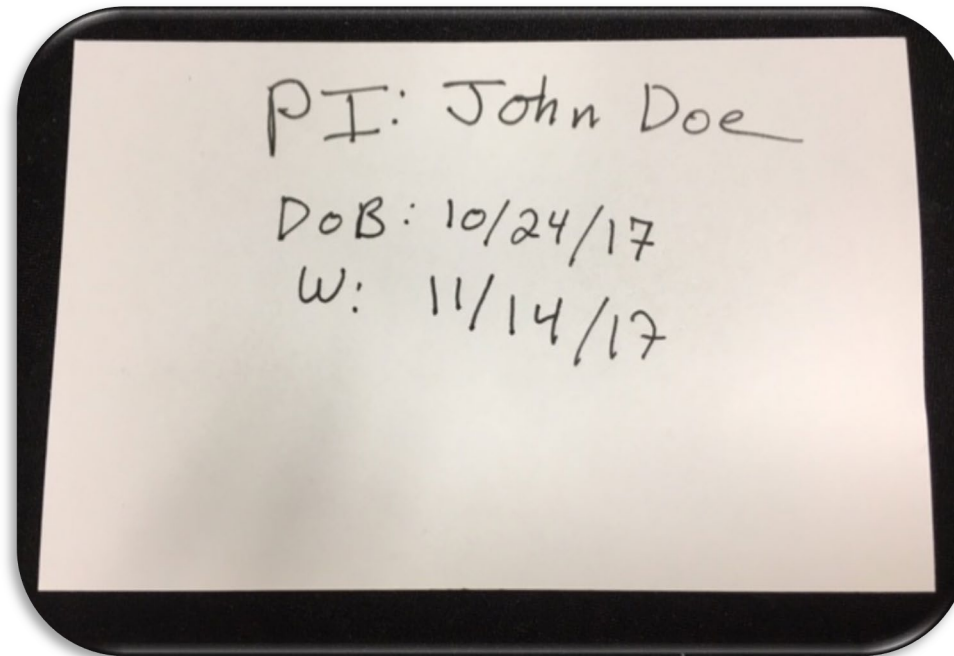
- Male mice are territorial
- Severe fight wounds may require euthanasia



What information is missing?



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- Protocol number
- Date card was requested

What's The Issue?



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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?



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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



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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	<u>5</u>	<u>4</u>

After Correcting the Cage Density:



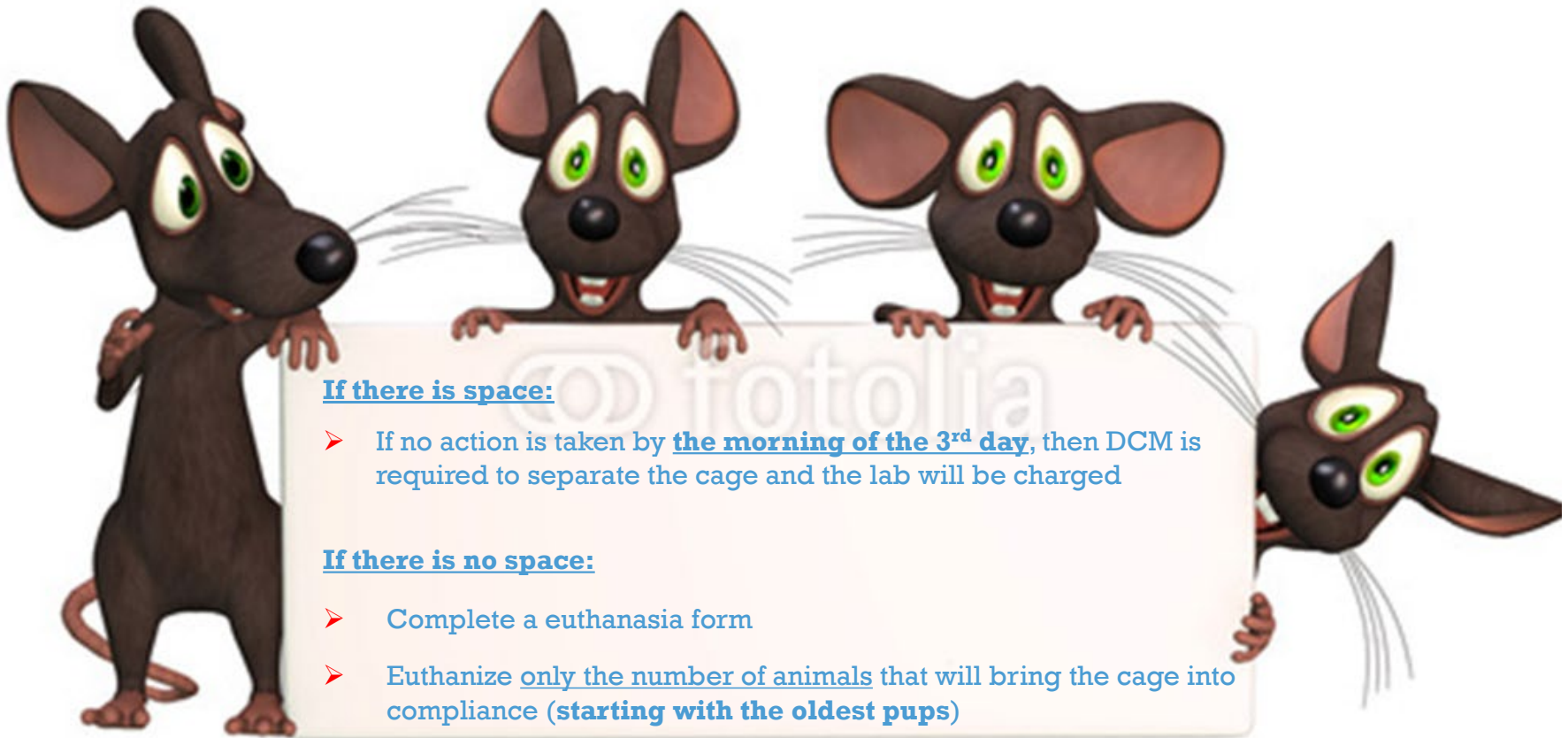
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- Flip over the Action Required card
- Throw the Action Required card away
- Add the Action Required card to your private collection

DCM Options



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Research Staff Should Know:



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- 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 **\$52 separation fee** for every cage separated by DCM

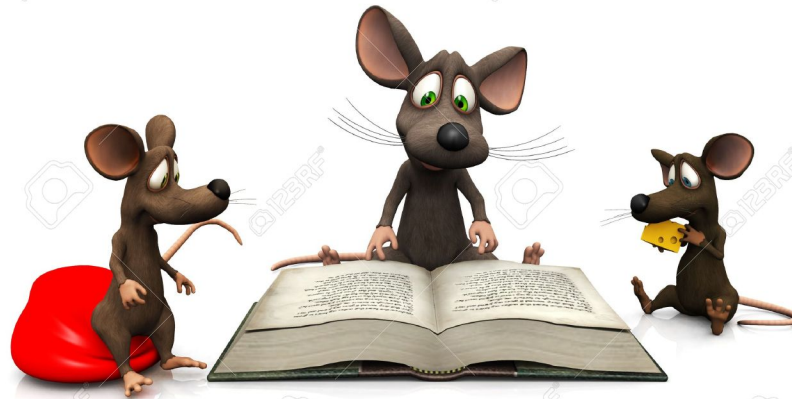


Your Lab Didn't Wean.... Now What?



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**(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?

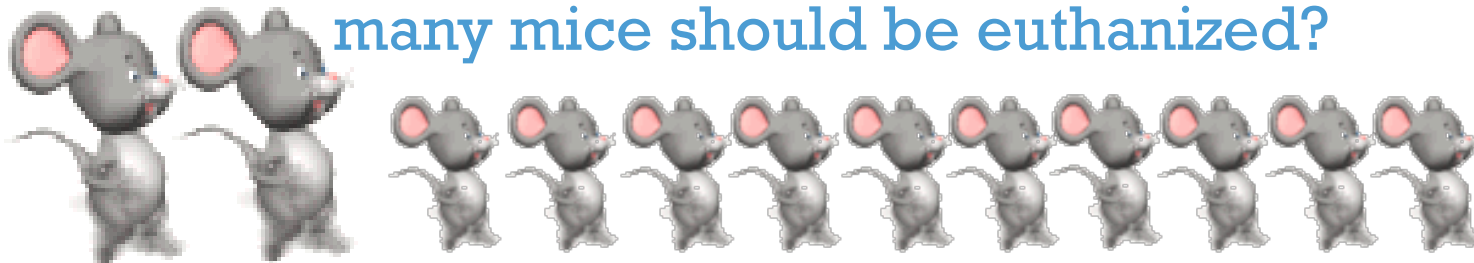
Maximum # of animals allowed in a Tecniplast cage= 5

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 15 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



DCM finds 3 adult mice (30g each) and 12 pups from 2 litters in a cage, one litter is 15 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

- 1 Adult Mouse
- 3 (15) 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

Euthanasia: Rodent Pups and Adults



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- The method of euthanasia of pups and adults must be described in the application.
- Ensure only methods approved in the application are used.
- The number of unweaned pups to be euthanized prior to weaning should be estimated in the 'unweaned' column of ACAP table 4.3. The PI/Lab is responsible for accounting and tracking the number of pups euthanized prior to weaning.
- Unweaned pups are vertebrate animals and must be treated humanely and in compliance with federal and institutional policies

Donated Animals



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



- A useful collection of people that exchange ideas and experiences. (*listserv*)
- Serves as the liaison that improves communication between PIs and the IACUC.
- <http://research.unc.edu/Offices/NLAC/index.htm>



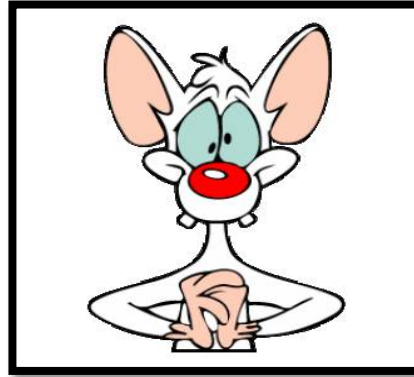
Colony Management Lecture



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



Animal Studies Core for Experimental and Colony Support

Charlene Santos: cmross@email.unc.edu

Office: 919-843-7988





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