Mouse Breeding and Colony Management





Social drug: Mice with any one working copy of

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Research Manager

Colony Management Core

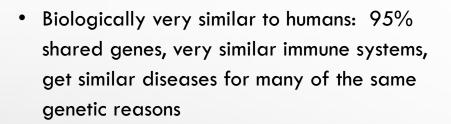


THE PLAN

- General mouse information
 - Basics
 - Reproduction
 - Basic genetic considerations
- Colony management:
 - Breeding schemes/strategies
 - Breeding performance factors
 - Weaning, ID and Tissue sampling options
 - Documentation
 - Breeding troubleshooting
- Common Health issues
- Long term colony management considerations
- resources

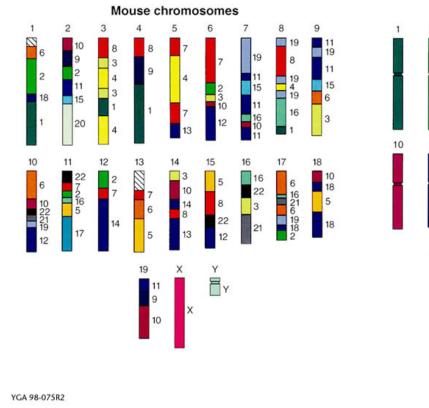


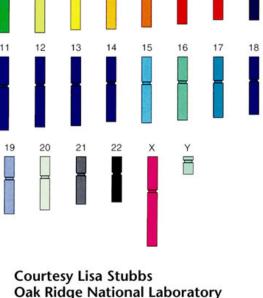
Why Use Mice as a Model



- Can manipulate the genome directly and model specific human diseases
- Inbred mice are available that are genetically identical to each other. Increases accuracy and reproducibility in experiments
- Have been used in research for more than 100 years. Very well understood and described
- Cost effective- small, reproduce quickly, easy to handle and transport

Mouse and Human Genetic Similarities





Human chromosomes





Mouse Basics

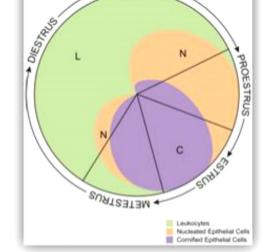


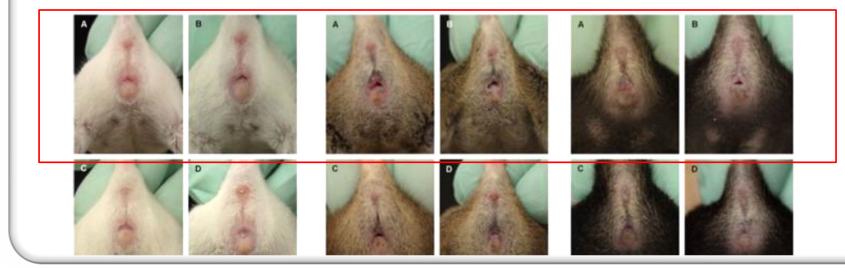


Lifespan	1.5 to 2.5 years
Weight	20 to 40 grams
Length (including tail)	6 to 7 inches (15 to 18 centimeters)
Mating Age	6-8 weeks of age
Estrous cycle	4 to 5 days
Duration of pregnancy	19 to 21 days
Litter size	2 to 12 young
Weaning age	21 to 28 days
Generation Time	12 weeks

Estrous Cycle Phases

Proestrus (A)		Ovarian follicular development phase
	Estrus (B)	Sexually receptiveOvulation
	Metestrus (C)	CL formation, eggs in oviduct
	Diestrus (D)	New wave of follicular development if not pregnant



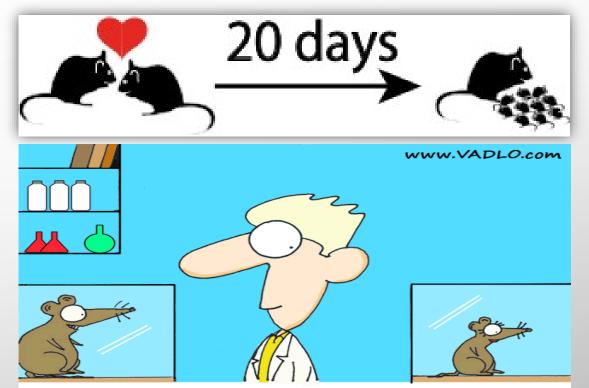


Article Source: <u>Mouse Estrous Cycle Identification Tool and Images</u> Byers SL, Wiles MV, Dunn SL, Taft RA (2012) Mouse Estrous Cycle Identification Tool and Images.

Postpartum Estrus



- Females come into estrus within 24 hours of parturation
- Can be impregnated if male is present
- Ideally first litter will be ready to wean when second litter is due
- Take into consideration when determining suitable breeding scheme



"When are you setting up the breeding?"





Laboratory Mouse

Strain: is a group of animals that is genetically uniform Substrain: colonies of same strain that have been separated for at least 20 generations

Ex: C57BL, SW, BALB/c, FVB etc

Ex: C57BL/6J vs C57BL/6N





Mouse Genetic Background



Genetic Background:

- <u>Inbred</u>: nearly genetically identical to each other due to at least 20 generations of brother/sister breeding (F1, F2...,F20)
 - Smaller litters, smaller pups at weaning, higher illness/birth defects, lower fertility, higher pup mortality
 - Ex: C57BL/6, DBA, BALB/c
- <u>Outbred</u>: genetically undefined; produced by random matings of breeding-age unrelated members of mouse colony
 - Long life spans, high disease resistance, early fertility, large and frequent litters, low pup mortality, rapid growth, large size
 - Ex: CD-1, Swiss Webster, ICR
- <u>Hybrid</u>: A mouse that is first generation (F1) crosses between 2 different inbred strains
 - Ex: B6129SF1/J





Mouse Mutants



Mutants (single gene):

- <u>Transgenic</u>: A mouse that contains stably inherited DNA which has been inserted randomly into the genome
 - Ex: tissue-specific, inducible Tg
- <u>Targeted Mutations</u>: A mouse that has a particular gene silenced
 - Ex: knockout, knock-in, conditional knockout



Inbred Strain Nomenclature

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- Strains indicated by all capitalized letters
 - AKR, CBA, DBA, FVB etc.
- Many exceptions to this rule since many strains named before standardized nomenclature rules
 - 129, C3H, BALB/c (the /c is part of the strain designation)
- C57BL/6J vs C57BL/6N
 - C57BL = strain designation (black offspring of female C57)
 - /6 = substrain designation
 - J = source (The Jackson Laboratory) vs N=NIH, subline designation also
 - microbiological status sometimes included in brackets
 - [BR] = barrier reared, [GF] = germ free, [GN] = gnotobiote, etc.





Inbred Strain Abbreviations

AKR = AK	BALB/c = C	CBA = CB	C3H = C3	C57BL = B
C57BL/6= B6	C57BL/10= B10	DBA/1 = D1	DBA/2 = D2	SJL = S or J
	SWR	= SW 129S1	/SvImJ= 29S	
			• O (



Inbred Strain Abbreviations





Choosing a Breeding Scheme

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- WHAT GENOTYPES DO I NEED?
 - How can I identify them?
- WHAT CONTROLS ARE AVAILABLE?
- LINKAGE CONSIDERATIONS
- X-linked or autosomal?
- MULTIPLE GENES
 - Linked or segregating independently?
- REPRODUCTIVE CONSIDERATIONS (STERILITY OR SUBFERTILITY?)
- EMBRYONIC OR POSTNATAL LETHALITY?





Mating Combinations <Always only one male per cage>

PAIR

TRIO

HAREM







1 **d** 3-4 **Q**





Monogamous Pair



- Ease of accurate record keeping
- Continuous breeding, i.e., male and female always together – takes advantage of postpartum estrus
- Parents are known
- Don't have to separate parents
- Reduces male burnout from excessive breeding
- Exact date of birth for each litter
- Continuous breeding allows for the generation of a max amt of litters per female during her breeding life span

CONS

- Higher number of males required
- Higher number of cages required
- If the male is aggressive, he may cannibalize pups and/or injure the female – not common
- If one of the breeding pair dies, it can be difficult to replace





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Polygamous Trio or Harem





Labor intensive

- Requires fewer male mice
- Fewer numbers of cages needed
- Can reduce exposure of pups and females to aggressive males
- Allows to produce a large number of litters quickly

- Accurate record keeping is more difficult
- IACUC cage density only allows 9 pups total between 2 litters when 3 adults are present

CONS

- Most times Male must be removed, and females separated because pups total between 2 litters exceeds 11
- Females must be removed from harems when obviously pregnant
- In both situations, Unable to take advantage of postpartum estrus and therefore fewer litters per female
- Higher incidence of male burnout

QUALITY

- Compliance to ideal cohort (e.g. sex, age, etc.)
- Health profile
- Frequency between cohorts
- Proper control animals

Triple Constraint of a Breeding Project

SPEED

- Assisted reproductive technology
- Cryopreservation to cohorts
- Peak vs. continuous breeding

COST

- Genotyping
- Animal husbandry
- Microbiological monitoring

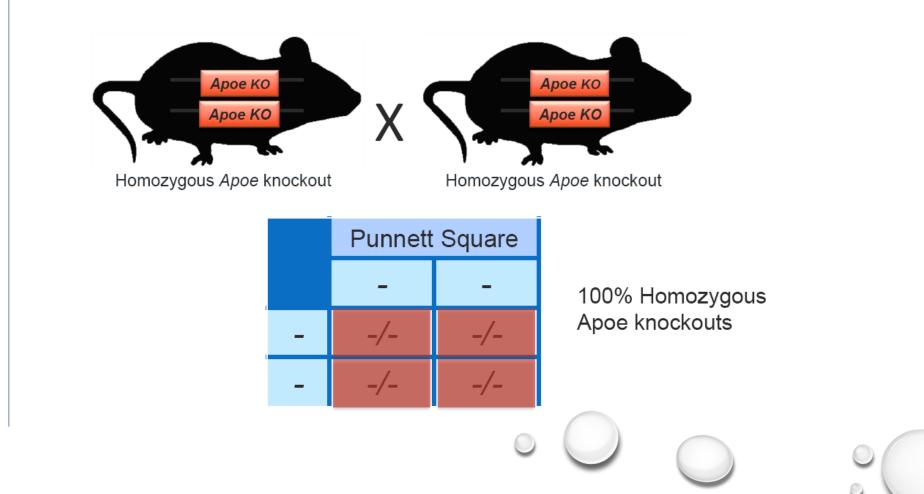


Homozygous mutant (-/-) x Homozygous mutant (-/-)

- Can be used if both genders are viable and fertile as homozygotes
- Offspring: 100% homozygous; genotyping not required
- Recommend regenotyping breeders before pairing or 1st offspring litter
- mutants should be backcrossed to the parental inbred strain every 10 generations to avoid genetic drift (i.e. random mutations effecting phenotype)
- If do not backcross, eventually will create mutants with a divergent inbred background (new substrain)
- Controls: wild type congenic strain or inbred strain mice

Ex 1: KO, Congenic Controls

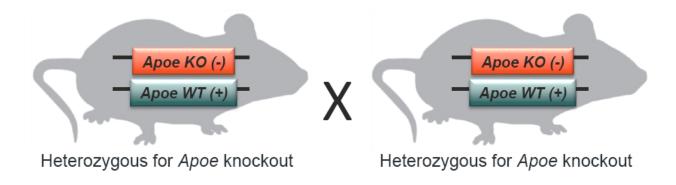
• Experimental Mice, Homozygous Apoe knockout

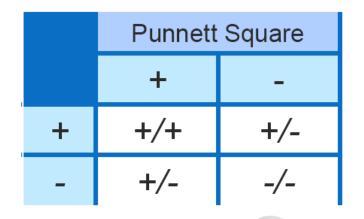


Heterozygous mutant (+/-) x Heterozygous mutant (+/-)

- Must be used if neither gender is viable or fertile as homozygotes
- Offspring: 25% homozygous; 50% heterozygous; 25% wildtype; genotyping required
- mutants should be backcrossed to the parental inbred strain every 10 generations to avoid genetic drift (EX: backcross a mutant on a C57BL/6J background to the standard C57BL/6J strain for 2 generations)
- Controls: +/+ or +/- siblings or inbred strain mice

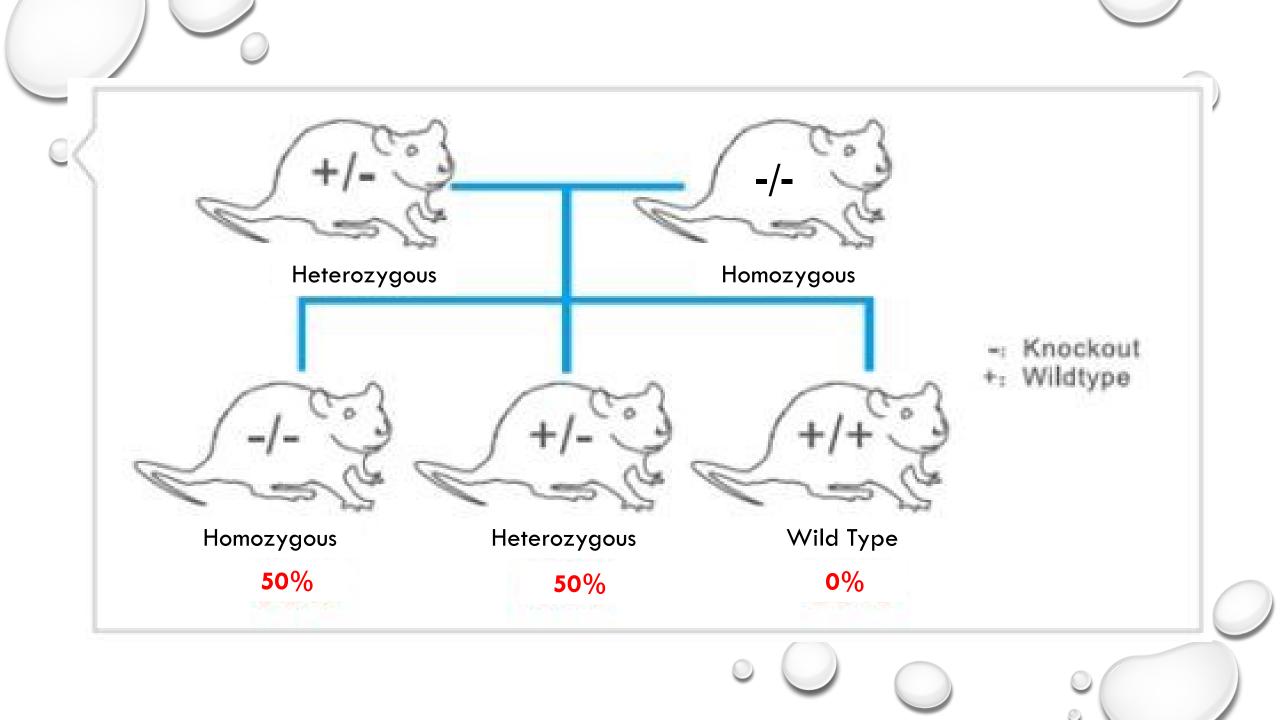
What percentage of the offspring will be homozygous for *Apoe* KO?



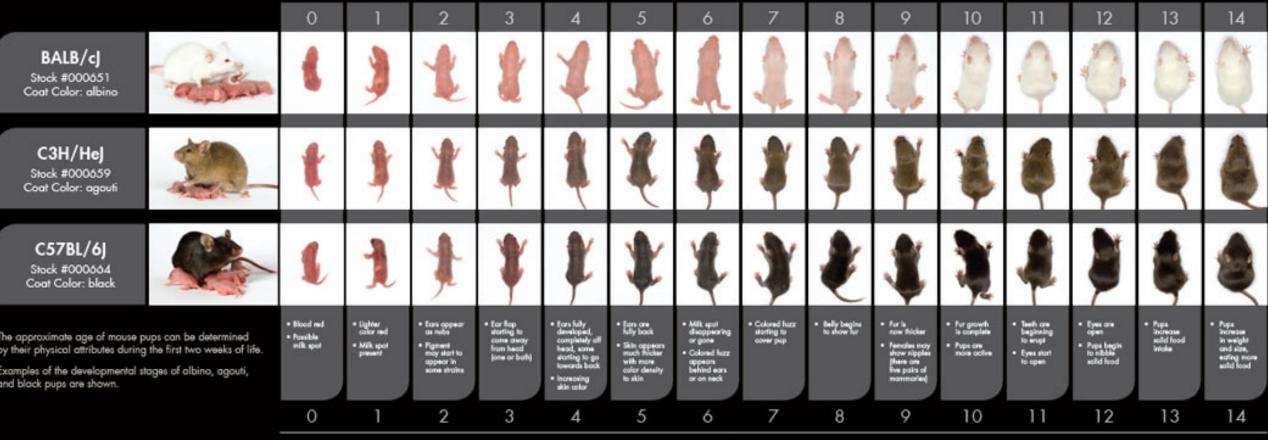


Heterozygous mutant (+/-) x Homozygous mutant (-/-)

- Can be used if only one gender is viable and fertile as a homozygote
- Offspring: 50% homozygous; 50% heterozygous; genotyping needed
- mutants should be backcrossed to the parental inbred strain every 10 generations to avoid genetic drift (EX: backcross a mutant on a C57BL/6J background to the standard C57BL/6J strain for 2 generations)
- Controls: +/+ congenic strain, normal phenotype +/- siblings, or inbred strain mice



JAX[®] Mice Pup Appearance by Age





Days of Age

To order JAX* Mice: 1-800-422-6423

orderquest@jax.org www.jax.org

jAX* is a registered teademark of The Jackson Laboratory

IACUC Requirements for Breeding Cards

CMC-DCM	PI:				Protocol #:
RFID:			Sam	ple:	
Strain:					Mating Date:
Male ID:		DOB:	1	Gen	otype:
Female ID:		DOB:		Gen	otype:
	#		Wea	aned	
DOB	Born	Date Weaned	M	F	Comments
Signation of the		The second second			
States Part			12.72		

CMC-DCM	PI:				Protocol #:
RFID:			Sam	ple:	
Strain:			E Start		Mating Date:
Male ID:		DOB:		Gen	notype:
Female ID:		DOB:		Gen	otype:
	#		Wea	ined	
DOB	Born	Date Weaned	M	F	Comments
				1	
Sector Sector					

- PI Name and protocol #
- Birthdate of pups if breeding cage (required within 3 days of birth) and a pink DCM card with litter DOB and DOW inserted
- Pre-printed cage card with IACUC protocol number (DCM RFID card)
- Note: temporary cards allowed, but must include all above, plus date the RFID card was ordered
- Cards should also include mouse strain name, genotype, DOB of the breeders, mouse IDs, sexes



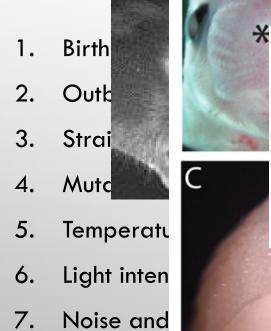
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Breeding Performance Factors

В

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С



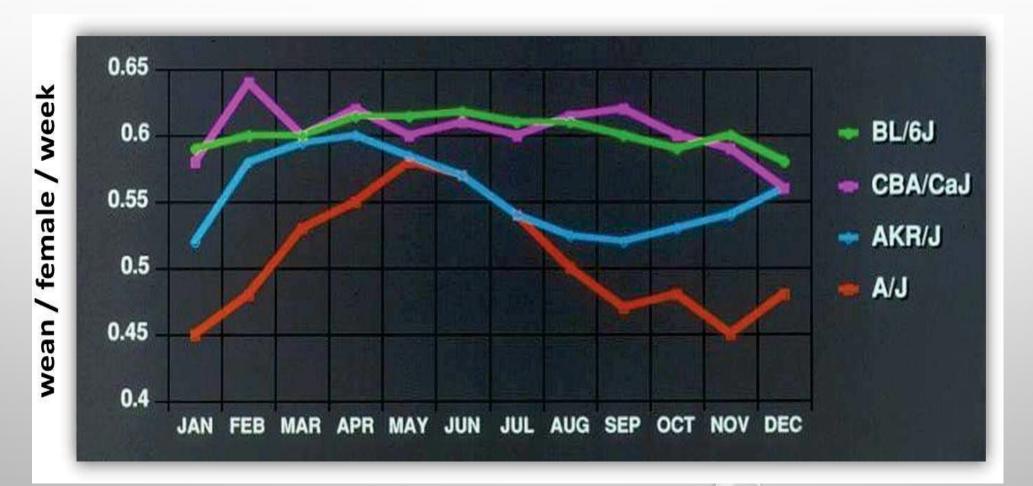


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MOUSE REPRODUCTION CAN CHANGE WITH THE SEASONS



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Strategies for Maximizing Productivity of a Breeding Colony

When to set up breeding• Mate mice when they are 6-8 weeks old.• Note that it is not unusual for the first litter to be smaller than the second or third, which are typically the largest.When to foster pups• Foster pups - if the mother does not "nest" the pups right away but leaves them scattered around the cage. - if milk spots do not appear in the pups by the time they are 24-hours old.• Note that it is not unusual for the first litter to be smaller than the second or third, which are typically the largest.• Men to foster pups• Foster pups - if the mother does not "nest" the pups right away but leaves them scattered around the cage. - if milk spots do not appear in the pups by the time they are 24-hours old.• If you need offspring from a female who is a poor mother, plan ahead to have foster mothers ready when she gives birth. • Housing 2 females together will often allow them to work together and successfully raise 2 litters.	Factors	Action to promote successful breeding	Comments
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Factors	Action to promote successful breeding	Comments	\bigcirc
When to replace breeding pairs for optimal performance	 Replace breeding pairs as their reproductive performance declines (typically 6–8 months of age) For a large colony, maintain a stable supply of breeding pairs at various ages by replacing a specific percentage on a weekly or monthly basis. 	breeders produces a more consistent quantity of pups than does a colony with	
When to replace individual female breeders	 Replace female breeders when they produce no litter within 60 days of pairing (unless this delay is normal for the strain), produce no litter within 60 days of their last litter, or produce litters but wean no pups for 2–3 litters. 		
When to replace individual male Breeders	 Replace male breeders when they reach 1 year of age, or if they are infertile with a young, fertile female. 		



Factors	Action to promote successful breeding	Comments
When to cull a litter	 Reduce the number of pups in a litter if the mother is having problems feeding the pups. 	 Some mothers may be unable to provide milk for more than a few pups. For segregating strains, remove unwanted pups as soon as they can be phenotyped or genotyped.
How to improve breeding Behaviour	 With young females, use experienced males. Isolate males for 2 weeks before pairing. Rotate males within a strain among cages. 	 Males mature later than females; therefore age at first litter for same-aged breeding pairs is often determined by the male.

Optimizing Breeding Performance



Replace breeders before their reproductive performance declines

- Replace non-productive breeders
- Mate mice early
- Use experienced males
- Keep meticulous and accurate breeding records
 - Investigate deviations in breeding performance
 - Compare your colony breeding performance to known (supplier, other labs, etc.)
 - Keep environmental conditions suitable and stable
 - Periodically verify the genotypes of breeders/offspring
- Litter fostering
- Breeder diet vs regular
- Mating numerous females simultaneously
- Use ART (Assisted Reproductive Methods)
 - Ex: IVF, Superovulation, Embryo transfer, etc.



Successful Fostering

- Choose foster mother with different coat color and great maternal behavior
- Ideally natural pups are born within 2 days of the foster ones
- Keep the litter the same (cull down natural litter)
- Take the mother out during transfer
- Rub some urine, dirty bedding from foster cage on the pups and then mix them with bedding in the nest
- Return the female to the cage and return the cage to the rack to avoid any more stress
- She should start nursing within 6 hours



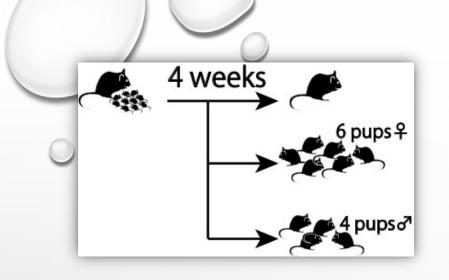
Timed Pregnancies



- 1. House males individually for 1-2 weeks prior to mating
 - Pick experienced, proven males If possible or use older (3-4 months old) over younger males
- 2. Use 8-15-week-old females
 - Typically sexually mature by 6-8 weeks
 - Avoid using virgin females older than 15 weeks, often mate less reliably
- 3. Group-house females and synchronize their estrous cycles prior to m
 - Lee-boot and Whitten effect
- 4. Verify estrous state of females prior to mating
 - Should be in proestrus or estrus
 - Inspect visually
 - Can confirm with cell morphology following vaginal swab
- 5. Add only 1-2 female(s) in each male cage in the afternoon
- 6. Check for vaginal plugs each the next morning
- 7. The date a plug is observed is gestational day 0 to 0.5



https://www.jax.org/news-and-insights/jax-blog/2014/september/six-steps-for-setting-up-timed-pregnant-mice



- Pups are to be weaned on day23 if they are 10grams
- Male and female pups are separated at weaning into separate cages with NO more than 5 mice per cage.
- If a litter contains a single animal of one sex, then place a gel cup in the cage with the pup

Weaning











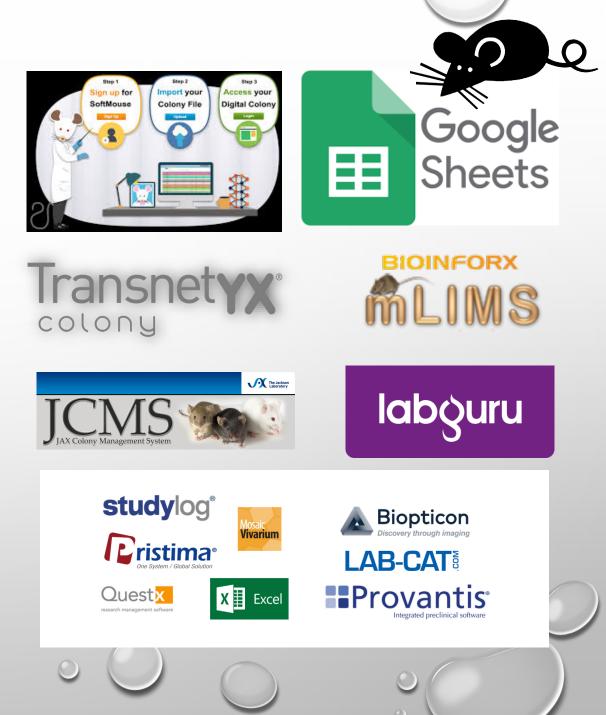
Weaning

- Place a few pieces of rodent chow on the floor of the cage with <u>ALL</u> newly weaned pups
- Add food to the hopper and provide a clean water bottle or toggle the lixit if on autowater
- Place a pink toggle card with wean date +7days to make sure the lixit is toggled daily and the mice are checked for signs of dehydration

CMC-DCM	PI:			Protocol #:	
Strain: DOB:		Sex:	Dam:	Sire:	
RFID #:			_		
Animal # ID		ID	Genotype	Comments	

Record Keeping Methods

- 1. **Paper/pen in facility:** update online later
- 2. Laptop/tablet
 - Databases many software options available for free/purchase
 - 2. **Excel-** can be difficult with multiple users
 - 3. **Google sheets:** shareable secure excel with editing capabilities and ability to restore changed information
 - 4. Keep database current





Identification Methods

It is important to select the appropriate identification method for each individual lab and research

This should be based upon the age of the animal, the number of characters one wishes to include, and the duration of the experiment

ID information needs to be recorded on the cage card in the event that clarification of the numbers or characters becomes necessary for any reason

Some ID methods can be combined with tissue collection for genotyping to minimize stress to the animal





Identification Methods Temporary

 Non-toxic, permanent markers can be used to temporarily mark the fur, tail, or skin of the animal

• This ink usually lasts 3-4 days without the need to remark

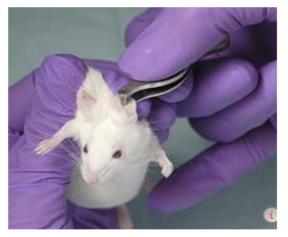


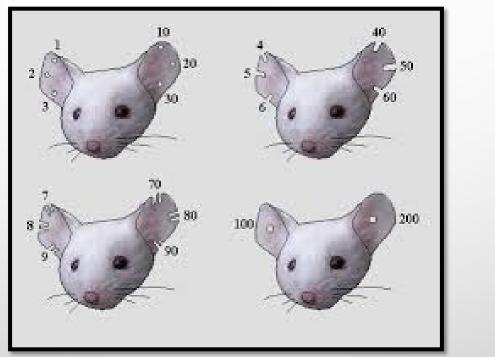
Identification Methods Ear Notch/Punch

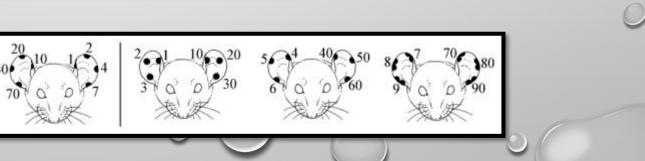


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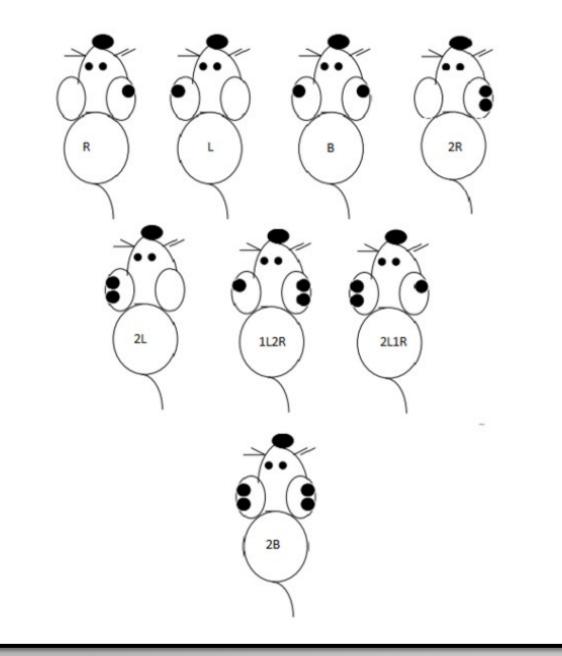








Alternative Mouse Numbering System (within one litter) Ear punch used to make notches. Option: to repeat these notches and discriminate by sex.

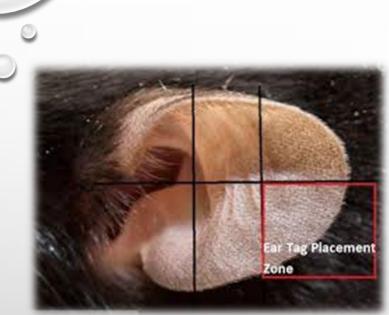


Identification Methods Most Common Ear Notch System

- Easy to Read
- Inexpensive
- Can use tissue for genotyping

However:

• Some strains of mice are known for ear mutilation



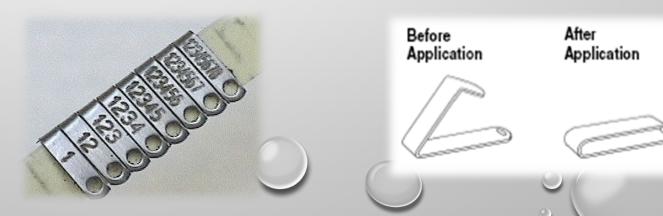


Identification Methods Ear Tag

- Easy to administer
- Can use long numbers

However:

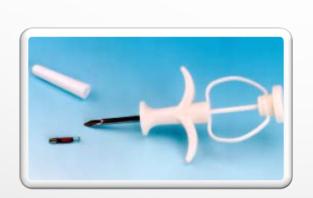
- Tags are often lost
- May cause ear irritation
- Must restrain a mouse to read the tag



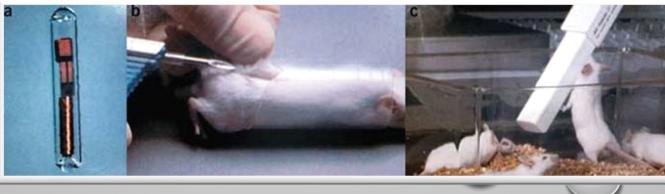
Improper Tag Placement



Identification Methods: Microchip Transponders



- Often called "Pit Tagging", they are implanted subcutaneously between the scapulae or on the lower back
- Each microchip is encrypted with a unique, non-replicable number and are read with a portable, hand-held scanner







Identification Methods: Ear RapID Tags

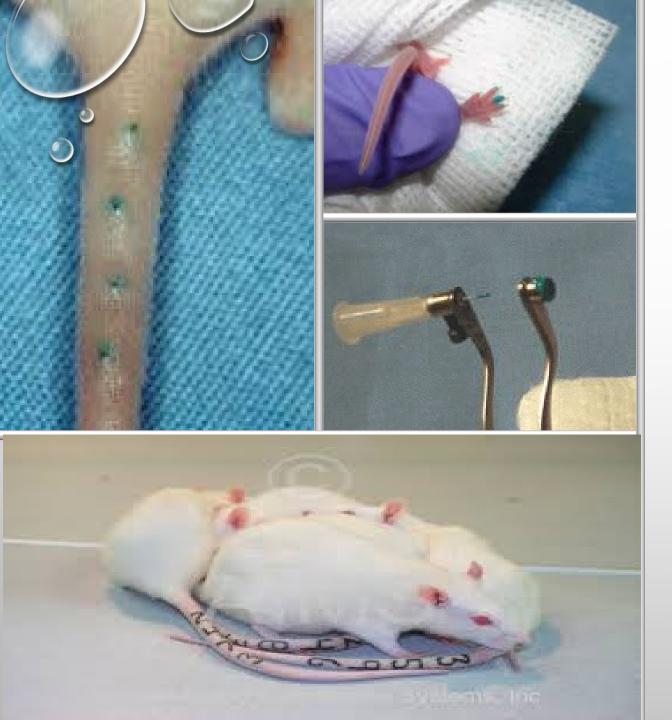


- Permanent ID method
- Easy to read
- Hypoallergenic
- Some systems are programmable and provide telemetry

<u>However</u>:

• Expensive!!!!





Identification Methods: Tattooing

- Low health risk
- Can be used on adults (tails, footpads, toes) and neonates (toes, footpads)
- Versatile

However:

- Somewhat difficult/time consuming to administer
- Requires skill, equipment and money
- Ink fades over time
- Must immobilize animal

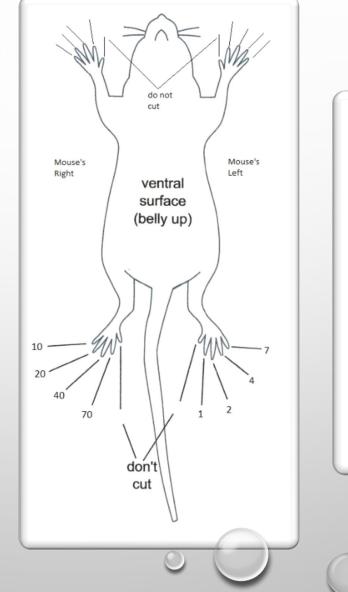


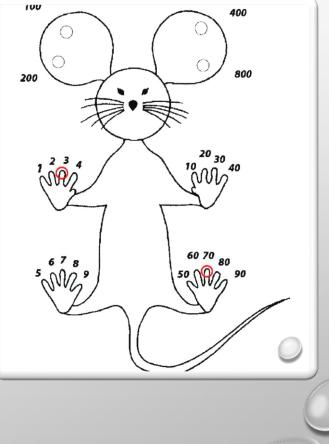
Identification Methods: **Toeing**

- Permanent
- Early combination of ID system and tissue sampling for genotyping(PD7-10)
- No more than 2 toes per foot or 4 toes total per animal

HOWEVER:.

- Requires scientific justification and iacuc approval
- Must pick up mice to read



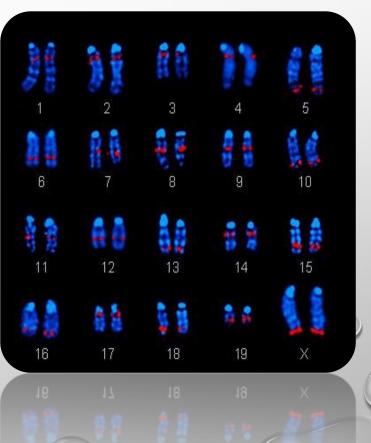




Genotyping







- Ear Notch/punch
- Toes
- Tail Snips
- Blood
- Hair
- Saliva
- Feces



Troubleshooting Breeding Problems(Breeding Problems and Possible Resolutions)

Source: (The Jackson Laboratory Handbook on Genetically Standardized Mice)

Problems	Possible Resolution	
 Mice are not breeding Females are not getting pregnant 	 A. Make sure you have male and female breeders. B. Add nesting material specifically designed for that purpose. C. Try a different diet, for example, one with more or less fat. D. Minimize stress-including human contact with the mice and activity and noise in the room. Construction-related vibrations, even outside the building, can disrupt breeding. If possible, move mice to a quieter area. E. Try a 14:10 light cycle. F. Make sure mice are healthy and able to breed (for example, not too obese or too old). Try healthier, younger animals. G. If you never see vaginal plugs, try a new male. H. Determine whether the problem is the male or female: pair a proven breeder female with the male and a proven breeder male with the female. I. Surgically evaluate the reproductive tract. If the female appears normal, try low dose gonadotrophins, ovarian transplant, IVF; if the male appears normal, try IVF. J. Research the strain reproductive characteristics. For a transgenic, check the effect of the transgene on breeding. For a strain carrying a mutation, check the effect of the mutation. Adjust the breeding strategy accordingly. 	

		5.0
Problems	Possible Resolution	
 Mice get pregnant but you never see pups (females are resorbing fetuses) 	D. Minimize stress—including human contact with the mice and activity and noise in the room. Construction-related vibrations, even outside the building, can disrupt breeding. If possible, move mice to a quieter area. The most common cause of fetal resorption is stress.	
 Individual breeders are not producing an expected number of pups 		

Oroblems	Possible Resolution
 Females give birth, but don't raise their pups Pups disappear or do not survive 	 D. Minimize stress—including human contact with the mice and activity and noise in the room. Construction-related vibrations, even outside the building, can disrupt breeding. If possible, move mice to a quieter area. J. Research the strain reproductive characteristics. For a transgenic, check the effect of the transgene on breeding. For a strain carrying a mutation, check the effect of the mutation. Adjust the breeding strategy accordingly. K. If you introduced a male to a cage with pups, he may have killed them. Wait to add a male until all pups have been weaned and removed.
• Colony productivity has dropped	L. Check for environmental factors that might have changed. Consider room conditions (temperature, vibrations, building construction, odours, etc.), caretaker, and diet. If more than one cage, area, or strain is affected, expand the search. If possible, restore environment to previous conditions.

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Unhealthy Mouse



- Signs that your mouse is sick:
 - Scruffy coat
 - Hunched, sunken at hips
 - Weight loss, low activity
 - Skin lesions
 - Labored breathing
 - Eye or nasal discharge
 - Abnormal behavior
 - Hypothermia/lethargy









Unhealthy Mouse=Bad for Breeding

• Example: malocclusion

- Teeth overgrow chronically, have to trim weekly
- Genetic predisposition
- Do not use as breeders





<u>Common in pregnant dams:</u>

- Prolapsed uterus/vaginal tissue
- Dystocia (prolonged/difficult labor)
- Dehydration









Cryo costs over time

Live colony costs

Breeding Colony Long Term Considerations

Cost-benefit of freezing down low use strains

Live animal

maintenance

6

Years

8

10

- Maintain Fou
 - backcros purchase
- Check for G
 - Consider
 - Dar
- Consider Cr
 - Freeze e
 - Saves \$3
 - Provides
 - Contact MMRRC@UNC for more information

50

40

30

20

10

0

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Cryopreserved sample

maintenance

Dollars

⁻housands of

Cost

Cumulative





RESOURCES

- FUNDAMENTALS OF BREEDING VIDEO:
 - HTTPS://WWW.JOVE.COM/VIDEO/10293
- JACKSON LABORATORIES:
 - <u>HTTPS://WWW.JAX.ORG/NEWS-AND-INSIGHTS/JAX-BLOG/2014/DECEMBER/FIVE-REASONS-WHY-YOUR-MICE-ARE-NOT-BREEDING</u>
 - <u>HTTPS://WWW.JAX.ORG/JAX-MICE-AND-SERVICES/CUSTOMER-SUPPORT/MANUALS-POSTERS-AND-</u> GUIDES/JMCRS-MANUALS-GUIDES/MANUALS-GUIDES-GENETICALLY-STANDARDIZED-MICE
 - <u>HTTPS://WWW.JAX.ORG/NEWS-AND-INSIGHTS/2011/SEPTEMBER/MOST-EFFICIENT-BREEDING-SCHEME-FOR-GENERATING-CRE-LOX-TISSUE-SPECIFIC-OR-IN</u>
 - <u>HTTPS://WWW.JAX.ORG/-/MEDIA/JAXWEB/FILES/JAX-MICE-AND-</u>
 <u>SERVICES/COLONYSIZEWKSHT.PDF?LA=EN&HASH=8C1CB11B342A9E8177B31DD2D7A7BFC6414628AC</u>
 - HTTPS://WWW.RESEARCH.UCI.EDU/FORMS/DOCS/IACUC/JAX-BREEDING-STRATEGIES.PDF

Colony Management Core at UNC (CMC)

Focus on establishing, maintaining, and continuously managing research colonies for investigator use

- Our services allow for research personnel to focus on science
 - reduce excessive mouse production
 - assist in establishing new strain lines
 - minimize breeding disruptions and stress to the animals
- Services are custom selected to suit each laboratories' need and budget
 - Aid with maintenance and production colonies
 - Able to handle large colonies with multiple strains/genotypes

- Skilled, dedicated breeding specialists perform daily tasks
 - Years of experience with transgenic colonies
 - Hands on at UNC
- Secure, shareable mouse database updated in real time
 - Currently use google sheets (can be viewed/edited by all added individuals)
 - Updated between 2 to 5 times a week depending on the size and needs of colony

ttps://research.unc.edu/cmc/





QUESTIONS?



