

- Mouse Tales: Introduction to Mouse Breeding and Colony Management



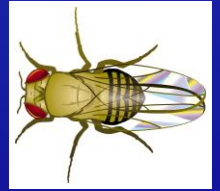
Jan Parker-Thornburg, Ph.D.
MD Anderson Cancer Center

Overview

- Why do we use mice?
- General information about the mouse
- GE mouse models and their upkeep
- Answers to elementary breeding questions

Genetically Engineered Animals

- Drosophila (fruit flies)
- Mice
- Rats
- Zebrafish (and other fish)
- Chicken
- Pigs
- Rabbits
- Sheep
- Cattle
- Etc.



Why Mice?

- Inexpensive
- Easily housed
- Easy to handle



Why Mice?

Large litters

generally 4-8 pups

Resistant to infection

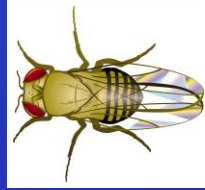
Inbred strains widely available

Inbred strains include BALB/c, C57BL/6, FVB, etc.

Extensive genetic history



Why Mice?



- **Small size**

- Birth weight: 1.0 – 1.5 grams
- Adult weight: 20-50 grams

- **Short maturation period**

- Weaning is generally by 21 days of age.
- Sexual maturity reached by 2 months of age.

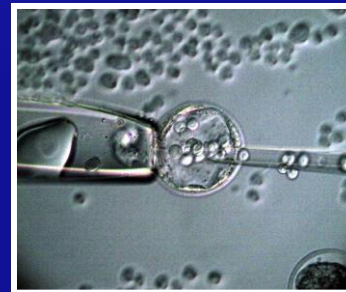
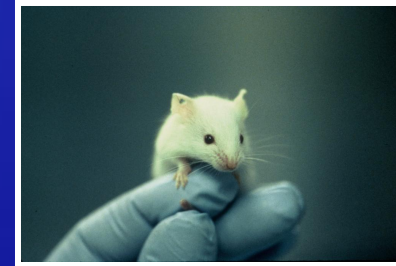
- **Relatively long lifespan**

- 1-3 years



Why Mice?

- Mammalian system
- Genetically malleable
- Embryos can be easily cultured and manipulated



This also now applies to RATS!!

About The Mouse

- 38 diploid autosomes
- 2 sex chromosomes
- XX is female
- XY is male



About The Mouse

- Food consumption: 3-5 g/day
- Water consumption: 6-7 ml/day



(picture from Edstrom Industries, Inc.)

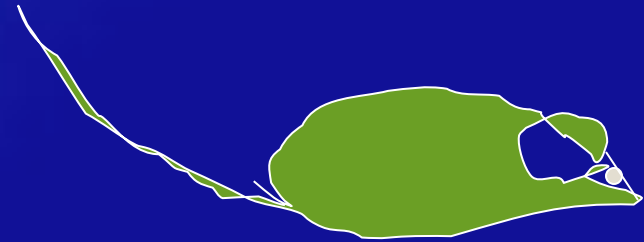
About The Mouse

- Obligate nose breather
- Large surface body area per gm body weight
- Temperature regulation by nesting
- Sensitive to high and low temperature extremes.



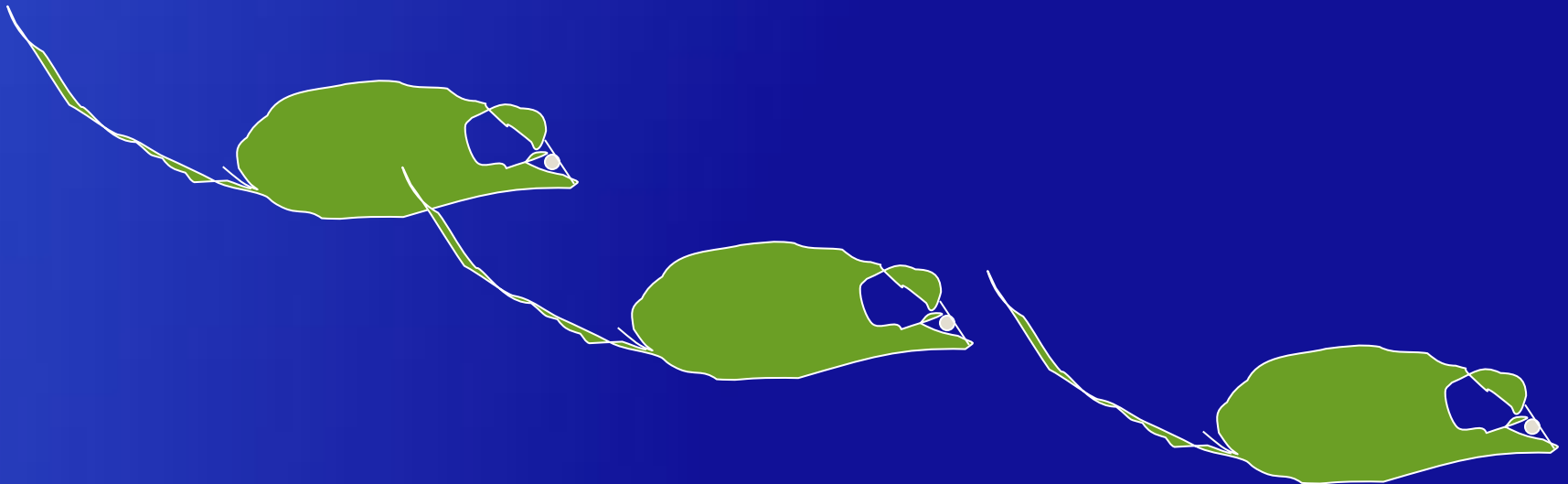
About The Mouse

- Nocturnal (most active in the dark)
- Poor eyesight
- Sensitive to smell
- Hearing range is from 0.5 – 120 KHz



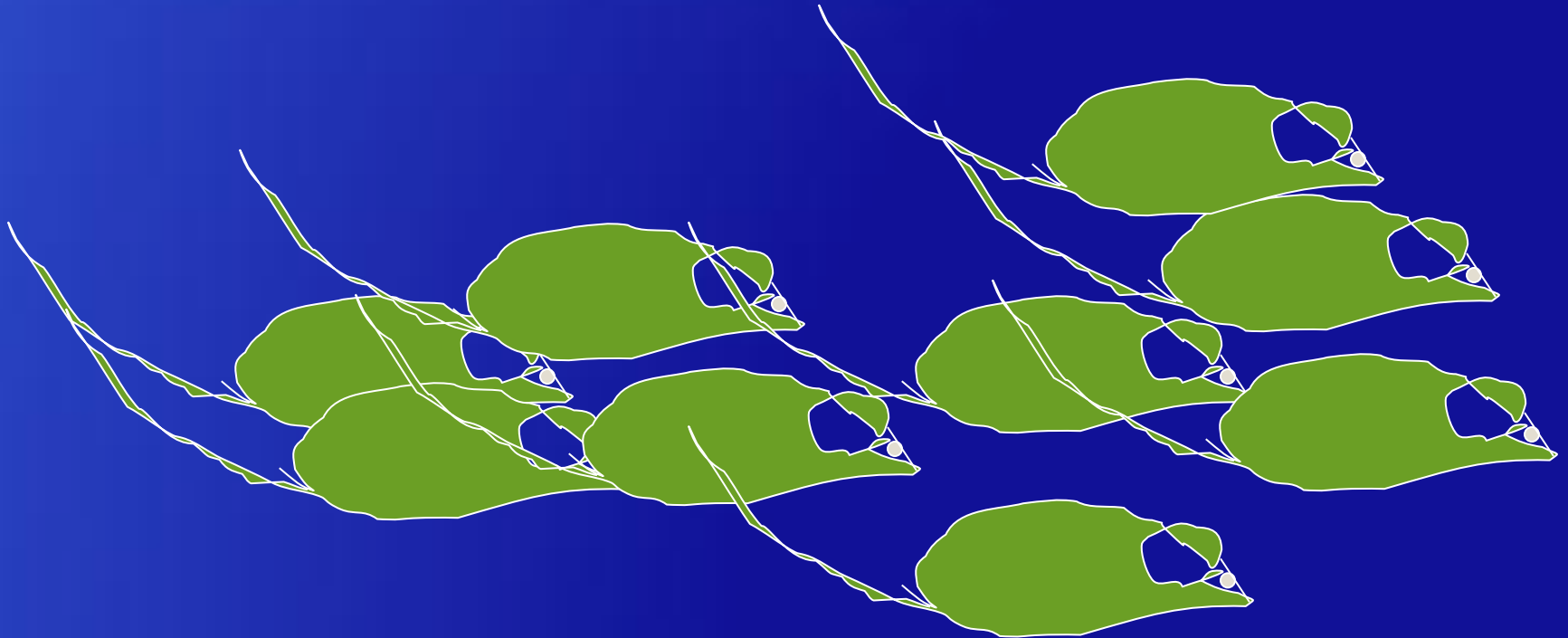
About The Mouse

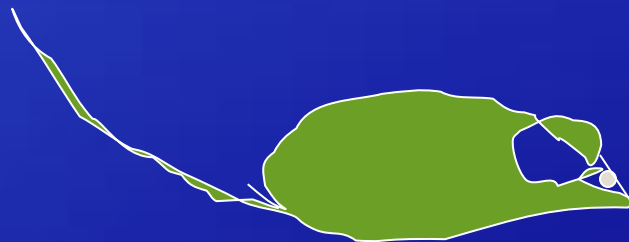
- Genome has been completely sequenced
- Knock-out and conditional alleles are being generated for “all” genes.



About The Mouse

- Mice sound easy!
- So, why are you here?





Project # 55555

Hello Dr. XXXXX,

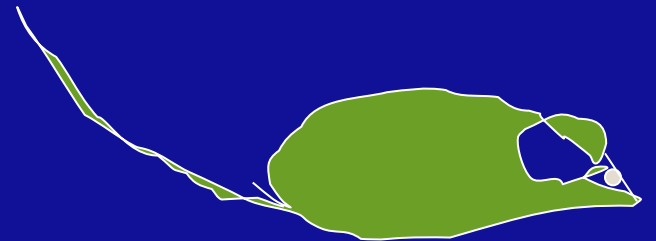
29 pups

I am sending you this notice to let you know that you have **29** pups for your project. They were born on 9.27.08 and will be weaned and ready to transfer on 10.18.08. Please note that you have 20 males and 9 females. In order to receive your animals you will need to fill out a *Request for Research Animals* form and submit it to Vet Med. On this form you will fill in the numbers/genders of your mice, where you want them to go, and the date you wish them to be transferred (weaning date). List GEMF as the vendor and \$0.00 as the cost. As it can take Vet Med 2 weeks to get this transfer done, you will need to submit the paperwork as soon as you receive this notification. Please don't hesitate to call me if you have additional questions.

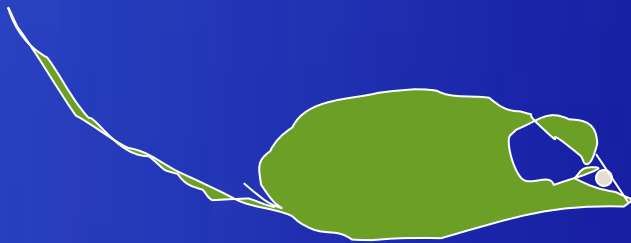
GEMF Technician

The PI Response:

“Fantastic! Mice! Why don’t I have results yet?”

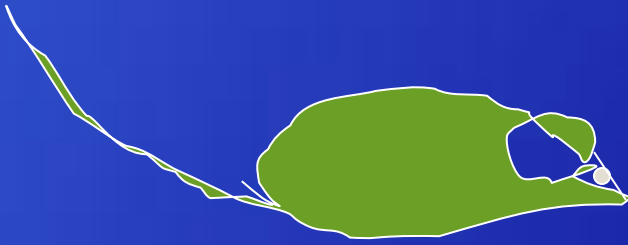


The Lab Tech Response:
“Ummm, mice! What do I do with them?”



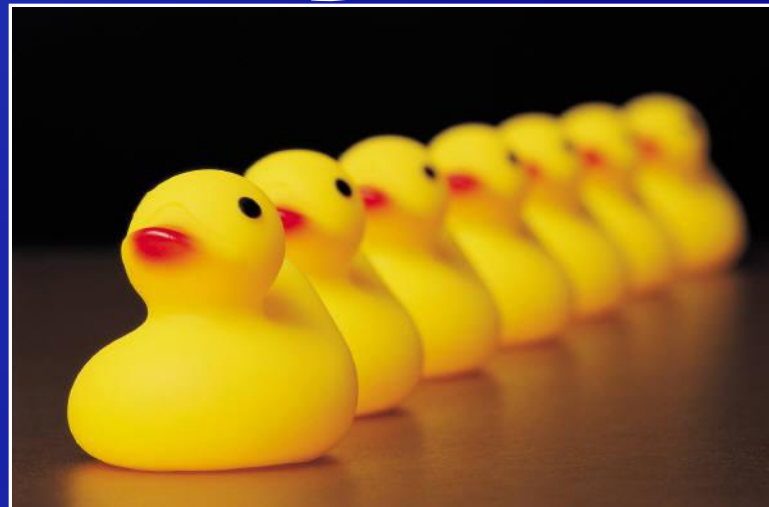
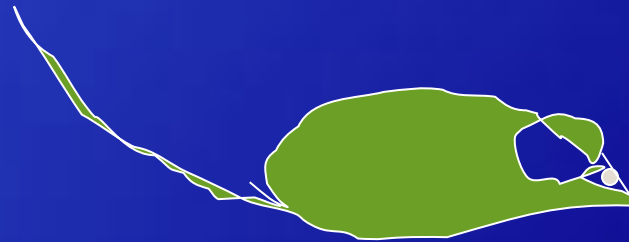
The Veterinarian Response:

“GEM mice. I wonder what is wrong with them?”



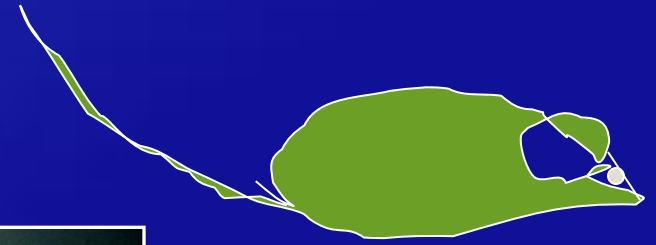
The Animal Tech Response:

“More mice???!!!!! AARGH!!!!”

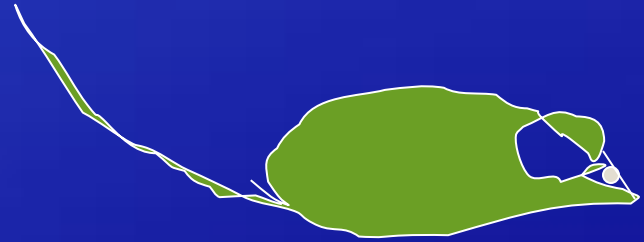


The Mouse Response:

“Just wait until you find out what I’m going to show you!”



The Mice Know!



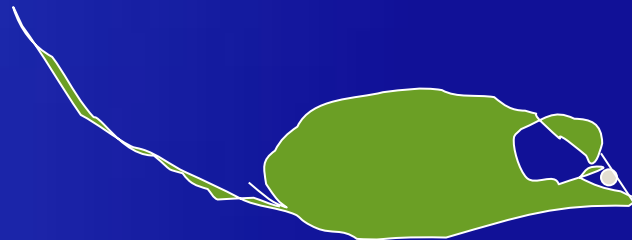
They are an investment.

And, unlike our economy, they generally expand exponentially.

So, you need to plan ahead!

Typical Questions:

1. What do I do with them?
2. How many do I need to keep?
3. How are they cared for?
4. How are they mated?
5. How do I tell a male from a female?
6. How do I keep track of them?



“So many mice, so little time”

Managing a GEMM colony



What do I do with the mice?

The answer is easy!

Determine which mice carry the gene you need, and then,

Establish lines for analysis.



(in practice, however, it is NOT that easy. . .)



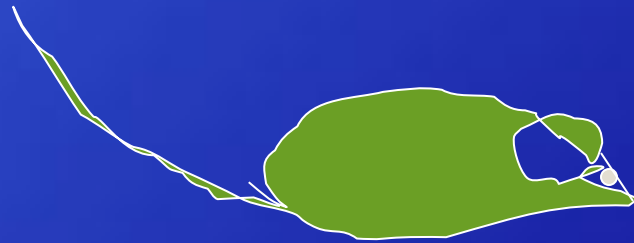
How many mice do I
need to keep?



What type of project is it?



How many mice do I need to keep?



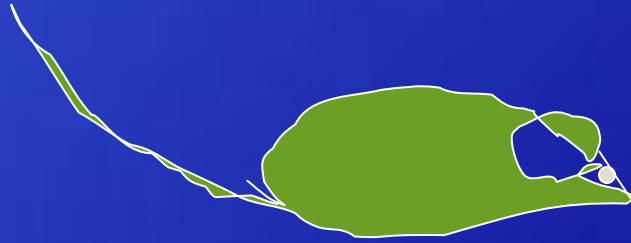
Knock-out or a **knock-in**?

Conditional knock-out or **conditional knock-in**?

The inserted DNA goes to a pre-determined place in the genome.

Experiment done in ES cells, which are then inserted into a blastocyst to generate “chimeras”.

How many mice do I need to keep?



Transgenic?

The DNA can insert anywhere in the genome.
This is a random event.

How many mice do I need to keep?

Chimeras



How many mice do I need to keep?

Chimeras



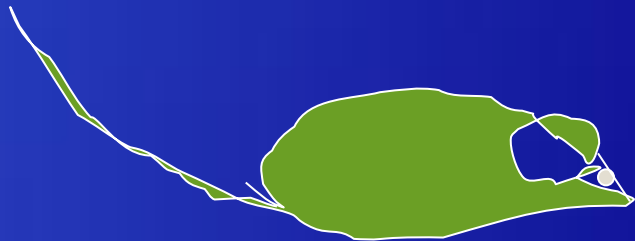
How many mice do I need to keep?

Gene Targeting? *KO or KI:*

Test all chimeric males (up to 10) for germline transmission;

5 wild type females/male at a minimum rotated through no more than 2 at a time; check plugs daily.

(Note: all males are single housed for mating)



How many mice do I need to keep?

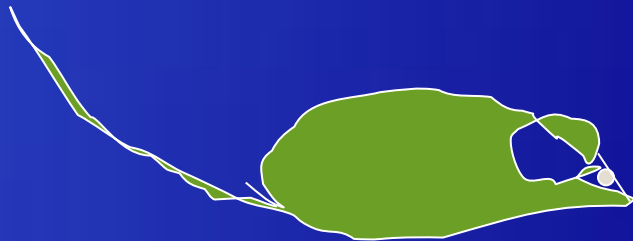
Gene Targeting?

If fewer than 2 males, test female chimeras as well (1 wt male/female).

The goal is to produce as many pups as possible in as short a time as possible!

After establishment of germline transmission, maintain mating pairs from at least 2 injected clones.

Why??

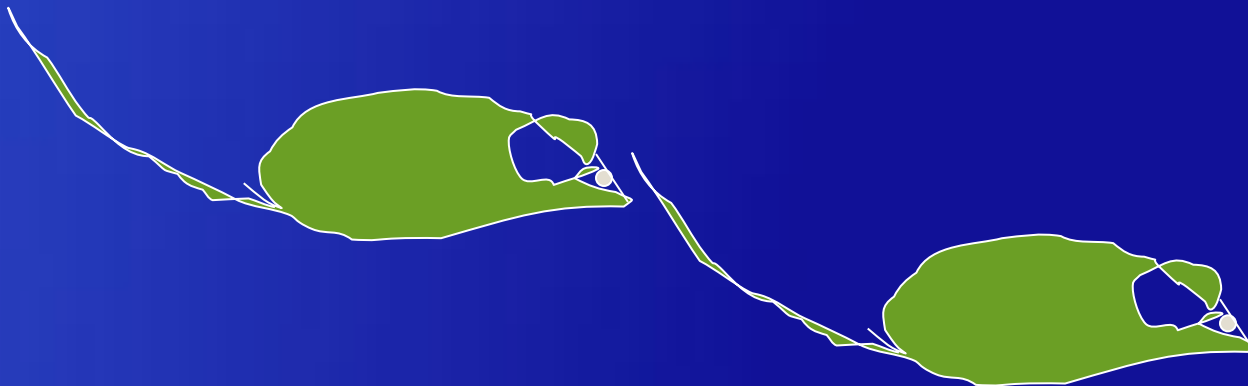


How many mice do I need to keep?

Gene Targeting?

CKO or CKI: Test all chimeric males (up to 10) for germline transmission;
5 wild type females/male at a minimum.

If fewer than 2 males, test female chimeras as well (1 wt male/female).



How many mice do I need to keep?

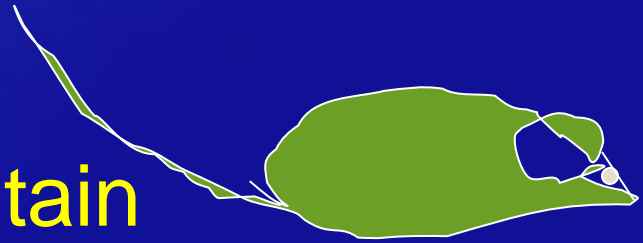
Gene Targeting? *CKO* or *CKI*:

Mate in the same manner as for KO and KI chimeras.

BUT:

You will also need to maintain stocks of deleter (Cre, Flp) mouse lines (2-3 mating pairs of each line).

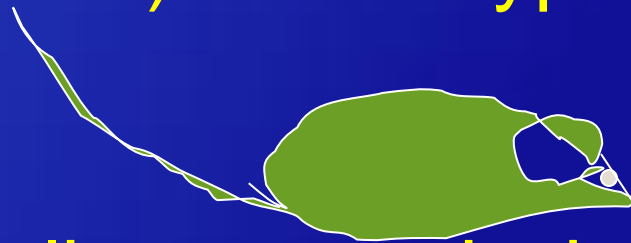
And, you will need to keep both undeleted and deleted lines.



How many mice do I need to keep?



Transgenic? Mate up to 7 founders
(prefer males) to wild-type females.
(WHY?)



After germline transmission, maintain at
least 3 independent founder lines.
(WHY?)

How many mice do I need to keep?

α -MyHC-MCP1 Transgenic Phenotypes



Mice exhibit a typical wasting phenotype prior to sacrifice.



Mice exhibit a swollen appearance.

How many mice do I need to keep?

α -MyHC-MCP1 Transgenic Phenotypes

Line 3: Heterozygous viable with strong phenotype
Homozygous lethal

Line 6: Heterozygous viable with no phenotype
Homozygous viable with moderate phenotype

Line 48: Heterozygous lethal

How should GEM mice be housed?

Gnotobiotic?

germ free

Barrier?

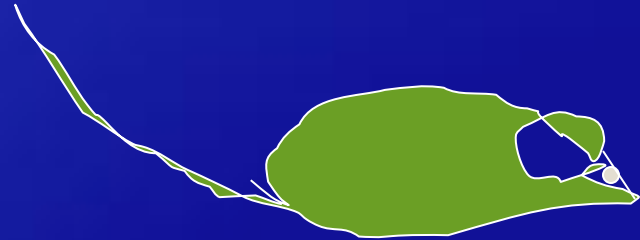
pathogen free

CSPF?

specific pathogen free

Conventional?

dirty . . .



It depends on the gene and the study.

How should GEM mice be housed?

Gnotobiotic (germ free): generally unnecessary

Barrier (specific pathogen free): used for any mouse that may be immunocompromised

CSPF (conventional specific pathogen free): used for most standard GE mice

Conventional (dirty): not recommended due to possible deleterious outcomes.



Mating GE mice

How can I tell a male from a female?

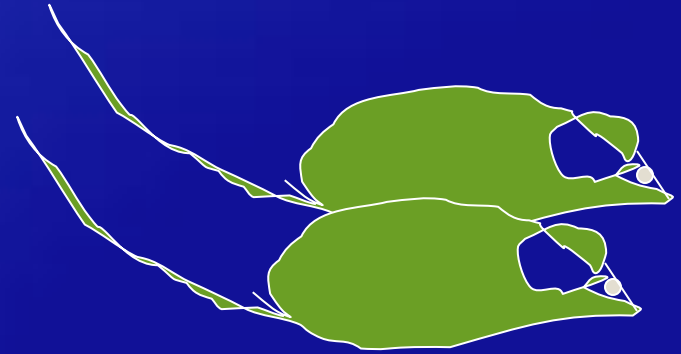


What if I can't tell?

Mating GE mice

Determine the age of your mice

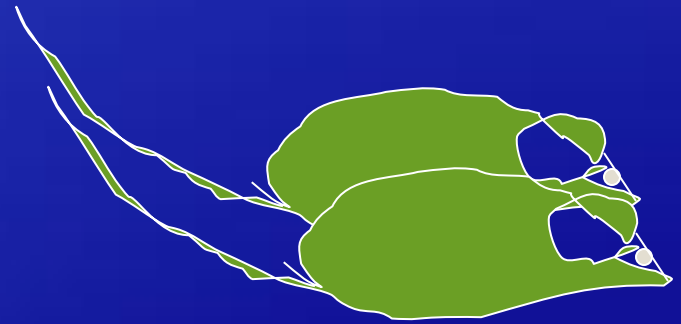
- Minimum breeding age:
 - males: 35-42 days
 - females: 21 days
- Maximum age for FIRST breeding:
 - males and females: 6 months



Mating GE mice

When to set up matings

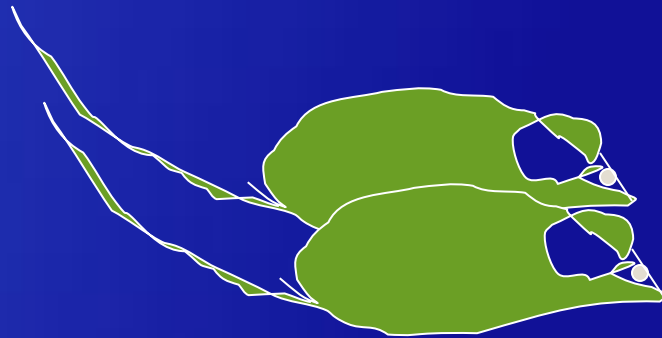
- Set up matings only when you know the mice will be carefully supervised over 1-2 days (NOT on Fridays).



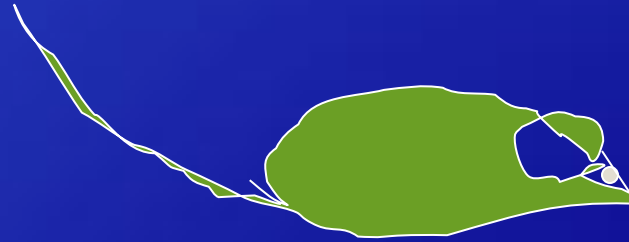
Mating GE mice

How to set up matings

- Put female into the male cage (allow the male to “dirty” the cage first)



Mating GE mice- Mating Problems



Age

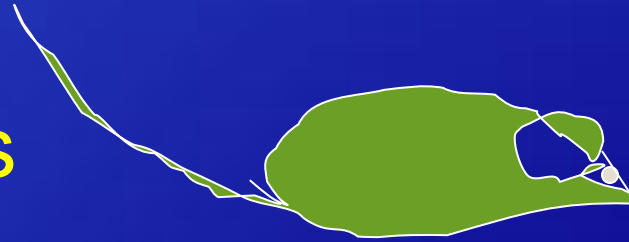
Fertility

Infections & Pathogens (rederivation)

Don't wait to mate. . .

Mating GE mice- Mating Problems

Seasonal Problems

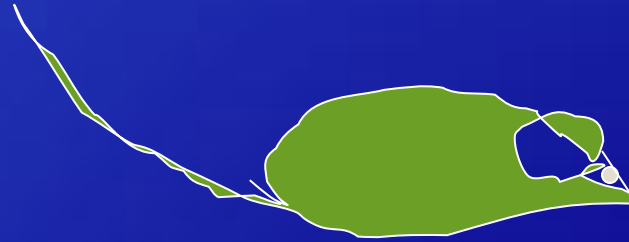


Light Cycles

Construction, Noise, Vibration

Mating GE mice- Mating Problems

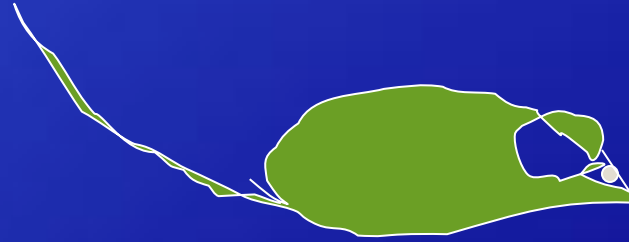
Air Changes



The Bruce effect

The Lee-Boot effect

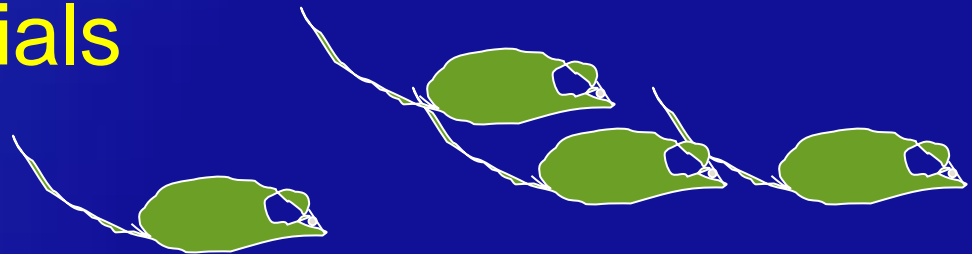
Mating GE mice- Mating Solutions



Timed matings-use to identify sterile males

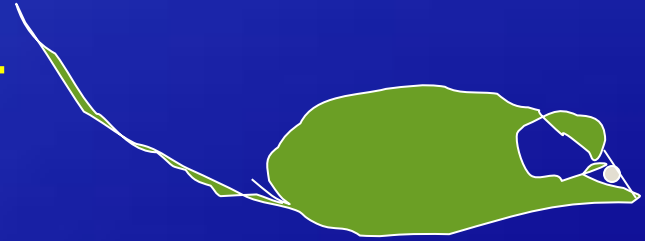
Change diet

Add nesting materials



Mating GE mice- Mating Solutions

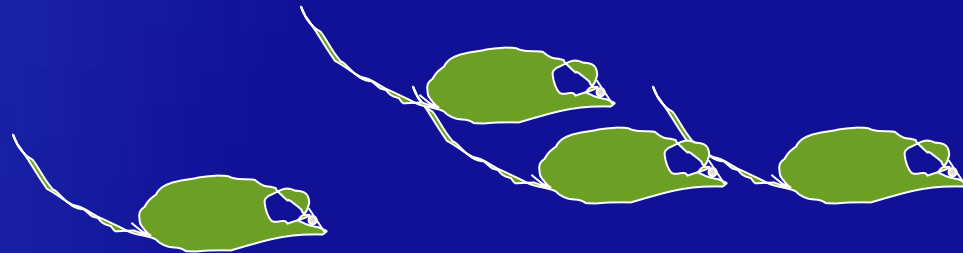
Test for sperm viability/IVF



Move to a different spot in the room.

Check lights, noise and vibration

Change type of housing (VMIs to static)



Identifying and Tracking Mice

Clipping

ears

toes

Tagging

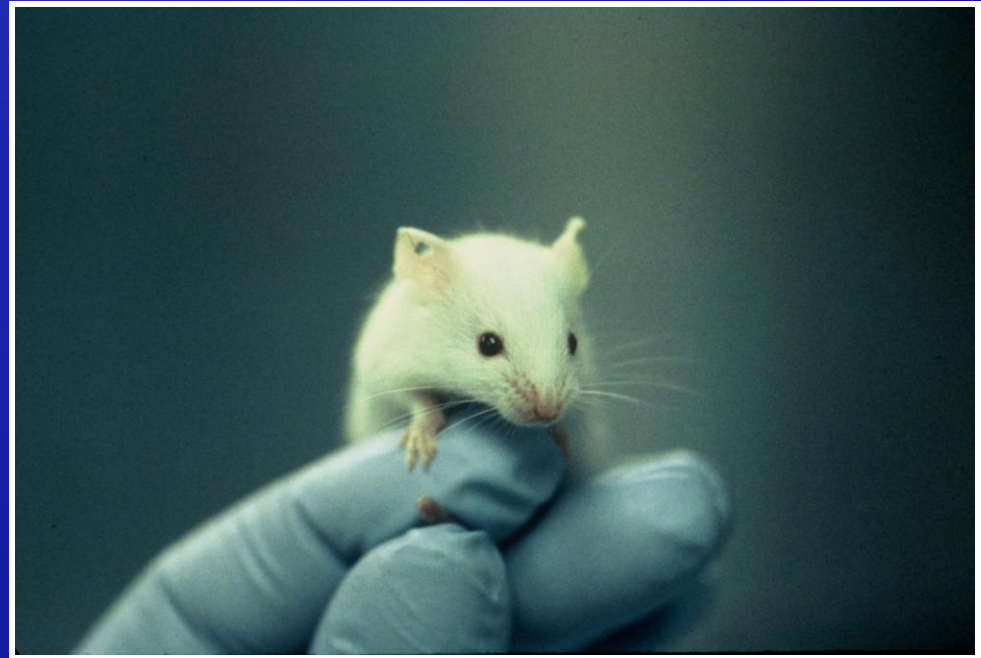
ears

Tattooing

ears

tails

Chipping



Identifying and Tracking Mice

Clipping for identification:

toes? generally not allowed except for specific circumstances.

ears? various easy clipping schemes.

tissues can be used for PCR identification.

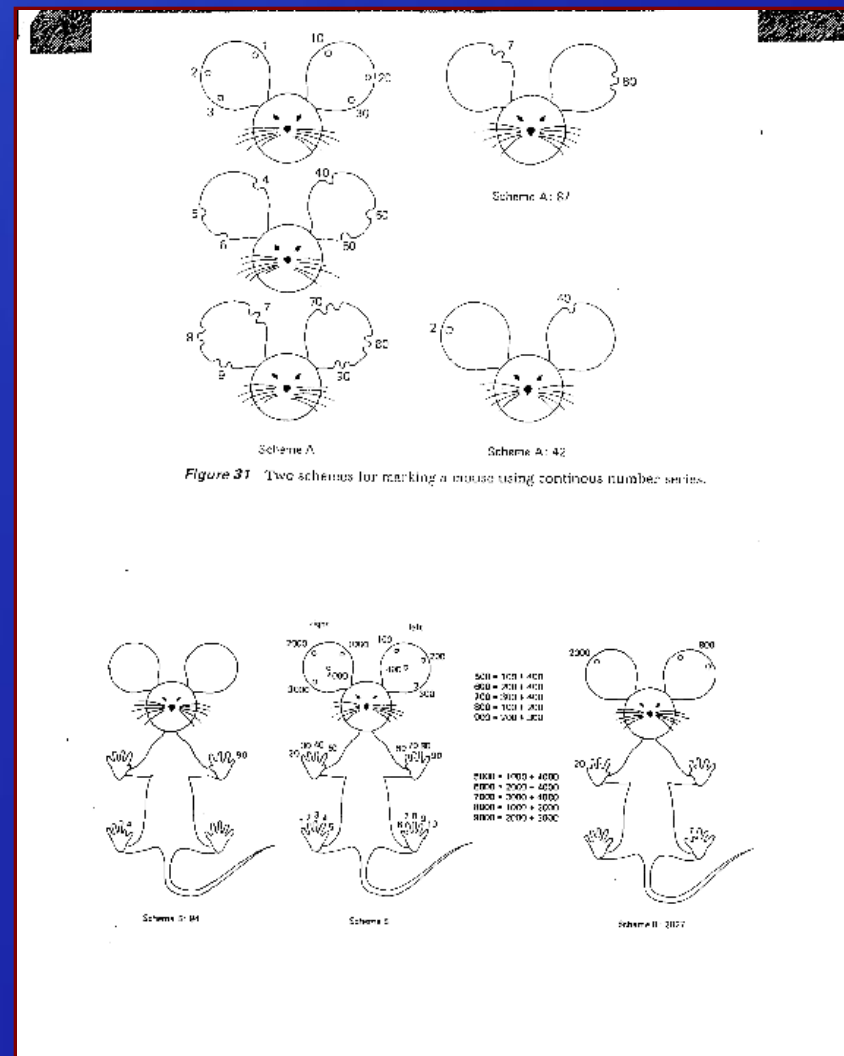
technicians DON'T generally like to do this.

Identifying and Tracking Mice

Ear tags? easy for the technician, not so nice for the mouse.

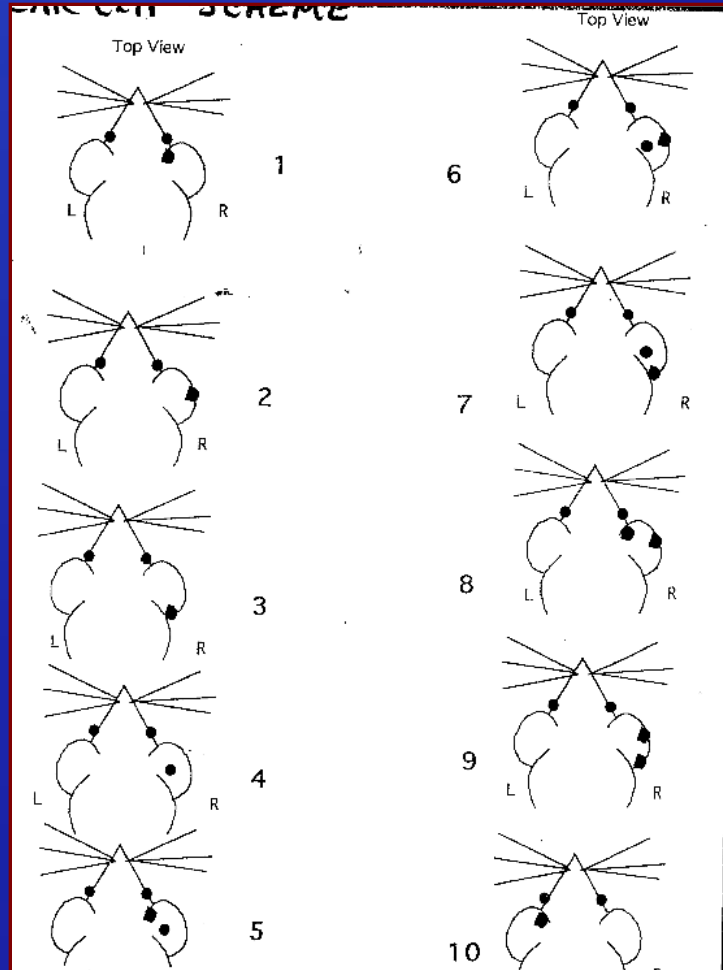


Combination ear/toe scheme



from: Manipulating the Mouse Embryo

Simple Ear Clip Scheme



How to track your mice

Commercial computer programs



Facility

- Colony management
- IACUC protocol processing
- Tracking of experimental data
- Maintenance of staff, cost, and health records

FACILITY software
the modern lab center. Whether desktop application supported on all r meet your organi to configure FACI financial manager match any of the Combine this cust networked system areas of benefit t



Big Bench software



[Biotechniques. 2001 Jan;30\(1\):130-2.MouseBank: a database application for managing transgenic mouse breeding programs.Hopley R, Zimmer A.SourceNational Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA. rlhopley@codon.nih.gov](#)

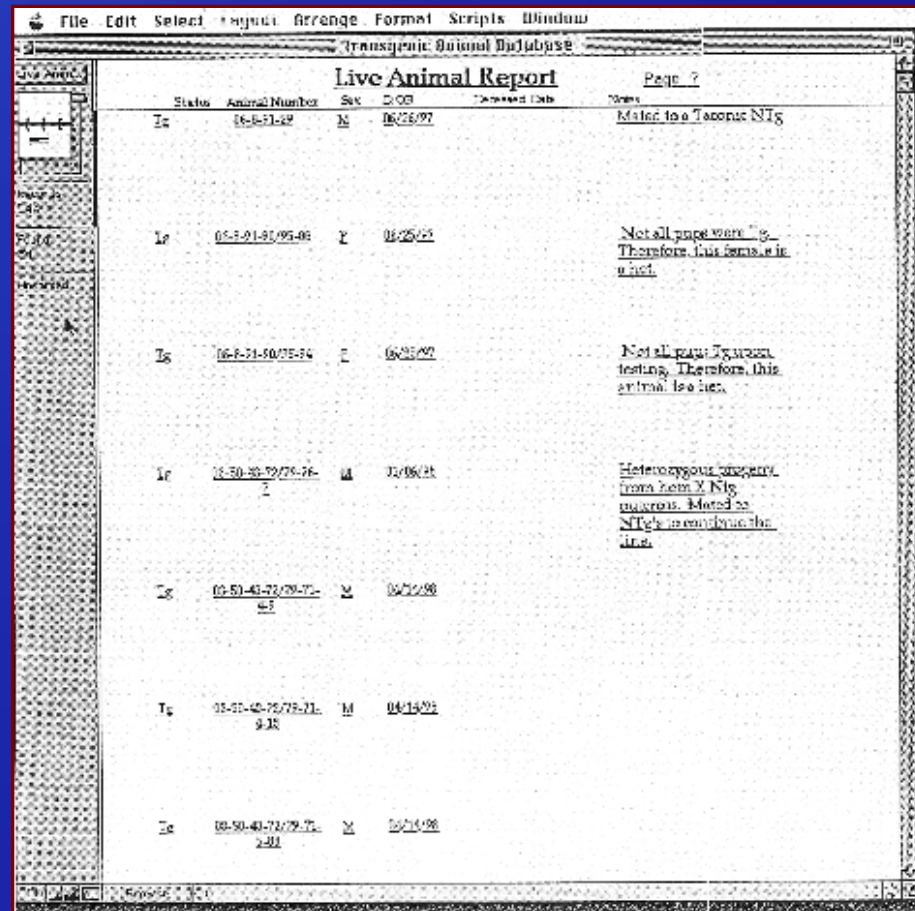


SoftMouseDB™

Transgenic Mouse Breeding & Colony Maintenance
Record-Keeping System

How to track your mice

Develop in-house computer programs



File Edit Select Window Arrange Format Scripts Window
Transgenic Animal Database

Live Animal Report Page 7

Status	Animal Number	Sex	Date	Notes
Tc	05-51-51-02	M	06/26/97	Mated to a Tacorac NTg
Lf	05-51-51-05-06	F	06/26/97	Not all pups were Tg. Therefore this female is not.
Tg	05-51-51-05-04	F	06/26/97	Not all pups Tg were tested. Therefore this animal is a hetero.
Lf	05-50-43-72/75-76-77	M	04/08/98	Heterozygous transgene from hetero NTg parents. Mated to NTg to continue the line.
Tg	05-50-43-72/75-76-77-78	M	04/14/98	
Tg	05-50-43-72/75-76-77-78-79	M	04/14/98	
Tg	05-50-43-72/75-76-77-78-79-80	M	04/14/98	

How to track your mice

Use cage cards

<u>Type</u>	<u>Date</u>	<u>Status</u>
Breed		Transgenic
Wean		Non-Transgenic
Investigator: _____		
Protocol # _____		
Construct _____		
Male Animal #(s) _____		

DOB	Sire #	Dam #
Female Animal #(s) _____		

DOB	Sire #	Dam #
# Born	DOB	# Animals In Cage
PUPS	DOW	Animal #'s

Different colors of cage cards can be used to easily denote status:

yellow for tg hold,

white for ntg hold,

blue for wean or unk.,

green for breed,

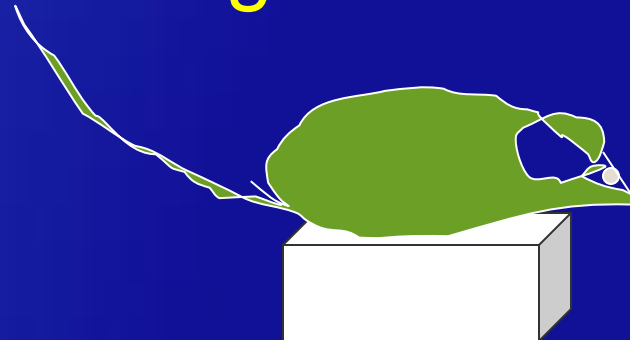
pink for studs)

Archive!

Preserving GEMM lines

Cryopreservation of embryos
need lots of males and females

Cryopreservation of sperm
need 2-4 fertile males
only preserving 1/2 of the genome

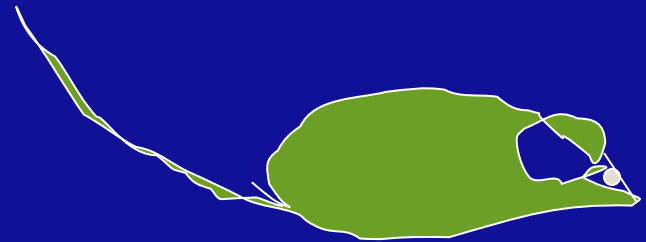
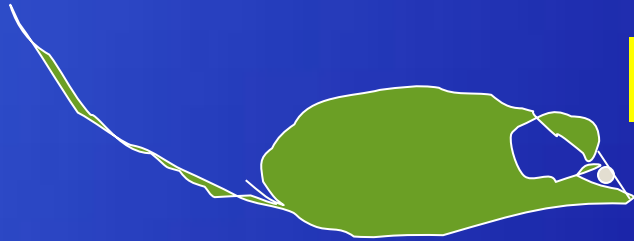


What does the investigator need from the technicians and care givers?

Observation

Information

COMMUNICATION!!!



Thanks to:s

- The meeting host: ACTS Training/Paula Clifford and William Singleton
- The meeting sponsors
 - Animal Specialties and Provisions
 - Pharmacal Research Laboratories, Inc.
 - Transnetyx
 - Fisher Scientific
 - Quip Laboratories
 - PharmaSeq
 - Charles River Laboratories
 - Taconic
 - The Jacson Laboratory
 - Somark
 - BioServ
 - World Courier
- The University of Pennsylvania
- Donors of pictures and information: ISTT, Elsa Flores, Laura Conour, Edstrom Industries
- UT-M. D. Anderson Cancer Center/Cancer Center Support Grant