

The Jackson Laboratory

Mouse Colony Management and Breeding Strategies



Overview

- **Mouse reproduction**
- **Factors affecting breeding performance**
- **Data collection and good colony management**
- **Breeding schemes**



Mouse Reproductive Milestones

- **Gestation: 18.5 to 21 days**
- **Litter size: 2 to 12+ pups**
- **Weaning age: 17 to 28 days**
- **Sexual maturity: 5 to 8 weeks**
- **Productive breeding life: ~ 7-8 months**

Reference: Silver LM. (1995). *Mouse Genetics: Concepts and Applications* Oxford University Press. Online at: www.informatics.jax.org/silver/

Genetic Background

Influence on Breeding Performance

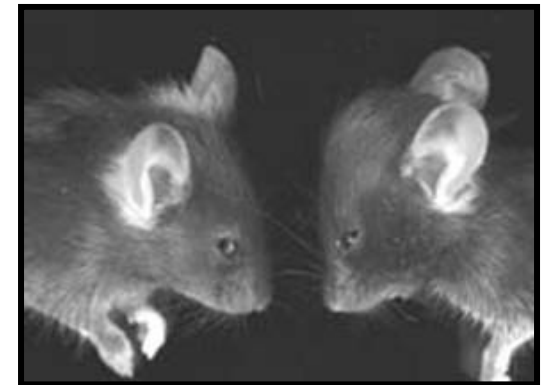
- Hybrid vigor
- Fecundity
- Postnatal defects
- Behavior

F1, F2 hybrids

BTBR *T+ tf/J*

C57BL/6J

SJL/J



Genetic Modification-Mutants and Transgenics

Influence on Breeding Performance

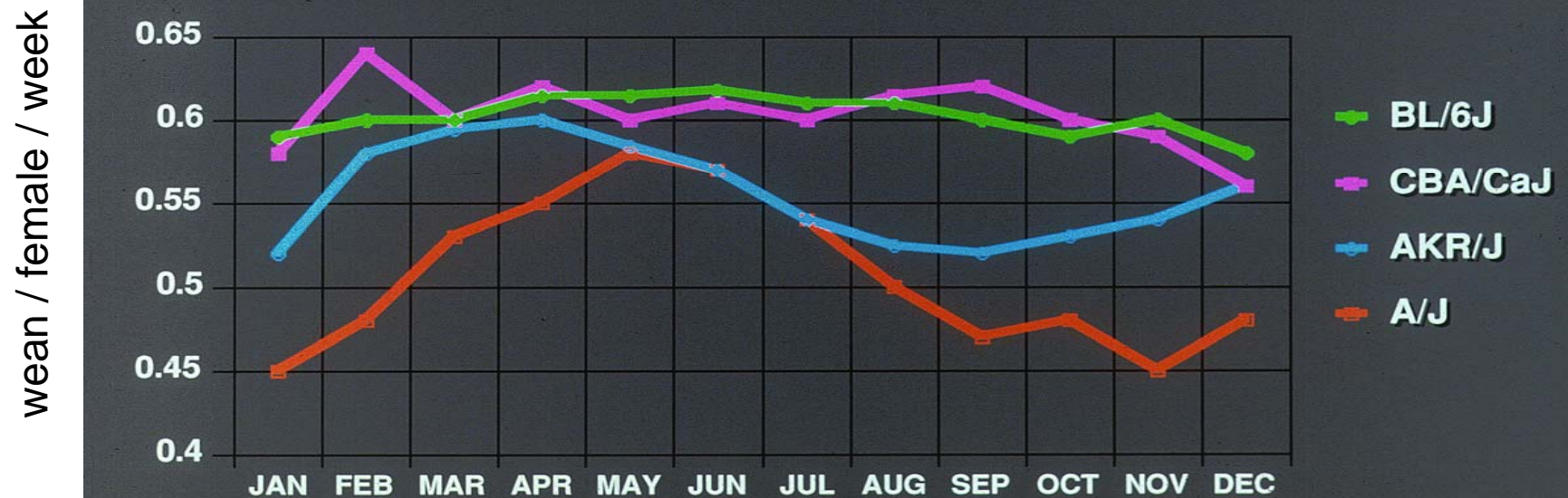
- **Embryonic lethality**
- **Infertility or subfertility**
- **Mammary function for lactation**
- **Abnormal behavior**
 - **poor mothering instinct**
 - **aggression**
- **Disease**
 - **tumor development**
 - **neurodegeneration**

Environmental Factors

Influence on Breeding Performance

- Temperature
- Light cycle and intensity
- Noise and vibrations (construction, equipment)
- Air pressure
- Odors (toxic fumes, perfume)
- Over handling - leave pregnant mothers alone!
- New caretaker
- Nutrition
- Health status
- Season

Seasonal variation in inbred mice



Signs of Sick Mice

- **Scruffy coat**
- **Tumors**
- **Hunched posture**
- **Weight loss**
- **Labored breathing**
- **Dermatitis, skin lesions**
- **Ocular or nasal discharge**
- **Abnormal behavior- isolation**

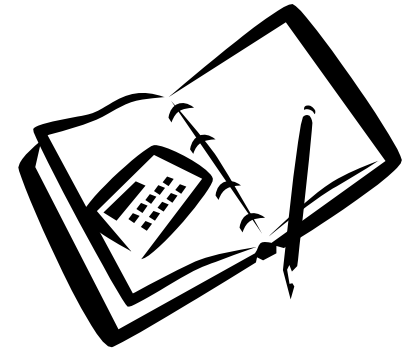
Data Collection & Record Keeping

Critical for successful colony management



Record Keeping: Pedigree Book

- **Strain history**
 - strain development
 - name changes
- **Litter information**
 - Mating pairs (dam & sire)
 - birth dates
 - litter number
 - number born
 - number weaned
 - animal number
 - sex of wean
 - pedigree number
 - genotypes (if necessary)
 - generation number



Record Keeping- Generation Number

N# = backcross generation number

F# = filial (brother X sister) generation number

p = cryopreserved

Generation: **N6F12 + F7**

(N5F25p)F17

?+ N10F5

Record Keeping Tips



- **Use pre-printed cards or labels**
- **Use different colors**
 - adjacent strains
 - matings vs. weanlings etc...
- **Separate strains with similar nomenclature**
- **Keep cages from a single strain together**
 - breeders and weanlings
- **Keep records in multiple locations**
- **Save all cage cards**

Colony Management Tips

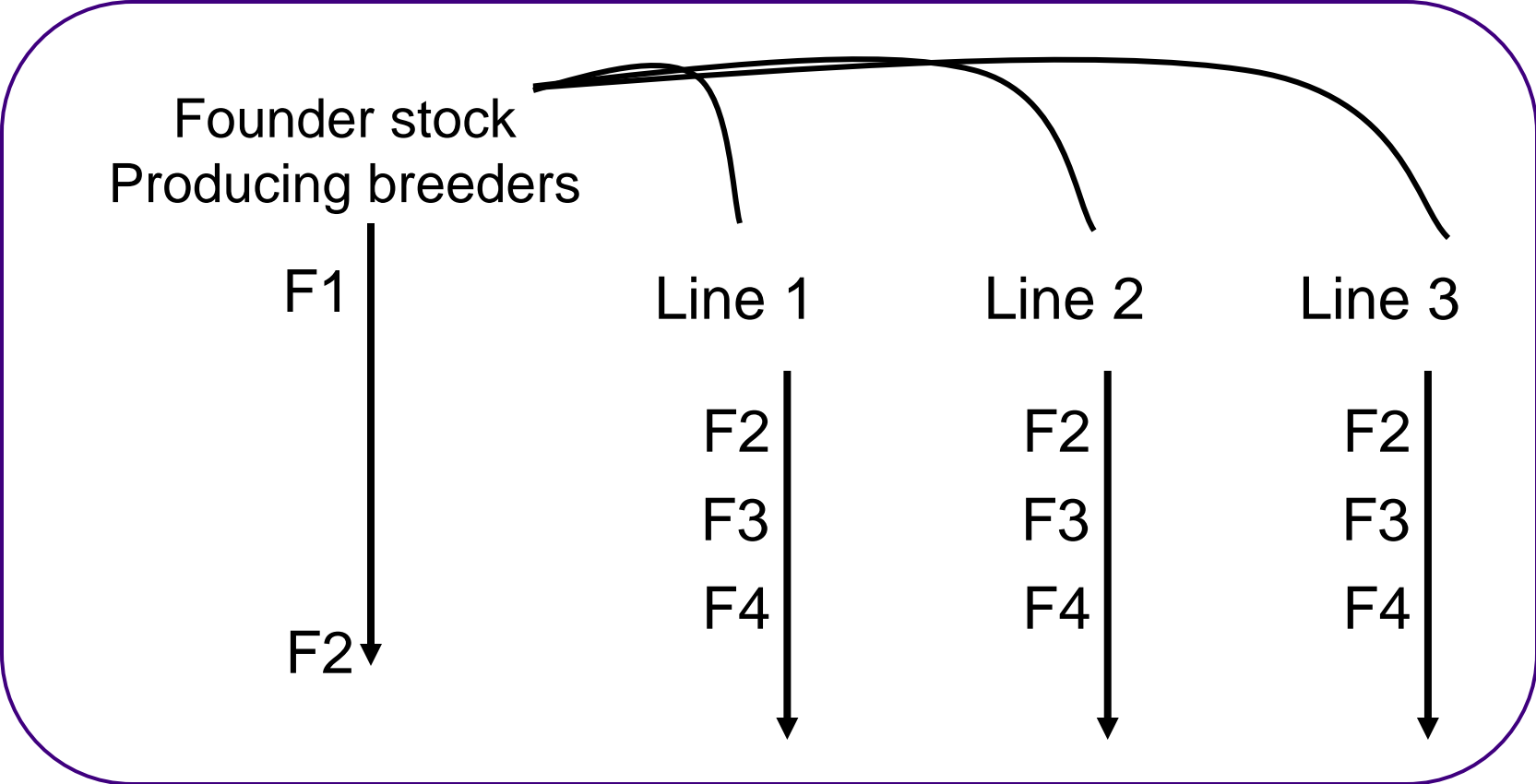
- **Obtain expected breeding statistics**
 - use inbred-specific data if mutant strain statistics not available
- **Mate early- 6-12 weeks of age**
- **Replace breeders on a rotation (monthly)**
 - breeding life span typically ~7- 8 months
- **Remove non-productive breeders ASAP**
- **Collect your own breeding statistics**
- **Evaluate your data regularly**

Colony Management Tips (cont.)

- **Record and investigate deviations**
 - environmental changes
 - check genotypes (breeding errors)
- **Expect changes on a new background**
 - keep older generations available
- **Choose breeders carefully**
 - avoid selection pressure
- **Keep founder stock and pedigreed colonies**
 - filial (brother X sister) matings

Pedigree Colony with Founder Stock

Single Established Colony -any strain type



Non-Productive Breeders

Criteria for Replacement

- No litter produced
 - 60 days after first mating
 - 90 days if slow starters
- No new litters
 - 60 days from last litter
- No weaned pups
 - 2-3 litters with no wean



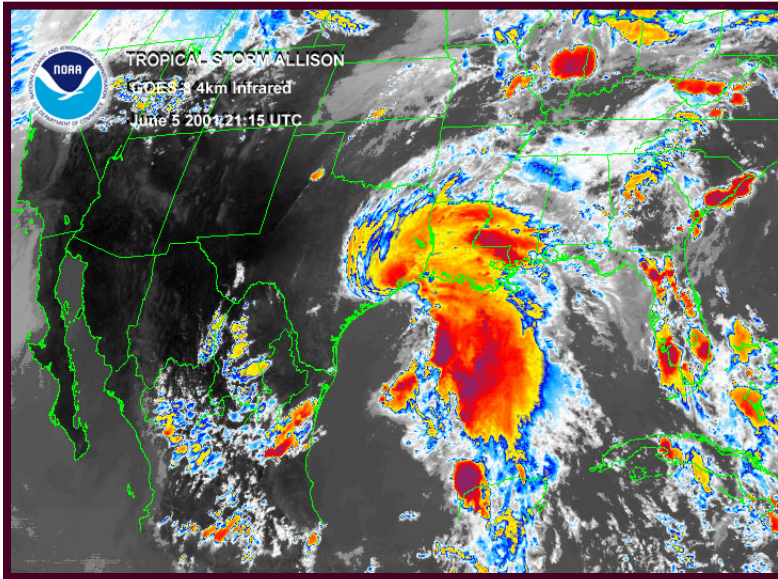
Breeding Tips for Low Producers

- Quiet place
- Ensure adequate darkness
- Minimal handling
- Use clean forceps or gloves
- Changing diet fat content
- Add nesting material
- Leave mating pairs together



Long-Term Colony Maintenance

- Maintain foundation stocks to minimize the number of generations per year
- Replace breeders from a trusted vendor each 5 to 10 generations
- Establish a genetic quality control plan
 - Genome scans- SNPs, MITs, and biochemical markers, remove spontaneous mutants
- Cryopreserve unique strains



In June of 2001, Tropical Storm Allison battered the Houston area flooding The Texas Medical Center. While many research animals were rescued, it was estimated that 30,000 or more mice were lost.

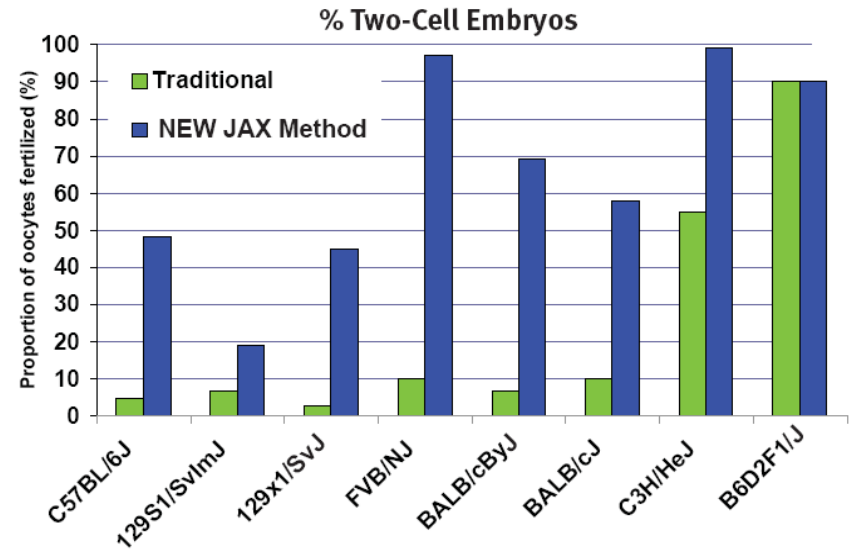
“Insurance and federal aid money should replace most of the lost material, but researchers can never get back the time they have invested. For example, some of the drowned transgenic mouse colonies at UTMSH took a decade to build, and only a minority can be regenerated from breeding pairs sent to other universities. This is a devastating loss.”
George Stancel, dean of the graduate school of Biomedical Science at UTMSH.”
Science 22 June 2001:Vol. 292. no. 5525, p. 2226

Sperm Cryopreservation

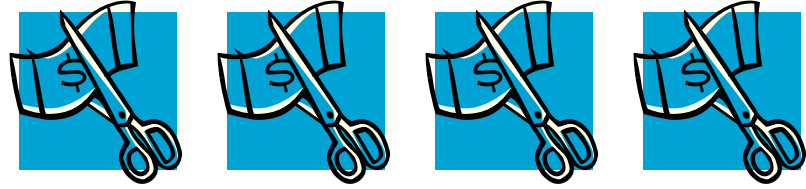
For colony “sleep”

For insurance and peace of mind....development and basic phenotyping of a GM mouse can take 2-3 years and cost more than \$100,000

Can you afford not to preserve?



Reducing Costs



- **Size colony for your needs**
- **Use both sexes in your experiments**
- **Use an age range of mice for your experiments (e.g. 4-8 weeks of age if used monthly)**
- **Rotate breeders on a strict schedule**
- **Replace nonproductive breeders ASAP**
- **Run a tight ship.... appoint a trusted colony manager to maximize your space!**
- **Cryopreserve unique and low use strains**

Mouse Breeding Schemes



B6.Cg-*m* +/+ *Lepr*^{db}/J

Choosing a Breeding Scheme

What genotypes do I need?

- most efficient way to produce them?

Will appropriate controls be available?

- genetic background

How are genotypes identified?

- phenotype?
- molecular assay (e.g. PCR)?
- breeding scheme?

Choosing a Breeding Scheme

- **What about Linkage?**
 - X-linked or autosomal?
 - Linked or multiple genes?
- **Reproductive issues (sterility)**
- **Phenotype issues (lethality)**

Homozygote (-/-) x Homozygote (-/-)

100% of offspring affected

Both genders viable and fertile as homozygotes

	-	-
-	-/-	-/-
-	-/-	-/-

Controls:

- Inbred and congenic: inbred
- Mixed background (e.g. C57BL/6, 129): F2
IMPORTANT! – F2 hybrid, only
***approximate* control**

Recommendation: Outcross to appropriate F1 hybrid every 10 generations.

Heterozygote (+/-) x Homozygote (-/-)

50% of offspring Homozygous

One gender NOT viable / fertile as homozygote

Controls:

- Inbred and congenic:
 - inbred
 - heterozygote siblings (if no phenotype)
- Mixed background (e.g. C57BL/6, 129):
 - heterozygote siblings
 - F2 hybrid (approximate)
 - outcross to F1 every 10 generations

	-	-
+	+/-	+/-
-	-/-	-/-

Heterozygote (+/-) x Heterozygote (+/-)

25% of offspring Homozygous

Neither gender viable / fertile as homozygote

Controls:

	+	-
+	+/+	+/-
-	+/-	-/-

- Inbred and congenic:
 - wildtype sibling
 - heterozygote sibling (if no phenotype)
 - Inbred
- Mixed background (e.g. C57BL/6, 129):
 - wildtype or heterozygote siblings
 - F2 hybrid (approximate)
 - outcross to F1 every 10 generations

Transgenic Mating Schemes

Expression of transgene may affect viability or fertility

Ex: B6.Cg-Tg(HDexon1)61Gpb/J

- Huntington's Disease model**
- tremors, seizures by ~ 9-11 weeks**
- reduced fertility (50% males infertile)**
- limited breeding window (3-4 weeks)**

Transgenic Mating Schemes

Insertional mutation

Ex: C57BL/6J-TgN(HBBHBG)40BCha
Hmga2^{pg-TgN40BCha}/J

- Inserted into *pygmy locus* (*Hmga2*^{pg})
- Homozygotes smaller in size; infertile

Evaluate phenotype of *multiple* founders to determine if effects are transgene- or insertion site-specific

Initial Transgenic Mating Scheme

wildtype (+/+) x **hemizygote (Tg/+)**
50% of offspring carriers

Avoid unwanted phenotypes (e.g. lethality, infertility)

Controls:

- **Inbred and congenic**
 - wildtype sibling
 - Inbred
- **Mixed background**
 - wildtype sibling

	Tg	+ (0)
+ (0)	Tg/+	+/+
+ (0)	Tg/+	+/+

Complex Breeding Schemes

Ex: B6C3Fe *a/a-Csf1^{op}/J*

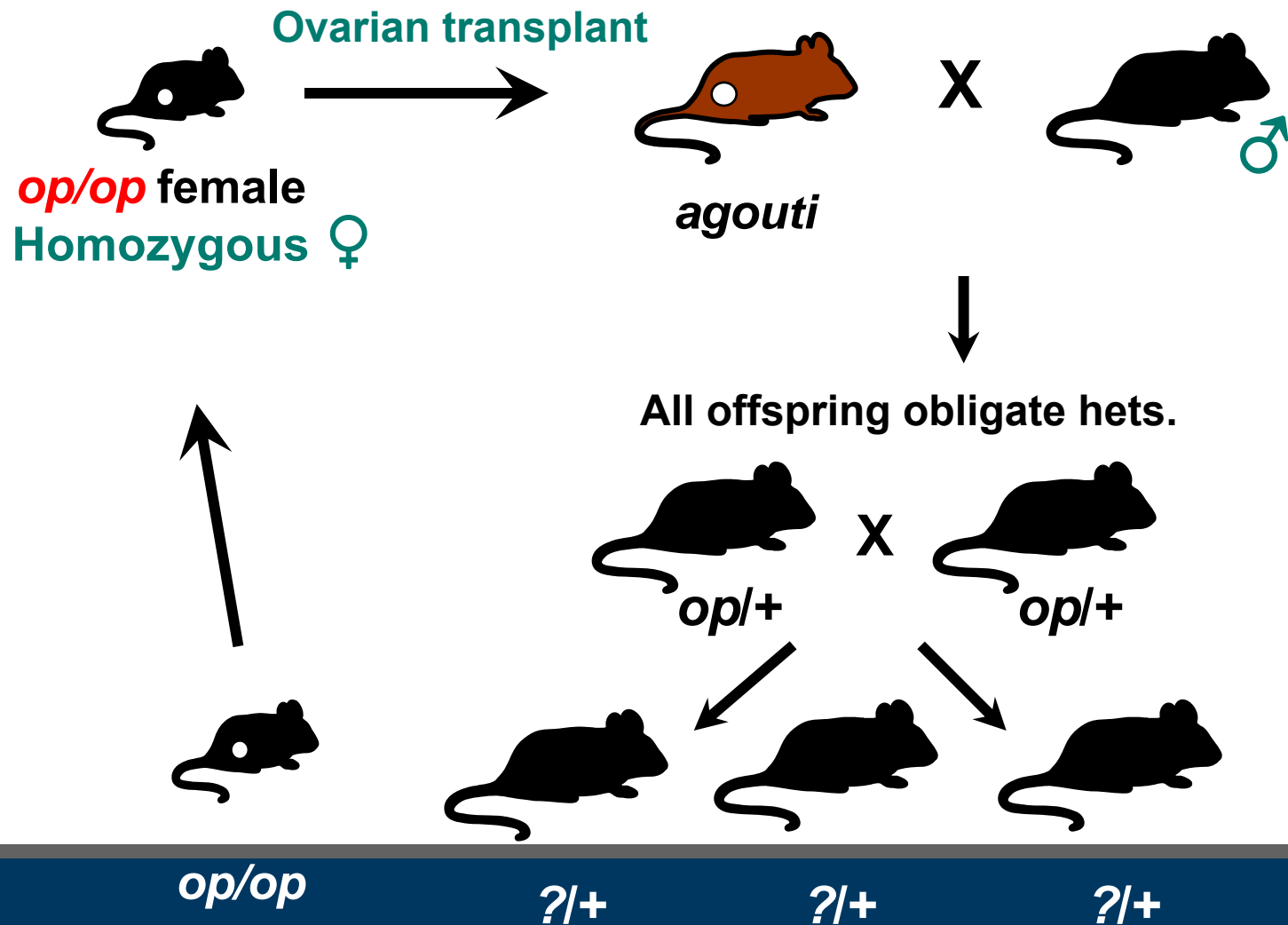
Homozygous (*op/op*) phenotype: osteopetrosis

- 1/3 smaller than unaffected siblings
- Long bones shorter in length
- Incisors fail to erupt; reduced viability
- Bone marrow deficiencies
- Reduced fertility

Heterozygote (*+/op*) x Heterozygote (*+/op*)

- 25% *op/op* Identify by phenotype
- 50% *op/+* Phenotype normal (breeders)
- 25% *+/+* Phenotype normal (controls)

Complex Breeding Scheme 1



Usefulness of Ovarian Transplantation

- **Circumvent genotyping difficulties / costs**
 - B6C3Fe *a/a-Csf1^{op}/J*
- **Female infertility or reduced fertility, but gametes viable**
 - mouse models of obesity and diabetes
- **Extend breeding life span**
 - Huntington's Disease models
 - mouse models of ALS

Complex Breeding Schemes 2

- **Double Mutants**

Ex: **WB/ReJ-Kit^W/+** x **C57BL/6J-Kit^{W-v}/+**



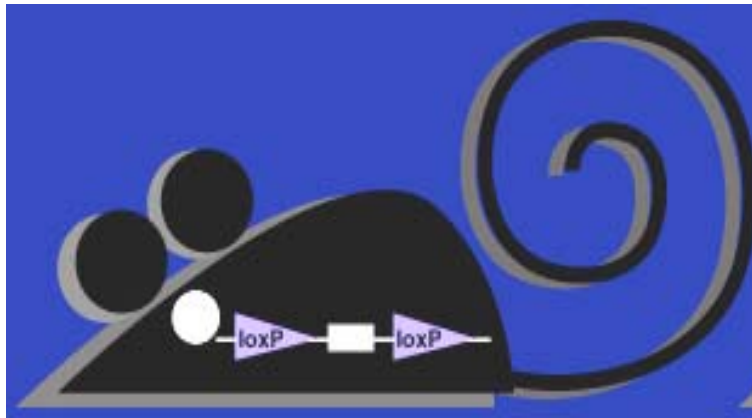
WBB6F1/J – Kit^W/Kit^{W-v}

- **Macrocytic anemia**
- **Mast cell deficiency**
- **Problems with infertility**

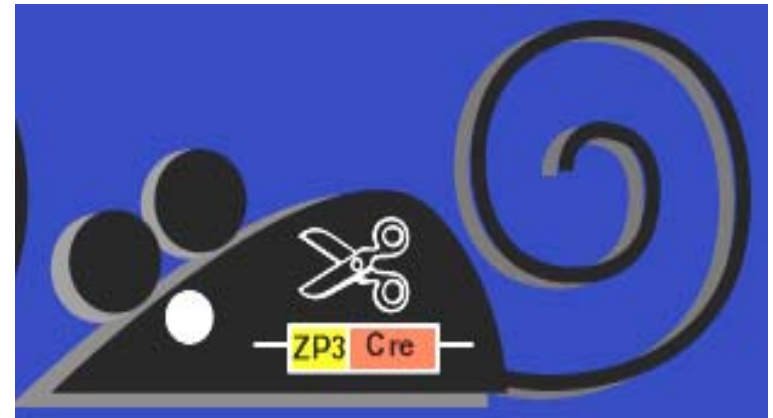
- ***Cre* – lox**

cre - *lox* Breeding Scheme

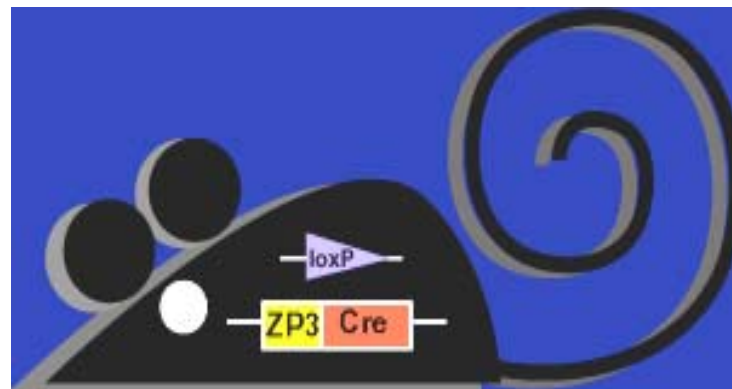
loxP flanked targeted gene



cre transgenic



X

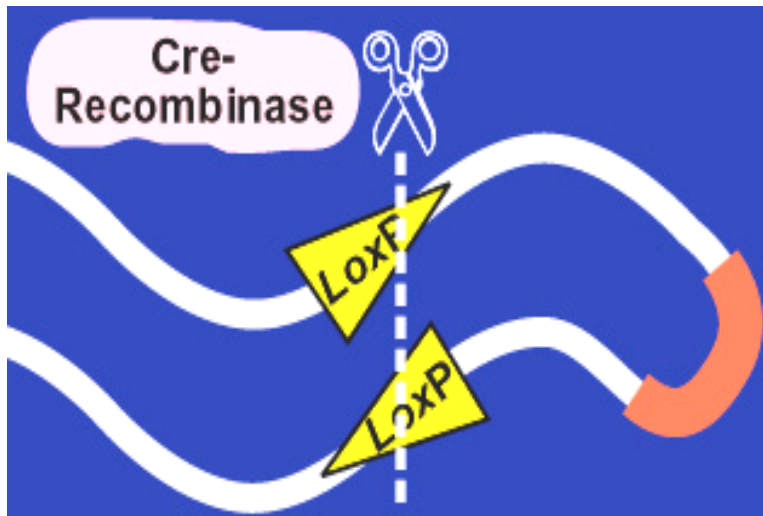


Tissue specific “knockout”

cre - lox Mechanism



Floxed target gene



Notable References

- Linder CC. 2003. Mouse Nomenclature and Maintenance of Genetically Engineered Mice. *Comp Med.* 53:119-125.
- Wolfer DP *et al.*, 2002. Knockout mice: simple solutions to the problems of genetic background and flanking genes. *Trends Neurosci* 25(7):336-340.
- Lake JP *et al.*, 1999. Dollars and sense: Time and cost factors critical to establishing genetically engineered mouse colonies *Lab Animal* 28:24-33.
- Silver LM. 1995. *Mouse Genetics: Concepts and Applications* Oxford University Press www.informatics.jax.org/silver/