Improving Mouse Colony Management Efficiency

Technical Information Services
The Jackson Laboratory

Leading provider of genetically defined mice and services including *In Vivo* pre-clinical research services

World renown non-profit genetics research institute and international training center

Bar Harbor, Maine

Sacramento, California.
The Laboratory’s Mission

**Research:** investigating genetics and biology of human disease

**Resources:** JAX® Mice & Services, bioinformatics data, technical publications and more...

**Education:** world-class courses, internships and other programs

www.jax.org/courses
JAX® Mice

The *Gold Standard* for Biomedical Research

- NIH funded resource
- 6,000 strains and growing
  - 2.9 million mice shipped annually
- Unsurpassed genetic quality & animal health
- Best characterized & referenced ~100 new pubs/week
- Common inbred strains (C57BL/6J, BALB/cJ, DBA/2J) support development/collection of specialty strains and other valuable community research resources
Online resources to expedite your research efforts

- JAX® Mice Database
  www.jax.org/jaxmice

- Mouse Genome Informatics
  www.informatics.jax.org

- Mouse Phenome Database
  www.jax.org/phenome

- And many more unique resources

www.jax.org/jaxmice/support/techsupport-index
Improving Mouse Colony Management Efficiency

Kathy J. Snow, Ph.D.
Technical Information Services
Overview

- Mouse reproduction
- Factors affecting breeding performance
- Data collection and good colony management
- Breeding strategies
Mouse Reproductive Milestones and Characteristics

- 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


8 day old NOD/ShiLtJ pups, stock # 001976
Breeding Performance
Background Effects

• Postnatal defects  C57BL/6J
• Behavior           SJL/J
• Fecundity          BTBR $T^+ \; tf \; /J$
• Hybrid vigor       F1, F2 hybrids

4 week old C57BL/6J mouse with hydrocephalus
Breeding Performance
Mutation & Transgene Effects

- Embryonic lethality
- Infertility or subfertility
- Mammary function (lactation)
- Abnormal behavior
  - poor mothering instinct
  - aggression
- Shortened breeding life span
  - tumor development (e.g. Trp53
tm1Tyj)
  - neurodegeneration (e.g. ALS, Huntington’s)

B6CBA-Tg(HDexon1)62Gpb/3J, stock number 006494
Breeding Performance

Environmental Effects

- Temperature
- Light cycle and intensity
- Noise and vibrations (construction, equipment)
- Handling (overhandling; caretaker changes)
- Odors (toxic fumes, perfume)
- Nutrition
- Health status
- Season

www.jax.org/jaxmice/support/husbandry/room-conditions
Seasonal variation in inbred mice
Data Collection & Record Keeping

Critical for successful colony management
– improve efficiency
– detect problems/mutations early
Record Keeping: Pedigree Book

Important information includes:

- **Strain data**
  - nomenclature
  - genetic background
  - history
- **Unique animal numbers**
- **Pedigree information**
  - parentage (dam and sire) & date mated
  - litter number, birth dates & pups born
  - weaned pups
  - number; (wean:born)
  - gender
  - genotypes (if applicable)
  - generation number
Record Keeping: Generation Number

N  =  Number backcross generation(s)
F  =  Filial (sister x brother) generation(s)
p  =  cryopreserved
+  =  separates generation information prior to importation to JAX
?  =  unknown generation number

Examples :  N6F12 + F8
            ? + N10F5

www.jaxmice.jax.org/support/husbandry/definitions
JAX Colony Management System

www.jax.org/jcms

• Create mating & litter records
• Track pedigrees and animal status
• Record genotypes
• Organize experimental data
• Prints cage cards
• Advanced database queries & data export
• Generate reports/updates
• Uses Microsoft Access®

Record Keeping Tips

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

- Neonates
  - tattoo (FDA approved pigment)
  - toe clip

- Weanlings and adults
  - ear tag
  - ear notching
  - micro-chip implant
  - JAXTag™

www.jax.org/jaxmice/support/husbandry/identification
Ear Notching

Example: Mouse #53

Numbering system from 1 to 99
Colony Management Tips

- Mate early (6-12 weeks)
- Choose breeders carefully
  - avoid selection pressure
- Replace breeders on a rotation (monthly)
  - mixed aged colony breeds more consistently
  - requires having young breeders available
- Replace non-productive breeders ASAP (60-90 days)
- Collect your own breeding statistics
  - evaluate regularly
Colony Management Tips (cont.)

• **Record and investigate deviations**
  – environmental changes?
  – breeding errors? (check genotypes)

• **Expect changes on a new background**
  – keep previous generations while evaluating

• **Refresh colony every ~ 10 generations**
  – replace breeders from a trusted vendor
  – backcross to inbred or F1 hybrid parent

• **Maintain pedigreed colonies**
  – filial (sister x brother) matings

www.jax.org/jaxmice/support/husbandry
Maintaining a Pedigree Colony

Single Established Colony - any strain type

*sister-brother mating only!*

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**Prevent substrain development - refresh every ~10 generations**
Mating Options

• Pair: one female x one male
• Trio: two females x one male (same cage)
  – “aunting” phenomenon
• Harem: single male, more than two females
  – NOT recommended
• Male rotation: two females x male (week 1), same male, two new females (week 2)
  – single mutant male, need many offspring
  – male has a very short lifespan (neuro. mutants)

www.jax.org/jaxmice/support/husbandry/colony-planning
Breeding Tips for Low Producing Strains

- Quiet place
- Ensure adequate darkness
- Minimal handling
- Use clean forceps or gloves
- Change dietary fat content
- Add enrichment
- Leave mating pairs together
How to Foster a Litter

• Select foster mother
  – different coat color
  – has successfully weaned a litter (ideal)
  – has a near age matched litter (ideal)
• Remove natural litter and reduce in size
• Combine foster and natural pups
• Gently mingle pups with soiled shavings from foster cage
• Place all pups in foster cage
• Do not disturb
• Pups gathered into the nest is a good sign

www.jax.org/jaxmice/support/husbandry/fostering-litters
Reducing Costs

- Size colony for your needs
- Use both sexes or an age range
- Consider ordering cohorts of mice
- Rotate breeders on a strict schedule
- Replace nonproductive breeders ASAP
- Run a tight ship…appoint a trusted colony manager
  - maximizes efficiency
- Cryopreserve unique and low-use strains

www.jax.org/jaxservices/cryopreservation/calculator.html
Cryopreservation

- For colony “sleep”
- For insurance and peace of mind
  - development and basic phenotyping of a genetically-modified strain can take 2-3 years and cost more than $100,000

Can you afford not to preserve your strains?
The Jackson Laboratory
Genetic Stability Program

Frozen embryos used to refresh foundation stock every five generations

US patents 7592501, 8110721

www.jax.org/jaxmice/genetichealth/stability
Choosing a Mouse Breeding Scheme

- 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?
Homozygote (-/-) x Homozygote (-/-)  
100% of offspring affected

Both genders viable and fertile as homozygotes

<table>
<thead>
<tr>
<th>Background</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>inbred or congenic</td>
<td>inbred*</td>
</tr>
<tr>
<td>mixed background (e.g. B6;129) outcross to F1 every ~10 generations</td>
<td>F2 hybrid <em>approximate</em> control*</td>
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</tbody>
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*Determine acceptable controls in your area of study
Genotype new breeders for insurance
Het (+/-) x Hom (-/-)  
50% homozygous offspring

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Het (+/-) x Het (+/-)  
25% homozygous offspring

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<td>+</td>
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<td>-</td>
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One (or both) genders not viable or fertile as homozygotes

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<tr>
<th>Background</th>
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</thead>
<tbody>
<tr>
<td>inbred, congeneric or mixed</td>
<td>wild-type or het sibling*</td>
</tr>
<tr>
<td>background</td>
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* if no phenotype

Genotyping typically required each generation
Transgenic Considerations
Founder lines & site of integration

- **Tg expression may affect viability or fertility**
  Ex: B6CBA-Tg(HD exon1)62Gpb/3J
  - Huntington’s disease model
  - tremors, seizures by ~ 9-11 weeks
  - females infertile & 50% males sterile
  - limited breeding window (3-4 weeks)

- **Insertional effects/mutations**
  Ex: B6.Cg-Hmga2\textsuperscript{pg}-Tg\textsuperscript{40BCha}/BmJ
  - transgene inserted into pygmy locus (Hmga2\textsuperscript{pg})
  - heterozygotes phenotypically normal
  - homozygotes smaller in size; infertile

**Evaluate multiple founders!**
Initial Transgenic Mating Scheme

Wildtype noncarrier (+/+) x Hemizygote (Tg/+ or Tg/0)
50% of offspring carriers

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<thead>
<tr>
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<th>Tg</th>
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<tbody>
<tr>
<td>+</td>
<td>Tg/+</td>
<td>+/-</td>
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<tr>
<td>+</td>
<td>Tg/+</td>
<td>+/-</td>
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Background Controls

<table>
<thead>
<tr>
<th>Background</th>
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<tbody>
<tr>
<td>inbred or mixed background</td>
<td>wild-type (noncarrier) sibling</td>
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Are homozygous (Tg/Tg) viable?
Undesirable phenotype?
Complex Breeding Schemes

• No standard PCR assay for genotyping
  – e.g. B6;C3Fe a/a-Csf1^{op}/J

• Homozygous \( \text{op}/\text{op} \)
  – long bones shorter in length
  – incisors fail to erupt
  – small, deficient bone marrow
  – reduced macrophage numbers
  – low fertility, reduced viability

• Heterozygous \( \text{op}/+ \) & wild-type \(+/+\)
  – phenotypically normal; indistinguishable

• Breeding options:
  – test mating (lost time and space)
  – ovarian transplant
Ovarian Transplantation

op/op → transfer ½ ovary

op/op × ovariectomized host → all obligate hets

op/+ × op/+ → incomplete ovariectomy

op/op
?/+  
?/+  
?/+
Usefulness of Ovarian Transplants

- Circumvent genotyping difficulties/costs
  - unknown or unmapped mutations

- Poor female reproduction but gametes viable
  - mouse models of obesity and diabesity

- Extend breeding span of severe phenotype strains
  - Huntington’s disease models
  - Amyotrophic lateral sclerosis (ALS) models
Complex Breeding Schemes: Cre-lox

GeneX

“floxed” target gene

Cre excision

GeneX

“knockout” allele
Complex Breeding Schemes: Cre-\(\text{lox}\)

- cre transgene with liver-specific expression
  Ex: B6.Cg-Tg(Alb-\text{cre})21Mgn/J

- Cre-\(\text{lox}\) mouse

- 50\% heterozygous for Gene X conditional knockout after 1 generation
Complex Breeding Schemes: Cre-\(\text{lox}\)

- heterozygous Cre-\(\text{lox}\) mouse

- \(\text{loxP}\) mouse - “floxed” gene/allele

25% Homozygous for conditional knockout after 2 generations
Complex Breeding Schemes: Cre-lox

Cre mouse – *cre* transgene (Tg) widespread expression promoter
FVB/N-Tg (EIIa-Cre)C5379Lmgd/J

*loxP* mouse - floxed gene/allele

Offspring: 50% heterozygous knockout after 1 generation
Complex Breeding Schemes: Cre-lox

Offspring 2\textsuperscript{nd} generation: 25\% homozygous knockout
Summary

• Be aware of the breeding characteristics of your strains
• Keep good records & evaluate them regularly
• Choose most effective breeding strategy to meet research needs
• Consider cost saving options
  – Use both sexes, age range
  – Cryopreservation
  – Use alternative approaches (not mice)
Complete support from start to finish.

- Common inbred & specialty JAX® Mice strains
- Basic, custom, and complex breeding capabilities
- Genome scanning and speed congenics
- Compound efficacy testing & phenotyping
- Cryopreservation and recovery

www.jax.org/jaxmice