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- Humanized NSG™ for Innovative Preclinical Research
  - August 11, 2016, 1:00 pm ET USA

- Essential Tips for New Mouse Researchers
  - September 8, 2016, 1:00 pm ET USA

- Generating Mouse Models Using CRISPR/Cas Technology
  - September 15, 2016, 1:00 pm ET USA

- Genetic Enhancement of NSG™/NRG Mice for Improved Human Disease Modeling
  - September 22, 2016, 1:00 pm ET USA
  - September 27, 2016, 9:00 am ET USA

www.jax.org/education-and-learning/webinars | THE JACKSON LABORATORY
MAKE YOUR LIFE IN THE LAB EASIER

BLOG POST | April 26, 2016

HOW TO PREVENT MOUSE BREEDING COSTS FROM DESTROYING YOUR RESEARCH BUDGET

Janine Low-Marchelli, Ph.D.

Are time and money being well-spent on generating experimental cohorts?

Anyone running a lab may be tempted to have technicians, students, postdocs, or senior scientists assume responsibility for in-house mouse breeding for experiments. “Certainly,” you must be thinking, “I don’t have the budget to buy experimental mice when I have lab staff who are perfectly capable of breeding them our facility.” In some instances, this may be true. However, traditional in-house breeding and mouse colony maintenance can be expensive, time-consuming, and not as straightforward as you might think.

So what can you do?

Step 1: Determine the financial costs of maintaining your colony

First, determine how many mice you need for your experiment. A common mistake in selecting sample size is underestimation. Variability in phenotype, even in genetically identical, inbred mice, should be accounted for. If you need some tips for determining mouse sample size, check out my blog article on experimental design.
The Jackson Laboratory’s Mission

“To discover precise genomic solutions for disease and empower the global biomedical community in the shared quest to improve human health.”

Performing Research
Investigating genetics and biology of human disease

Providing Resources
JAX® Mice Clinical & Research Services, online data resources, technical publications, and more

Educating Scientists
World-class courses, internships, and other programs
JAX® Mice
The Gold Standard for Biomedical Research

- NIH-funded resource
- >8,000 strains and growing
  - 2.7 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 Research

- **JAX® Mice Database**
  [www.jax.org/mouse-search](http://www.jax.org/mouse-search)

- **Mouse Genome Informatics**
  [www.informatics.jax.org](http://www.informatics.jax.org)

- **Mouse Phenome Database**
  [www.jax.org/phenome](http://www.jax.org/phenome)

- **Others, including:** JAX-Clinical Knowledgebase, Mouse Tumor Biology Database
Learning Goals

- Review reproductive characteristics to inform breeding expectations and handling
- Determine mouse sex
- Recognize and detect reproductive stages in female mice
- Troubleshoot when mice are non-productive
1. What is Your Role?

Which of the positions below best describes your role at your institution?

- Principal Investigator
- Veterinarian
- Colony Manager
- Postdoc
- Research Technician
- Animal Care Technician
- Other
Reproductive Characteristics

- Sexual maturity: 5 - 8 weeks
- Estrous cycle: ~4 days; spontaneous
- Number of eggs ovulated: 6 - 16 (variable by strain)
- Gestation: 18.5 - 21 days
- Litter size: 2 - 12+ pups
- Productive breeding life: ~7- 8 months

Sex Determination: Adults

MALE

FEMALE
Sex Determination: Weanlings

MALE

FEMALE
2. What is the Gender of this Mouse?

- Male
- Female

Picture from: WikiHow / Animal Handling/MSAT deck
Sex Determination: Newborns

MALE

FEMALE
Male Anatomy

Adapted, with permission, from Cook MJ. 1965. The Anatomy of the Laboratory Mouse. www.informatics.jax.org/cookbook
Shown as appears in “Comparative Anatomy and Histology: A Mouse and Human Atlas. Edited by Treuting, Dintzis, Liggitt and Frevert

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Sperm Production

- Diploid spermatogonial stem cells – lifetime supply
- 12 stages of spermatogenesis in the seminiferous tubules – 35 days
- Transport to epididymis for storage and maturation (motility)
- For IVF, collect sperm from the epididymis

SG=spermatogenic cells; SC=spermatocytes; ST=spermatids; SZ=spermatozoa; S=non-spermatogenic cells (Sertoli cells); L=interstitial cells (Leydig cells)

H&E image from "Comparative Anatomy and Histology: A Mouse and Human Atlas. Edited by Treuting, Dintzis, Liggitt and Frevert"
Female Anatomy


“Comparative Anatomy and Histology: A Mouse and Human Atlas. Edited by Treuting, Dintzis, Liggitt and Frevert

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Female Anatomy

- Gonadal adipose tissue
- Ovary
- Oviduct
- Uterine horn
- Uterine horns
- Adipose tissue
- Bladder
- Clitoral gland
- Vagina
**Oocyte Production**

- Females born with finite number of oocytes
- At 6 weeks, each ovary has 10,000 oocytes
- 6-16 oocytes ovulate over 2-3 hours every 4-5 days in mice


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Reproductive States of Adult Female Mice

- Cycling
- Pregnant
- Pseudopregnant
- Anestrus
  - e.g., seasonal non-cycling
- Reproductively senescent

Estrus & Ovulation under Neuroendocrine Control

Hypothalamus → Pituitary → Ovary → Uterus

- Hypothalamus: (GnRH)
- Pituitary: (FSH & LH)
- Ovary: (E & P)
- Uterus: (E & P)
  - Inhibin
  - Pg

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The Estrous Cycle

Stages (~4 days per cycle)
- Proestrus (13 hrs)
- Estrus- ovulation (15 hrs)
- Metestrus (13 hrs)
- Diestrus (56 hrs)

Cycle Interruption
- Mating
- Pheromones
- Exogenous hormones
- Environment

Select proestrus/estrus females for
- Timed matings
- Pseudopregnant females
  - vasectomized males required

Detecting Estrous Cycle Stage

- **Proestrus**: (cornified epithelial cells)
  - Images showing cornified epithelial cells.

- **Estrus**: (polymorphonuclear leukocytes)
  - Images showing polymorphonuclear leukocytes.

- **Metestrus**:
  - Images showing a transition phase.

- **Diestrus**:
  - Images showing a resting phase.


Detecting Estrous Cycle Stage


Detecting Estrous Cycle Stage


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3. Estrus Review

Which stage are these cells from?

- Pro-estrus
- Estrus
- Metestrus
- Diestrus
Estrous Cycle Interruption: Mating

Female in proestrus  Vaginal plug after mating

- Best observed early morning
- Produced by seminal vesicle and coagulating gland
- Prevents mating with other males
- Plug does not guarantee pregnancy
- Mating stimulation (not plug) induces pseudopregnancy
Hormonal Changes During Pregnancy

- Progesterone
- Estrogen
- LH
- FSH

Mating

Pregnancy/Pseudopregnancy

Time

Hormone concentration
Mouse Embryology


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Estrous Cycle Interruption: Pheromones

- Females in high-density group housing isolated from males tend to cease cycling (Lee-Boot, 1955)
- Pheromones in male urine induce estrus in females (Whitten, 1956)
- Exposure of pregnant female to genetically different males results in pre- or post-implantation failure (Bruce, 1959)
- Adult males accelerate puberty in females (Vandenberg, 1969)

Becker and Hurst, 2009 PMID: 2660991

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Estrous Cycle Interruption
Superovulation with Exogenous Hormones

- Pregnant Mare Serum Gonadotropin (PMSG)
  - Induces follicular development, similar to FSH
  - Follicles begin to degrade around 48 hrs

- Human Chorionic Gonadotropin (hCG)
  - Given before follicles degrade, usually mid-afternoon
  - Induces ovulation, similar to LH, within 12 hrs

- Considerations
  - Mating occurs in proestrus/early estrus ~mid-night (dark cycle)
  - Superovulation success is strain specific
  - Size/age
## Reproductive Characteristics Survey

### Table 4.1. Reproductive performance for selected strains of JAX® Mice.

<table>
<thead>
<tr>
<th>Strain of JAX® Mice (stock number)</th>
<th>Number of breeding pairs</th>
<th>Number of pups weaned per female (mean)</th>
<th>Number of litters per female (mean)</th>
<th>Maternal age (days)</th>
<th>Litter size (pups)</th>
<th>Percent weaned: born</th>
<th>Percent females weaned</th>
<th>Maternal age (months) when last litter weaned (mean, range)</th>
<th>Dates of data generation (mo/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>129P3/J (000690)</td>
<td>48</td>
<td>13.4</td>
<td>3.2</td>
<td>18-30</td>
<td>80</td>
<td>4.6</td>
<td>4.2</td>
<td>86 (2.9-10.5)</td>
<td>12/06-5/08</td>
</tr>
<tr>
<td>129S1/SvImJ (002448)</td>
<td>47</td>
<td>22.5</td>
<td>4.6</td>
<td>17-30</td>
<td>67</td>
<td>5.3</td>
<td>4.9</td>
<td>89 (3.8-11.3)</td>
<td>12/05-2/08</td>
</tr>
<tr>
<td>129X1/SvJ (000691)</td>
<td>50</td>
<td>20.7</td>
<td>4.5</td>
<td>19-50</td>
<td>77</td>
<td>5.1</td>
<td>4.6</td>
<td>89 (3.2-9.8)</td>
<td>1/06-2/08</td>
</tr>
<tr>
<td>A/J (000646)</td>
<td>50</td>
<td>21.4</td>
<td>4.3</td>
<td>17-35</td>
<td>74</td>
<td>5.3</td>
<td>5.0</td>
<td>88 (5.2-9.2)</td>
<td>12/05-1/07</td>
</tr>
<tr>
<td>AKR/J (000648)</td>
<td>49</td>
<td>18.6</td>
<td>3.4</td>
<td>20-33</td>
<td>65</td>
<td>5.5</td>
<td>5.4</td>
<td>96 (3.6-7.6)</td>
<td>12/05-11/07</td>
</tr>
<tr>
<td>B6(Cg)-Tyr-e-2J/J (000058)</td>
<td>19</td>
<td>21.6</td>
<td>3.4</td>
<td>29-62</td>
<td>70</td>
<td>6.6</td>
<td>6.3</td>
<td>92 (2.9-8.9)</td>
<td>6/07-6/08</td>
</tr>
<tr>
<td>B6.129P2-Apoel1Unc/J (002052)</td>
<td>50</td>
<td>17.3</td>
<td>3.9</td>
<td>25-31</td>
<td>74</td>
<td>5.2</td>
<td>4.5</td>
<td>83 (5.0-9.5)</td>
<td>12/05-8/07</td>
</tr>
<tr>
<td>BALB/cByJ (001026)</td>
<td>50</td>
<td>19.8</td>
<td>3.8</td>
<td>18-62</td>
<td>84</td>
<td>5.4</td>
<td>5.2</td>
<td>96 (5.4-10.3)</td>
<td>12/05-9/07</td>
</tr>
<tr>
<td>BALB/cJ (000651)</td>
<td>50</td>
<td>21.1</td>
<td>4.1</td>
<td>18-26</td>
<td>92</td>
<td>5.5</td>
<td>5.4</td>
<td>98 (5.9-9.1)</td>
<td>3/06-7/07</td>
</tr>
<tr>
<td>C3H/HeJ (000659)</td>
<td>50</td>
<td>17.3</td>
<td>3.5</td>
<td>18-41</td>
<td>73</td>
<td>5.2</td>
<td>5.0</td>
<td>92 (3.7-7.2)</td>
<td>12/05-6/07</td>
</tr>
</tbody>
</table>
Data in the Mouse Phenome Database

Mouse strain: BALB/cJ

Available phenotype data for this strain:

- appearance and coat color
- behavior
- blood-clinical chemistry
- blood-hematology
- blood-lipids
- blood-xenobiotics
- body composition
- body weight size and growth
- bone
- brain
- cardiovascular
- development
- ear
- endocrine
- eye
- gallbladder
- immune system
- kidney
- liver
- metabolism
- muscle
- nervous system
- neuroendocrine
- reproduction
- respiratory
- spleen

All phenotype measurements
- projects / data sets
- aging-related studies
- in larger initiatives
- as outlier / exceptional
- sex differences
- compare vs. other strains
- in your collection

Intervention studies [info]
- atenolol, isoproterenol
- Bacillus anthracis lethal toxin
- cadmium
- cocaine
- ethanol
- fluoxetine
- high-fat diet
- high-fat diet and ethanol
- imipramine
- lithium
- methamphetamine
- valproate
- guineleanore
- R-6-Br-APB
- sucrose

Also available for this strain:

- Photos and appearance alleles
- SNPs / genotypes
- BALB/cJ and other strains
- Polymorphic
- List genes polymorphisms strains across database based on Sanger database
- Genes / promoters
- BALB/cJ is a survey general

Mouse Phenome Database

Phenotype data: BALB/cJ strain > reproduction

Select one:
- development
- mobility — male
- all

Investigators, papers, protocols

http://phenome.jax.org/ | THE JACKSON LABORATORY
BALB/cJ-Assisted Reproduction
Poor Oocyte Yield Following Superovulation

Phenotype data > BALB/cJ strain > reproduction

Options:
- development
- morphology—male
- physiology

Focus: physiology

Measurements:
- About these BALB/cJ averages (♂♀)
- Exit BALB/cJ mode

reproduction — physiology — fecundity

- superovulation [♀]:
  - females recovered: 97.8
  - oocytes per donor: 15.5
  - live oocytes: 92.9
  - dead oocytes: 1.85
  - fragmented oocytes: 4.12

- Jax3:
  - female fertility:
    - age at first litter: 75.8
    - number of litters: 3.60
    - pct nonproductive matings: 44.4

http://phenome.jax.org/
Reproductive Characteristics Can Inform Strategy

- Plan for...
  - Number of breeding cages
  - Frequency to set up new breeder cages
  - Number of females needed for superovulation (IVF, cryopreservation)
Reproductive Success
Environmental Effects

- Nutrition
- Light cycle and intensity (14hr light/10hr dark)
- Stress… noise, vibrations, odors, over-handling
  - Breeding cessation
  - Resorption of fetuses
  - Cannibalism of litters
- Health status
- Seasonal effects

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
  - Depends on the strain

- **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 (mice with neurological mutations)
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

www.jax.org/jaxmice/support/husbandry
Troubleshooting Non-productive Mice

First, define the problem…

- Don’t get pregnant
- Get pregnant, but never give birth
- Give birth, but pups die
- Not enough pups

Troubleshooting Non-productive Mice

Second, determine the cause…

- Has anything in the room changed?
- Do the animals appear healthy?
- Are nearby strains having problems?
- What is the breeding history?
- Is this strain prone to problems?

Strategies for Non-productive Mice

- Mate new males to new females
- Check for copulation plug
- Check for milk spots

Interventions

- *In vitro* fertilization (IVF)
- Ovary transplant
- Fostering
- Artificial Insemination
- Intracytoplasmic sperm injection (ICSI)
- Low dose gonadotropins

4. Breeder Troubleshooting Review

Which factors can cause breeding difficulties in mice? (Select all which apply.)

- Noise
- Diet
- Over-handling
- Health problems
How to Foster a Litter

- Select foster mother
  - Different coat color (helpful)
  - Has successfully weaned a litter (ideal)
  - Has a near age matched litter (ideal)
- Reduce natural litter size of foster mother
- Gently mingle pups with soiled shavings from foster cage
- Place all pups in foster cage
- Monitor, but do not disturb
- Pups gathered into the nest is a good sign

Ways to Reduce Costs

- Size colony for your needs
- Use both genders or age range of mice
- Mate early, rotate breeders regularly & replace non-productive breeders ASAP
- Consider purchasing cohorts of mice
- Cryopreserve unique and low-use strains
Benefits of Cryopreserving Low-Use Strains

- Reduce genetic drift, improve experimental reproducibility
- Reduce maintenance costs
Strain Rescue Program

- For small colonies threatened by old age, breeding cessation, or poor health

- Advanced techniques used to attempt to rescue your strain, including
  - Superovulation, sperm collection & *in vitro* fertilization
  - Ovarian transplantation
  - Hysterectomy derivation

- Successfully rescued ~100 strains from extinction

- Call us before it is too late!
Don’t Let Genetic Drift Derail Your Research

JAX® Patented Genetic Stability Program
Consistent performance and data reproducibility
JAX® Mice GSP is the only program that effectively limits genetic drift. Through rigorous refreshing of foundation stock every five generations from a 25 year supply of cryopreserved embryos, GSP ensures your data lasts for generations to come.

- Common inbred & specialty JAX® Mice strains
- Basic, custom & complex breeding capabilities and speed congenics
- Genome Scanning
- Cryopreservation & recovery
- Compound efficacy testing
5. Are you interested in learning more about any of these services or models? (Select all which apply.)

- On-site breeding
- Off-site breeding
- Genome scanning
- Cryopreservation
- Model generation
- In vivo pharmacology
- PDX mouse models
- Humanized mice
- On-site seminars
Thank you!

Do you have unique strains which may be at risk in the event of disaster, contamination or genetic drift?

Contact your Regional Representative:
www.jax.org/jaxmice/support/regionalcontacts

Contact Technical Support:
micetech@jax.org

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