Guidelines for Sizing Experimental Mouse Colonies



Technical Information Services

October 5, 2017



Leading the search for tomorrow's cures

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





Online Resources to Expedite Research

- JAX[®] Mice Database <u>www.jax.org/mouse-search</u>
- Mouse Genome Informatics <u>www.informatics.jax.org</u>
- Mouse Phenome Database <u>www.jax.org/phenome</u>
- Others, including: <u>JAX-Clinical Knowledgebase</u> <u>Mouse Tumor Biology Database</u>









JAX[®] Mice

The Gold Standard for Biomedical Research

- NIH-funded resource
- >9,500 strains and growing
 - >3 million mice shipped annually



- Unsurpassed genetic quality & animal health
- Best characterized & referenced ~100 new pubs/week
- Common inbred strains (<u>C57BL/6J</u>, <u>BALB/cJ</u>, <u>B6.SJL-*Ptprc*^a</u> <u>*Pepc^b*/BoyJ</u>) support development/collection of specialty strains and other valuable community research resources



Learning Goals

Breeding performance data collection

Define research needs

Calculate # of breeders (females) needed

- Steady state
- How decisions impact colony size
- Adjusting research needs to existing resources



Reproductive Characteristics

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



8 day old NOD/ShiLtJ pups (001976)

Silver LM. (1995) *Mouse Genetics: Concepts and Applications,* Oxford University Press. Available online at <u>www.informatics.jax.org/silver</u>



Data Collection & Record Keeping

Critical for successful colony management

- Improve efficiency
- Detect problems/mutations early





Record Keeping: Pedigree Book / Cage Cards

- Weaned pups per female per week
 - Average number of pups weaned per litter
 - Total pups weaned / # of litters
 - Average number of litters per female
 - Total # of litters / Total # of females
 - Including non-productive
 - Breeding rotation
 - Look for when litter sizes and/or frequency declines
- Genotype ratio (if segregating)
- Sex ratio
- Evaluate every ~6 months!!!!



Breeding Productivity Ranges for Common Backgrounds (WPF)

Strain	Wean/Female/Week
C57BL/6	0.54 - 0.83
BALB/c	0.52 - 0.60
C3H	0.69 - 1.21
B6;129 mixed	0.62 - 1.76
FVB/N	1.47 - 1.71
129	0.35 - 0.74
NOD	0.53 - 1.50

Assumptions

- Existing Colonies
 - Not covering building new colonies
- Mixed age breeders
- Breeding with pairs or trios
 - We very rarely use harem matings (1 male + more than 2 females)
- Close to even sex ratio
 - Very few strains show significant skewing
- Estimates based on averages
 - Breeding is biological!



Breeding Productivity vs. Age



AGE OF BREEDERS



Consistent Breeding Performance





Research Needs – Steady State Colony

- Number of mice
 - Can you take less?
- Genotypes
 - Homozygotes
 - Heterozygotes
 - Hemizygotes
- Sex(es)
- Age range

• Frequency

- Breeding Scheme
 - $\,\circ\,$ Hom x Hom
 - Het x Het
 - Het x Wild Type
- Controls
 - From colony
 - Another strain/colony



Standard KO: Congenic C57BL/6

Experimental Mice: Homozygous for GeneX KO allele

- Viable & fertile
- Breed Hom x Hom



- Control Mice: C57BL/6, wild type for GeneX
 - From vendor as needed
 - Own colony



Homozygous GeneX wild type





- 20 mixed sex homozygotes
- 5-8 weeks of age (4 week age range)
- Monthly (every 4 weeks)



- Average # of pups weaned per litter = 5
- Average number of litters per female = 2.8
- Breeding rotation = 26 weeks

General Steps

Calculate number of mice needed weekly

- Calculate wean/female/week
- Calculate number of female breeders needed
- Replacement breeders
 - Frequency
 - Factor in?





Line 2: What age range? Enter 1 if all need to be born in same week Enter 2 if 2 week range, 3 if three week range, *etc.* Line 3: How frequently do you need mice? Enter 1 for weekly, 2 for every other week, 4 for monthly Line 4: Divide Line 1 by smaller of Lines 2 or 3

Needed Weekly

Line 1: How many mice do you need?

Calculate # of Mice

Hom x Hom 20 Mixed Sex 4 week range Monthly

4

5

Calculate # of Mice Needed Weekly

Line 5: What sex of mice?

Enter 1 if using both, enter 2 if using one sex

Line 6: What Breeding Scheme?

If Hom x Hom, enter 1 If Het x Hom (or recip), enter 2 If Het/Hemi x WT (or recip.), enter 2 If Het x Het, enter 4

Line 7: Can you use fewer mice?

If yes, enter 1

If no, enter "fudge factor" (1.1 for 10% over, 1.2 for 20%, etc.)



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Hom x Hom 20 Mixed Sex 4 week range Monthly





1

Calculate # of Mice Needed Weekly

Hom x Hom 20 Mixed Sex 4 week range Monthly

5

Line 8: Calculate # mice you need weekly

Multiply Line 4 X Line 5 X Line 6 X Line 7 Round *up* to nearest whole number (5 * 1 * 1 * 1)

5 mice per week x 4 weeks = 20 mice/month!

Calculate wean/female/week (breeding performance)



Calculate Wean per Female per Week

Hom x Hom 20 Mixed Sex 4 week range Monthly

Line 9: Average number of pups/litter? Line 10: Average number of litters/female? Including non-productive Line 11: Breeding Rotation (in weeks) Line 12: Calculate wean per female per week Divide Line 10 by Line 11, then multiply by Line 9 Round to nearest hundredth





Breeding Productivity Ranges for Common Backgrounds (WPF)

Strain	Wean/Female/Week
C57BL/6	0.54 - 0.83
BALB/c	0.52 - 0.60
C3H	0.69 - 1.21
B6;129 mixed	0.62 - 1.76
FVB/N	1.47 - 1.71
129	0.35 - 0.74
NOD	0.53 - 1.50

Calculate # of Females Needed

Hom x Hom 20 Mixed Sex 4 week range Monthly

10

Line 13: Calculate number of breeding females Divide Line 8 by Line 12 Round *up* to nearest whole number (5 / 0.54)

10 females x 0.54 (wean/female/week) = 5.4 pups/week

What happens when breeders get old?



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Hom x Hom 20 Mixed Sex 4 week range Monthly

Line 14: Replacement females needed weekly

0.5

Divide Line 13 by Line 11 (10 / 26)

Round up to nearest half

Do we have to factor in additional breeders?



Hom x Hom 20 Mixed Sex 4 week range Monthly

May not need to factor in:

- When experimental mice and breeders have *different* genotypes
 - Heterozygote x heterozygote, using homs
 - Heterozygote x homozygote, using one sex
 - Heterozygote/hemizygote x WT, using one sex

- OR age range (weeks) < cohort frequency (weeks)
 - EX: 1 week age range needed every 4 weeks

Hom x Hom 20 Mixed Sex 4 week range Monthly

Need to factor in when: Yes

- Experimental mice and breeders have same genotype
 - Homozygote x homozygote
 - Heterozygote x homozygote, using both sexes
 - Heterozygote/hemizygote x WT, using both sexes
- AND age range (weeks) ≥ cohort frequency (weeks)
 - EX: 4 week range needed every 4 weeks

Hom x Hom 20 Mixed Sex 4 week range **Monthly**

Line 14: Rep	lacement females neede	d weekly	0.5
Divide Line Round <i>up</i> to	13 by Line 11 <mark>(10 / 26)</mark> o nearest half		
Line 15: Add	litional females needed		2
Multiply Line Round <i>up</i> to (0.5 * 2 / 0.5	e 14 by 2 then divide by Line 1 o nearest whole number 54)	2	
Line 16: Fina	al number of females ne	eded	12
Add Line 13	and Line 15		
(10 + 2)	JAX® Breeding & Husbandry Support TF	E JACKSON LABORATORY	





How Much Box Space Is Needed?

Will need additional space for experiments and controls!

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Hom x Hom



Do you have that much space?



Costs Per Mouse

Hom x Hom 20 Mixed Sex 4 week range Monthly

Pairs

- 76 box/pen weeks
 - 12 Breeding boxes x 4 weeks/cohort = 48 weeks
 - Holding for experiments/replacement = 28 weeks
 - 2 boxes held for 5 weeks each (3-8 weeks) = 10
 - 2 boxes held for 4 weeks each (3-7 weeks) = 8
 - 2 boxes held for 3 weeks each (3-6 weeks) = 6
 - 2 boxes held for 2 weeks each (3-5 weeks) = 4
- \$400/cohort*
- \$20/mouse*

Costs Per Mouse

Hom x Hom 20 Mixed Sex 4 week range Monthly

Trios

52 weeks

- 6 Breeding boxes x 4 weeks/cohort = 24 weeks
- Holding for experiments/replacement = 28 weeks
 - 2 boxes held for 5 weeks each (3-8 weeks) = 10
 - 2 boxes held for 4 weeks each (3-7 weeks) = 8
 - 2 boxes held for 3 weeks each (3-6 weeks) = 6
 - 2 boxes held for 2 weeks each (3-5 weeks) = 4
- \$275/cohort*
- \$13.75/mouse*

Altering Research Needs

- Age Range
- Sex
- Number of mice
- Breeding Scheme





- 20 mixed sex homozygotes
- 5-8 weeks of age (4 week age range)
- Monthly (every 4 weeks)



- 20 mixed sex homozygotes
- 8 weeks of age (1 week age range)
- Monthly (every 4 weeks)

2(

Changing Age Range

Hom x Hom 20 Mixed Sex 1 week range Monthly

Line 1: How many mice do you need?

Line 2: What age range?

Enter 1 if all need to be born in same week Enter 2 if 2 week range, 3 if three week range, *etc.*

Line 3: How frequently do you need mice?

Enter 1 for weekly, 2 for every other week, 4 for monthly

Line 4: Divide Line 1 by smaller of Lines 2 or 3





5
Hom x Hom 20 Mixed Sex 1 week range Monthly

Line 5: What sex of mice?

Enter 1 if using both, enter 2 if using one sex

Line 6: What Breeding Scheme?

If Hom x Hom, enter 1 If Het x Hom (or recip), enter 2 If Het/Hemi x WT (or recip.), enter 2 If Het x Het, enter 4

Line 7: Can you use fewer mice?

If yes, enter 1

If no, enter "fudge factor" (1.1 for 10% over, 1.2 for 20%, etc.)

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Hom x Hom 20 Mixed Sex 1 week range Monthly

Line 8: Calculate # mice you need weekly



Multiply Line 4 X Line 5 X Line 6 X Line 7 Round *up* to nearest whole number (20 * 1 * 1 * 1)

Hom x Hom 20 Mixed Sex 1 week range Monthly

Line 9: Average number of pups/litter? Line 10: Average number of litters/female? Including non-productive Line 11: Breeding Rotation (in weeks) Line 12: Calculate wean per female per week Divide Line 10 by Line 11, then multiply by Line 9 Round to nearest hundredth







Hom x Hom 20 Mixed Sex 1 week range Monthly

Line 13: Calculate number of breeding females

38 10

Divide Line 8 by Line 12 Round *up* to nearest whole number (20 / 0.54)

38 females x 0.54 (wean/female/week) = 20.5 pups/week



Hom x Hom 20 Mixed Sex 1 week range Monthly

Line 14: Replacement females needed weekly

0.5 1.5

Divide Line 13 by Line 11 Round *up* to nearest half (38/26)

Do we need to factor in replacement breeders?



Replacement Breeders

Hom x Hom 20 Mixed Sex **1 week range** Monthly

May not need to factor: NO

- When experimental mice and breeders have different genotypes
 - Heterozygote x heterozygote
 - Heterozygote x homozygote, using one sexes
 - Heterozygote/hemizygote x WT, using one sexes

OR age range (weeks) < cohort frequency (weeks)

• EX: 1 week age range needed every 4 weeks



How Much Box Space Is Needed?

Hom x Hom 20 Mixed Sex 1 week range Monthly



Costs Per Mouse

Hom x Hom 20 Mixed Sex 1 week range Monthly

Pairs

- 178 box/pen weeks
 - 38 Breeding boxes x 4 weeks/cohort = 152 weeks
 - Holding for experiments/replacement = 26 weeks
 - 4 boxes held for 5 weeks each (3-8 weeks) = 20
 - Cohort
 - 2 boxes held for 3 weeks each (3-6 weeks) = 6
 - Replacement breeders
- \$935/cohort*
- \$46.75/mouse*

Costs Per Mouse

Hom x Hom 20 Mixed Sex 1 week range Monthly

Trios

- 102 box/pen weeks
 - O 19 Breeding boxes x 4 weeks/cohort = 76 weeks
 - Holding for experiments/replacement = 26 weeks
 - 4 boxes held for 5 weeks each (3-8 weeks) = 20
 - Cohort
 - 2 boxes held for 3 weeks each (3-6 weeks) = 6
 - Replacement breeders
- \$536/cohort*
- \$26.80/mouse*



- 20 mixed sex homozygotes
- 5-8 weeks of age (4 week age range)
- Monthly (every 4 weeks)



- 20 female homozygotes
- 5-8 weeks of age (4 week age range)
- Monthly (every 4 weeks)

Hom x Hom 20 **Female** 4 week range Monthly

Line 5: What sex of mice?

Enter 1 if using both, enter 2 if using one sex

Line 6: What Breeding Scheme?

If Hom x Hom, enter 1 If Het x Hom (or recip), enter 2 If Het/Hemi x WT (or recip.), enter 2 If Het x Het, enter 4

Line 7: Can you use fewer mice?

If yes, enter 1

If no, enter "fudge factor" (1.1 for 10% over, 1.2 for 20%, etc.)

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Hom x Hom 20 **Female** 4 week range Monthly

Line 8: Calculate # mice you need weekly



Multiply Line 4 X Line 5 X Line 6 X Line 7 Round *up* to nearest whole number (5 * 2 * 1 * 1)

10 mice per week x 4 weeks = 40 mice/month!

20 females, 20 males

Calculate the number of breeding females needed



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Hom x Hom 20 **Female** 4 week range Monthly

Line 13: Calculate number of breeding females

10 19

Divide Line 8 by Line 12 Round *up* to nearest whole number (10 / 0.54)

19 females x 0.54 (wean/female/week) = 10.3 pups/week



Hom x Hom 20 **Female** 4 week range Monthly

Line 14: Replacement females needed weekly

0.5 1

Divide Line 13 by Line 11 (19 / 26)

Round up to nearest half

Do we need to factor in replacement breeders?



Hom x Hom 20 **Female** 4 week range Monthly

Need to factor in when: YES

- Experimental mice and breeders have same genotype
 - Homozygote x homozygote
 - Heterozygote x homozygote, using both sexes
 - Heterozygote/hemizygote x WT, using both sexes
- AND age range (weeks) ≥ cohort frequency (weeks)
 - EX: 4 week range needed every 4 weeks

Hom x Hom 20 **Female** 4 week range Monthly

Line 14: Replacement females needed weekly 0.5

Divide Line 13 by Line 11 (19 / 26)

Round up to nearest half

Line 15: Additional females needed

Multiply Line 14 by 2 then divide by Line 12 Round *up* to nearest whole number (1 * 2 / 0.54)

Line 16: Final number of females needed

Add Line 13 and Line 15

(19 + 4)

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1

12 23



How Much Box Space Is Needed?

Hom x Hom 20 **Female** 4 week range Monthly



Homozygote X Homozygote



- 20 mixed sex homozygotes
- 5-8 weeks of age (4 week age range)
- Monthly (every 4 weeks)

Homozygote X Homozygote



- 100 mixed sex homozygotes
- 5-8 weeks of age (4 week age range)
- Monthly (every 4 weeks)

100 4

Line 1: How many mice do you need? Line 2: What age range? Enter 1 if all need to be born in same week Enter 2 if 2 week range, 3 if three week range, etc. Line 3: How frequently do you need mice? Enter 1 for weekly, 2 for every other week, 4 for monthly Line 4: Divide Line 1 by smaller of Lines 2 or 3

Changing Number

Hom x Hom **100** Mixed Sex 4 week range **Monthly**





4

Hom x Hom **100** Mixed Sex 4 week range Monthly

Line 8: Calculate # mice you need weekly



Multiply Line 4 X Line 5 X Line 6 X Line 7 Round *up* to nearest whole number (25 * 1 * 1 * 1)

25 mice per week x 4 weeks = 100 mice/month!

Calculate the number of breeding females needed



Hom x Hom **100** Mixed Sex 4 week range Monthly

Line 13: Calculate number of breeding females

10 47

Divide Line 8 by Line 12 Round *up* to nearest whole number (25 / 0.54)

47 females x 0.54 (wean/female/week) = 25.4 pups/week



Hom x Hom **100** Mixed Sex 4 week range Monthly

0.5

Line 14: Replacement females needed weekly

2

Divide Line 13 by Line 11 (47 / 26)

Round up to nearest half

Do we need to factor in replacement breeders?



Hom x Hom **100** Mixed Sex 4 week range Monthly

Need to factor in when: YES

- Experimental mice and breeders have same genotype
 - Homozygote x homozygote
 - Heterozygote x homozygote, using both sexes
 - Heterozygote/hemizygote x WT, using both sexes
- AND age range (weeks) ≥ cohort frequency (weeks)
 - EX: 4 week range needed every 4 weeks

Hom x Hom **100** Mixed Sex 4 week range **Monthly**

2

12

Line 14: Replacement females needed weekly 0.5 Divide Line 13 by Line 11 (47 / 26) Round *up* to nearest half Line 15: Additional females needed Multiply Line 14 by 2 then divide by Line 12 Round *up* to nearest whole number (2 * 2 / 0.54)

Line 16: Final number of females needed

Add Line 13 and Line 15

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55

2

8



55 females x 0.54 = 29.7 pups/week

Hold all pups (4-6 boxes)

Replace 2 oldest breeders

Hold all pups (4-6 boxes)

Replace 2 oldest breeders

Hold all pups (4-6 boxes)

Final Colony

Week 1

Week 2

Hom x Hom **100** Mixed Sex 4 week range Monthly

How Much Box Space Is Needed?

Hom x Hom **100** Mixed Sex 4 week range Monthly







- 5-8 weeks of age (4 week age range)
- Monthly (every 4 weeks)

Changing Breeding Scheme

- Heterozygote X Heterozygote
 - Mixed genetic background
 - Homozygotes infertile
 - Area of research/journals require colony controls
- 20 mixed sex homozygotes
- 5-8 weeks of age (4 week age range)
- Monthly (every 4 weeks)



Changing Breeding Scheme

Het x Het 20 Mixed Sex 4 week range Monthly

Line 5: What sex of mice?

Enter 1 if using both, enter 2 if using one sex

Line 6: What Breeding Scheme?

If Hom x Hom, enter 1 If Het x Hom (or recip), enter 2 If Het/Hemi x WT (or recip.), enter 2 If Het x Het, enter 4

Line 7: Can you use fewer mice?

If yes, enter 1

If no, enter "fudge factor" (1.1 for 10% over, 1.2 for 20%, etc.)

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Changing Breeding Scheme

Het x Het 20 Mixed Sex 4 week range Monthly

Line 8: Calculate # mice you need weekly

5 20

Multiply Line 4 X Line 5 X Line 6 X Line 7 Round *up* to nearest whole number (5 * 1 * 4 * 1)

20 mice per week x 4 weeks = 80 mice/month!

Calculate the number of breeding females needed



Breeding Scheme

Het x Het 20 Mixed Sex 4 week range Monthly

10

Line 13: Calculate number of breeding females

38

Divide Line 8 by Line 12 Round *up* to nearest whole number (20 / 0.54)

38 females x 0.54 (wean/female/week) = 20.5 pups/week



Changing Breeding Scheme

Het x Het 20 Mixed Sex 4 week range Monthly

Line 14: Replacement females needed weekly 0.5

1.5

Divide Line 13 by Line 11 Round *up* to nearest half (38 / 26)

Do we need to factor in replacement breeders?


Replacement Breeders

Het x Het 20 Mixed Sex 4 week range Monthly

May not need to factor: NO

- When experimental mice and breeders have *different* genotypes
 - Heterozygote x heterozygote
 - Heterozygote x homozygote, using one sexes
 - Heterozygote/hemizygote x WT, using one sexes

- OR age range (weeks) < cohort frequency (weeks)
 - EX: 2 week age range needed every 4 weeks



Changing Breeding Scheme

Het x Het 20 Mixed Sex 4 week range Monthly

38 females x 0.54 = 20.5 pups/week

- 5 Homozygotes
 - Hold for experiments
- 10 Heterozygotes
 - Replacement breeders
 - Discard remaining
- 5 Wild type
 - Hold for controls





How Much Box Space Is Needed?

Het x Het 20 Mixed Sex 4 week range Monthly



Controls included!



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Your Total Costs

Monetary

• Per Diems



- If supplemented, overhead from grants may be funding
- Technician salary
- Genotyping
- Time used to manage colonies
 - Collecting/analyzing breeding data
 - Planning
- Space for colonies

Colony Management Tips

 Mate early (4-12 weeks)



- Choose breeders carefully
 Avoid selection pressure
- Replace breeders on a rotation (monthly)
 - Mixed-age colony breeds more consistently
 - Requires having young breeders available
- Replace non-productive breeders ASAP (60-90 days)
- Collect your own breeding statistics
 Evaluate regularly



Other Factors That Impact Breeding

- Pedigree/linage tracking
- Health status
- Good quality diet
 - Q/C ensuring consistency
 - Variation can impact breeding and weights
- Technician training and experience
 - Detect problems earlier
- Consistency (SOPs)
 - Following protocols





Review of Learning Goals

Breeding performance data collection

Define research needs

Calculate # of breeders (females) needed

- Steady state
- How decisions impact colony size
- Adjusting research needs to existing resources





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- Aged C57BL/6J Mice (25-78 wks)
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- The Basics of Mouse Nomenclature
 - Oct. 11, 2017, 8:00 pm ET
 - Oct. 12, 2017, 12:00 am GMT; 8:00 am AWST & CST;
 9:00 am JST & KST; 11:00 am AEST
- Guidelines for Sizing Experimental Mouse Colonies
 - Oct. 19, 2017, 9:00 am ET; 1:00 pm GMT
- Key Differences among B6 Mouse Substrains and the Research Impact
 - Oct. 26, 2017, 1:00 pm ET; 5:00 pm GMT





Thank you!

In need of mouse breeding and colony management expertise to advance your research?

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