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HOW TO PREVENT MOUSE BREEDING COSTS FROM DESTROYING YOUR RESEARCH BUDGET

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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.

Second, estimate how many breeder female mice are needed to generate these experimental cohorts. This JAX Breeding Colony Size Planning Worksheet lets you calculate breeder females based on desired experimental mouse age range and sex, frequency of experiments, mating scheme employed, and breeding characteristics. In addition, this JAX Colony Planning Resource offers additional breeding and colony considerations.

Third, calculate annual personnel and caging costs associated to generate these mice. This JAX Cost Savings Calculator allows you to account for salaries and benefits for personnel, per diem rates in the mouse facility (roughly $1/cage/day depending on your institute), and genotyping needs for the mouse strain. As a side note, the JAX Cost Savings Calculator also helps weigh the financial costs to maintain a low-use mouse strain live versus cryopreserving it.

Finally, factor in the number of months needed to generate the mice. A general rule of thumb is to account for approximately 3 months per breeding generation. The number of generations needed to generate the mice will depend on the number of starting breeder mice, mating scheme employed (some homozygous mutants may be subfertile), and the number of crosses needed to generate a mutant model (in the case of making a double, triple etc mutant mouse).

Now, you have calculated a total monetary cost to generate the desired number of mice *for one experiment*. How many mouse experiments of this mouse strain might be needed to publish a paper? How soon do you need data? How soon is your funding ending?

Step 2: Make lab morale a high priority

In Step 1, you calculated financial costs to get a single experimental cohort of mice. While this figure may be shocking on its own, remember that associated with financial costs, your research personnel costs include people's time, brain power, and physical efforts needed to care for the mice.

Consider the following: would you rather lab staff...

- clip mouse tails, label tubes, optimize genotyping reactions?
- wait months for mice to breed?
- analyze ambiguous data from underpowered experiments?

or

- read and evaluate current literature?
- write grants and papers?
- design experiments and perform actual research?

Hopefully your answers lie in the right-hand column. It's easy to take for granted how much energy traditional in-house breeding diverts away from research activities. Positive lab morale is priceless and contributes to data quality.

Step 3: Promote ethical animal use and reduce time to generate experimental cohorts

You have determined how many mice you need to perform your experiment (and placed high value on your lab staff's abilities). However, keep in mind that while you are expanding your colony to generate experimental mice, you are also generating mice along the way that may not be usable (wrong genotype or will be too old to use by experiment time). These mice contribute to your animal use protocol numbers and to colony maintenance costs.

To reduce animal use and costs, consider alternative strategies that also reduce the time to generate experimental cohorts. For instance, some strains are maintained more efficiently using ovarian transplantation, especially if the strain has difficulties breeding. Ovaries from a homozygous mutant female with reduced breeding ability are transplanted into phenotypically normal females. The recipient females then breed with male mice to generate a large number of heterozygous mice. The heterozygotes can then be breed together to generate homozygotes.

Another strategy to reduce time and number of animals used, is *in vitro* fertilization (IVF). Sperm, collected from 1-2 mutant males, and oocytes, collected from super-ovulated wild type females, are incubated in a dish to generate 2-cell embryos. These embryos are then transferred to several recipient pseudopregnant females, who will deliver offspring about 3 weeks later. Reproductive sciences cores and organizations like JAX utilize a combination of traditional breeding and IVF as a means to expand a colony to produce a large number of age-matched mice in a relatively short amount of time.

Step 4: Make wise decisions based on your calculations

Here is a simplified case study to summarize my point:

Let's assume you want to test a therapeutic intervention on your colony of Abcb11 knockout mice, a model for intrahepatic choleostasis. Between your control and experimental groups, you determine that you need 24 homozygous mice at 4 weeks old. The homozygous (hom) mice have reduced fertility, so to maintain the colony, the mice must be bred as heterozygotes (het).

Using a traditional breeding approach and working backwards from what you need to what you have on hand:

- Mendelian genetics predict that a het x het mating generates 25% hom offspring
    - To generate 24 hom mice, you need 96 offspring (24= 25% of 96)
- Assume a breeding female averages 6 pups/litter
    - You need 16 het breeding pairs (96 pups/6 pups per litter = 16 litters) or
    - 16 female and 16 male het mice minimum to generate one experimental cohort in one round of breeding (3 months)

Do you have 16 heterozygous females and 16 heterozygous males ready to breed? Probably not.
Here is how you might generate these 16 breeder pairs, again working backwards:

- Mendelian genetics predict that a wild type (WT) x het mating generates 50% het offspring
    - To generate 32 het mice, need 64 offspring (32=50% of 64)
- Need ~11 WT x het breeding pairs (64 pups/6 pups per litter = 10.7 litters)

  - To generate 11 het mice, need 22 offspring (11=50% of 22)

- Need ~4 WT x het breeding pairs (22 pups/6 pups per litter = 3.7 litters)

Do you have 4 heterozygous and 4 wild type mice ready to breed? This is probably more reasonable.

Therefore, starting with 4 WT x het breeder pairs, the 24 homozygous mice would be ready in about 9 months (from 3 rounds of breeding). For one experiment, you will have generated approximately 212+ mice, approximately 45-50+ mouse cages and associated housing or veterinary costs, hundreds of dollars in genotyping reagents, and several cumulative days or weeks of technician time and focus.

In this example, I did not include a “safety buffer” of extra time and extra mice. You would want to build in a pretty significant buffer since, as a general rule, not all matings will be successful and since this particular strain is also known to have reduced fertility. In this case, the 6 pups/litter is a wishful and naïve overestimate. Rounding up, you may be looking at 12-15 months of traditional breeding (and maybe more than 212 mice and associated costs) to generate what initially seemed like a reasonable experimental cohort of 24 mice.

Alternatively, using IVF and starting with 2 heterozygous males and several wild type females, you could generate the 16 het females and 16 het males (or more, given your safety buffer) in about 3 months, and the final cohort of 24 homozygous mice in another 3 months.

You may find that IVF, or even outsourcing your breeding to another party is a better use of your budget and your laboratory resources than you originally thought. Why don’t you find out?

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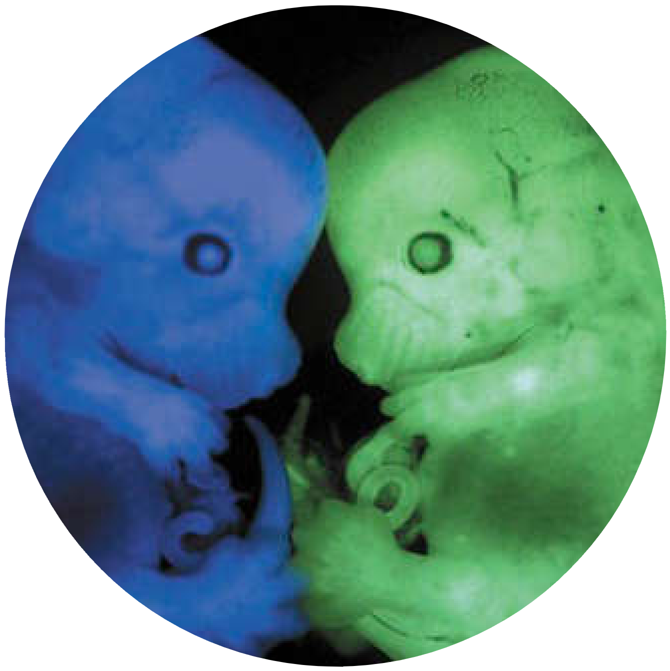

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