

# Optimizing Screening and Mating Strategies for Phenotype-driven Recessive *N*-Ethyl-*N*-nitrosourea Screens in Mice

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Phenotype-driven *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis screens in the mouse are being used to elucidate gene function and develop disease models. Many of the earlier screens focused on identifying dominant mutations, whereas many newer mutagenesis programs have arisen that focus on identifying recessive mutations. Recessive screens require more complex breeding and phenotyping procedures, yet little information is available on the optimal breeding and phenotyping strategies for identifying recessive mutations. Optimization involves minimizing the numbers of mice that must be bred and subjected to phenotypic screens while maximizing the number of mutant phenotypes that can be identified. Analysis of expected frequencies of mutants has been used to determine which of the typically used mating and screening strategies will produce the best returns in terms of identifying recessive phenotypes. As a general guideline, to minimize the number of mice to be screened, the optimal strategy is to mate a single generation 2 ( $G_2$ ) female and  $G_1$  male and screen either 11 or 17  $G_3$  offspring to obtain at least 1 or 2 homozygous mutants, respectively. When the expense of producing and housing the mice is the greatest cost factor and the phenotype is so robust that a single outlier will suffice, then the optimal strategy is to mate 2  $G_2$  sisters with the  $G_1$  male parent and screen a single litter from each. Intercrossing of  $G_2$  brothers and sisters is not an efficient method for maximizing returns from ENU screens.

**Abbreviations:** ENU, *N*-ethyl-*N*-nitrosourea; G, generation; SEQ, screening efficiency quotient

Phenotype-driven mutagenesis is a powerful technique to generate new mouse disease models and to determine gene function, thereby contributing to developing a functional map of the mouse genome.<sup>4</sup> To this end several large-scale projects have begun to mutate various genes in the mouse genome by gene trap–knockout, chemical mutagenesis, and other approaches and then phenotypically analyze the resulting mutant mice.<sup>2,3,6</sup> *N*-ethyl-*N*-nitrosourea (ENU) has become the standard mutagen for phenotype-driven chemical mutagenesis in the mouse.<sup>33</sup> This technique has certain advantages over the gene trap–knockout approach. First, the random nature of ENU mutagenesis means that no assumptions need be made about gene function—the genes involved are revealed by the mutant phenotype. Second, ENU mutagenesis does not create only null alleles that act recessively; it also yields dominant hypermorphic, hypomorphic, and antimorphic alleles—approximately 51% of human disease alleles do not act in a recessive fashion.<sup>27</sup> Third, ENU mutagenesis can be used to create an allelic series for any gene; this information is useful in mapping functional domains. Fourth, ENU mutants may reveal drug targets more easily than other techniques, because ENU mimics drug action more closely than do gene knockouts—many drugs do not inactivate proteins entirely.<sup>31</sup> Finally, the parallel sperm–DNA archives that have been created mean that, if required, mutations can be recovered in any gene of interest.<sup>1,24,29,34</sup>

Compared with other mutagens, ENU is more than an order of magnitude more mutagenic in stem cell spermatogonia.<sup>32,33</sup> Reported mutation rates vary between 1 mutation per 1 to

2.69 Mb of genomic DNA.<sup>1,24,29,34</sup> This frequency results in an estimated 20 to 100 mutations that have phenotypic effects in each  $G_1$  mouse.<sup>8,25</sup> The ability of ENU to efficiently mutate spermatogonial stem cells, which give rise to new sperm throughout the breeding life of the animal, allows many mutant offspring to be generated from a single ENU-treated male mouse. Large ENU mouse mutagenesis programs generally have used common laboratory mouse strains such as C3HeB/FeJ, BALB/c, and C57BL/6J.<sup>15,25,26</sup> In all of these strains, a repeated dosing regimen results in the highest mutation frequency,<sup>12</sup> typically with 3 weekly doses of 90 to 100 mg ENU/kg being optimal.<sup>17</sup> Because ENU is both toxic and mutagenic, the optimal dose is that which induces the highest mutation rate without rendering the animal infertile. The most common mutations are A:T to T:A transversions.<sup>5,18</sup> In the vast majority of cases, the mutations that lead phenotypic effects occur in the coding region or splice sites of genes and rarely in non-protein-coding DNA.<sup>28</sup>

Many of the initial large-scale ENU mutagenesis screens were designed to detect dominant-acting mutations.<sup>15,26</sup> Dominant screens are relatively simple in terms of breeding strategy, because only a single generation of breeding from ENU-injected male mice is required to reveal mutant phenotypes. Each  $G_1$  animal produced from such breeding is unique in terms of its mutation profile. However, the notion that more than 90% of mutations are recessive to wild type<sup>38</sup> emphasizes the importance of carrying out recessive screens.

In contrast to screens aimed at detecting dominant mutations, recessive screens require a further 2 generations of breeding to bring mutations carried by generation 1 ( $G_1$ ) mice to homozygosity. This need means that recessive screens are not only costly, but they also require good organization, given the complex nature of the breeding strategies and the high-throughput nature

Received: 17 May 2007. Revision requested: 19 Jun 2007. Accepted: 2 Jul 2007.

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of the phenotype screens, with many thousands of mice typically being screened for an individual phenotype. Despite the growing use of recessive screening,<sup>9,13,21,28,37</sup> little information on optimal mating and screening strategies is available. Breeding of mice should be minimized, whereas cost considerations mean that the returns from the animals bred should be maximized. To assist in planning and estimating the cost of recessive ENU mutagenesis screens, we analyzed the expected frequencies of mutants from different mating schemes to derive guidelines on the optimal breeding strategy as well as the number of mice that have to be screened in order to identify recessive phenotypes.

## Materials and Methods

**Statistical analysis.** Binomial distribution probabilities were calculated by using the equation

$$P_{(k \text{ out of } n)} = (n! / (k!(n-k)!)) p^k q^{n-k},$$

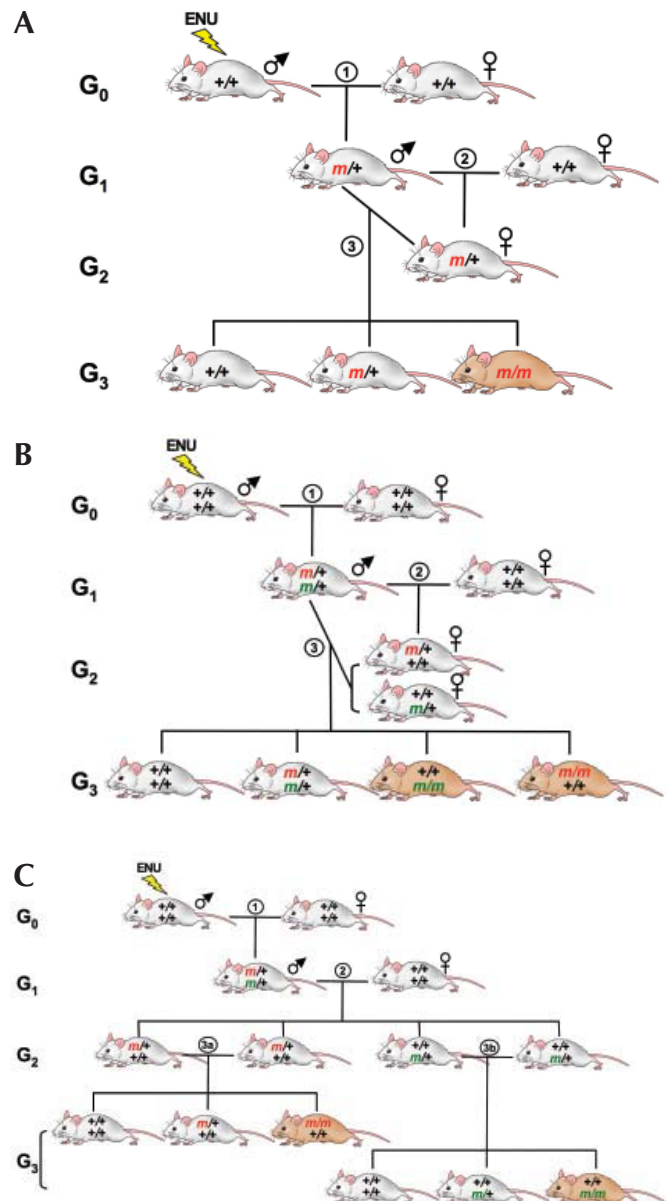
where  $n$  is the number of offspring,  $k$  is the number of homozygotes,  $p$  is the probability of obtaining a homozygote, and  $q$  is the probability of not obtaining a homozygote. An online binomial calculator<sup>10</sup> was used for all binomial probability calculations.

**Estimating the average number of homozygous mutations per pedigree.** As an example, assuming that each  $G_1$  mouse carries 50 loss-of-function mutations, then in a  $\text{G}_2 \times \text{G}_1$  mating, the binomial probability of obtaining at least 1 mutant mouse for a mutation represented in a litter of 5  $G_3$  mice (the typical size of a litter of C57BL/6J mice in our facility) is  $P = 0.763$ . Therefore, if a  $G_2$  female and  $G_1$  male share 25 mutations on average, then there will be 19.1 (that is,  $0.76 \times 25$ ) mutations on average for which at least 1  $G_3$  homozygous mutant will be recovered. In the case of a  $2\text{G}_2 \times \text{G}_1$  mating, on average 37.5 mutations are shared between the  $G_1$  male and at least 1 of the  $G_2$  females, of which 25 mutations are represented in at least 1 of 8  $G_3$  mice. Because  $P(0.95) = 0.74$  for a total of 10  $G_3$  offspring, then 18.5 (that is,  $25 \times 0.74$ ) mutations typically are brought to homozygosity in a single pedigree. For the other 12.5 mutations, which are represented at 1 in 4, because  $P(0.95) = 0.94$ , then on average 11.8 (that is,  $12.5 \times 0.94$ ) mutations are brought to homozygosity. Therefore, a total of 30.3 ( $18.5 + 11.8$ ) mutations likely will be homozygous in at least 1  $G_3$  mouse.

## Results

**Optimal animal numbers and mating strategies in genome-wide mutagenesis screens for recessive phenotypes.** In a typical project to identify ENU-induced mutant alleles, male mice are injected with ENU and mated to wild-type females. The  $G_1$  male offspring, which carry mutations from the ENU-treated male parent, then are mated to wild-type females. One or 2 female  $G_2$  offspring from these matings are backcrossed to the  $G_1$  parent to generate  $G_3$  mice, some of which will be homozygous for mutant alleles shared by the  $G_1$  male and  $G_2$  female.<sup>3</sup> The  $G_3$  mice then are screened for the phenotype of interest. These mating strategies are depicted in Figure 1 A, B.

A common question is how many  $G_3$  mice should be screened per pedigree (where ‘pedigree’ refers to all the offspring from a single  $G_1$  male) to maximize the chance of detecting mutants while keeping the number of  $G_3$  mice screened as low as possible. Therefore, we generated estimates for animal numbers and mating strategies for 2 broad classes of phenotyping assays—those with a high signal:noise ratio (where the mutant phenotype can easily and reproducibly be differentiated from the wild-type phenotype) and those with a low signal:noise ratio (where the phenotypes in nonmutant and mutant mice vary widely and overlap).



**Figure 1.** Schematic diagram of several of the common breeding strategies used in phenotype-driven ENU mutagenesis to discover recessive mutations. (A) Mating strategy where 1  $G_2$  female mouse is backcrossed to the  $G_1$  male. (B) Mating strategy where 2  $G_2$  female mice are backcrossed to the  $G_1$  male. (C)  $G_2$  micropedigree strategy. Example mutant alleles ( $m$ ) induced at 2 separate loci are shown in red and green. Two mutant alleles are shown in panels B and C, compared with 1 in panel A, to emphasize that more mutant alleles are brought to homozygosity in the strategies illustrated in panels B and C. Circled numbers indicate the sequential matings set up within a pedigree. The mouse images were obtained from reference 16.

**Phenotypes with a high signal:noise ratio.** Consider a phenotype that can be identified by a single  $G_3$  outlier (that is, not requiring confirmation by a second affected  $G_3$  mouse in the same pedigree). Clearly, such phenotypes have a high signal:noise ratio because mutant phenotypes can be detected with a fair degree of reliability without further confirmation from  $G_3$  littermates. Examples include coat-color phenotypes<sup>30</sup> and other dysmorphic abnormalities such as polydactyly<sup>7</sup> and dwarfism<sup>23</sup> as well as extreme phenotypes in which a cell type is substantially reduced or completely absent. For example,

reduction or absence of a hematopoietic cell population<sup>35</sup> or absence of a response to stimulation with lipopolysaccharide.<sup>14</sup>

For comparative purposes, we assumed that each  $G_1$  male carries (on average) 50 loss-of-function mutations that will have a phenotypic effect in the homozygous state. Estimates of the number of loss-of-function mutations carried in  $G_1$  mice vary from approximately 20 to 100 per  $G_1$  mouse.<sup>8,25</sup> The exact estimate used in calculations does not alter the comparisons we made because the rankings of different strategies remain the same regardless of the actual mutation rate. In a standard mating scheme (Figure 1 A), each  $G_2$  female mouse inherits, on average, 25 of loss-of-function mutations. If 2  $G_2$  females are backcrossed to a  $G_1$  male in each pedigree ( $2\text{♀}G_2 \times \text{♂}G_1$ ; the mating strategy depicted in Figure 1 B), the  $G_2$  females share, on average, 12.5 mutations, whereas 12.5 mutations will be unique to each female. Therefore, a  $2\text{♀}G_2 \times \text{♂}G_1$  mating brings, on average, 37.5 mutations to homozygosity in each pedigree. The 12.5 mutations common to both  $G_2$  females yield an average of 1 in 4  $G_3$  mice in this pedigree that is homozygous for an individual mutation, whereas the other 25 mutations would be unique to one or another  $G_3$  mouse and, on average, 1 in 8  $G_3$  mice screened in the pedigree is homozygous for any of these mutations.

The probability of finding at least 1 homozygote for any mutation present in the  $G_1$  male can be estimated as an exact binomial probability. Figure 2 provides these probabilities for different numbers of  $G_3$  mice screened in both scenarios (that is, expected homozygosity at 1 in 4 or 1 in 8). Eleven  $G_3$  mice must be screened to have a 95% chance of generating at least 1 homozygote for a mutation common to the 2  $G_2$  females (Figure 2 A). However, for the non-shared mutations, 23  $G_3$  mice must be screened to find at least 1 homozygous mutant at  $P = 0.95$  (Figure 2 B). Therefore, screening 23  $G_3$  mice gives a 95% chance of bringing each of the 37.5 mutations to homozygosity at least once.

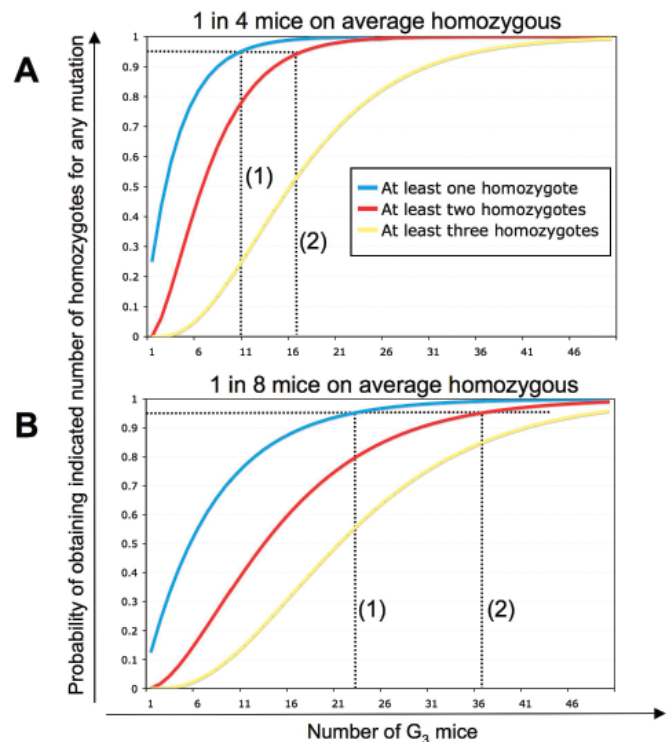
To compare the efficiencies of different mating and screening scenarios used in a recessive ENU mutagenesis screen, we propose the 'screening efficiency quotient' (SEQ):

$$\text{SEQ} = \frac{\text{No. of mutations that can be homozygous}}{\text{No. of } G_3 \text{ mice that must be screened at } P = 0.95 \text{ to have at least } n \text{ homozygotes}}$$

where  $n$  is the minimum number of homozygotes desired for a particular mutation. The higher the  $\text{SEQ}(n)$ , the more efficient the strategy. For the mating type  $2\text{♀}G_2 \times \text{♂}G_1$ , the  $\text{SEQ}(1)$  is 1.6 (that is,  $37.5/23$ ). A comparison of SEQs calculated for different mating strategies and screening numbers is shown in Table 1.

Now consider a second mating scheme in which rather than mating 2  $G_2$  female mice to the  $G_1$  male, only a single  $G_2$  female is used ( $\text{♀}G_2 \times \text{♂}G_1$ ; mating strategy 2, Figure 1 A). This strategy brings 25 mutations, on average, to homozygosity, and all mutations would be represented on average in 1 in 4  $G_3$  offspring. Therefore, 11  $G_3$  would have to be screened for a 95% chance of obtaining at least 1 homozygous mutant (Figure 2 A), with an  $\text{SEQ}(1)$  of 2.3 (that is,  $25/11$ ; Table 1). Therefore, for clear, reproducible phenotypes with a high signal:noise ratio, where a single  $G_3$  homozygous mutant is sufficient for reasonable certainty of obtaining a mutant phenotype, the optimal strategy is to backcross a single  $G_2$  female with the  $G_1$  male and then screen 11  $G_3$  offspring. In contrast, in light of the sole criterion of screening numbers and ignoring the cost of producing these animals, the often-used strategy of backcrossing 2  $G_2$  females to the  $G_1$  male is less efficient for mutant detection.

**Phenotypes with low signal:noise ratios.** In certain screens, a



**Figure 2.** Binomial probabilities of the chance of obtaining the indicated number of homozygous mutants among  $G_3$  mice. The distributions are plotted for the expected number of  $G_3$  homozygotes for an individual mutation carried by the  $G_1$  male, assuming that (A) 1 in 4 or (B) 1 in 8  $G_3$  mice is homozygous. The dotted lines indicated the number of  $G_3$  mice that must be screened to obtain at least 1 or at least 2 homozygotes for each  $G_1$  mutation with a probability of 95%.

single phenotypic outlier in a pedigree is insufficient to be confident that the deviant phenotype reflects a true mutant. Inherent variability between mice, in addition to inherent variation in assays, means that often more than 1 mutant in the pedigree is needed to ensure that a mutant phenotype has been uncovered. Examples of such phenotypes include impairment in hearing<sup>20</sup> and some neurobehavioral disorders.<sup>19</sup>

When 2  $G_2$  females are mated to a  $G_1$  male (Figure 1 B), 17  $G_3$  must be screened for a 95% chance of generating at least 2 homozygotes with a single mutation that is common between the 2  $G_2$  females (Figure 2 A). However, for non-shared mutations, 37  $G_3$  must be screened (Figure 2 B). Therefore, screening 37  $G_3$  mice gives a 95% chance of bringing each of the 37.5 mutations to homozygosity at least twice: the  $\text{SEQ}(2)$  is 1.0 (that is,  $37.5/37$ ; Table 1). In the case of backcrossing a single  $G_2$  female to the  $G_1$  male, 17  $G_3$  would need to be screened to achieve a 95% chance of obtaining at least 2 homozygotes for an individual mutation, and the  $\text{SEQ}(2)$  is 1.5 (that is,  $25/17$ ; Table 1). Therefore, even for less-reproducible mutant phenotypes, where there is inherent variation in the phenotyping assay, screening the offspring from a single  $G_2$  female mouse backcrossed to the  $G_1$  male may be most efficient. In contrast, the commonly used system of crossing 2  $G_2$  females to a parent  $G_1$  male and screening 20 to 24  $G_3$  offspring is less efficient in terms of mutation detection: this scheme has a probability of only 0.82 of obtaining at least 2 homozygotes for any mutation.

We did not consider X-linked mutations in the scenarios presented because they do not alter the conclusions. If the mating  $\text{♀wild-type} \times \text{♂}G_1$  is replaced with a  $\text{♀}G_1 \times \text{♂}G_1$  mating to allow recovery of X-linked mutations, the number of



**Table 1.** Screening efficiency quotients (SEQs) for various mating strategies to generate  $G_3$  mice

Minimal number of homozygotes required	Mating type <sup>a</sup>	Average potential mutations brought to homozygosity <sup>b</sup>	No. of $G_3$ mice to be screened <sup>c</sup>	SEQ <sup>d</sup>
1	$2fG_2 \times mG_1$	37.5	23	1.6
	$fG_2 \times mG_1$	25	11	2.3
	$fG_2 \times mG_2$	68	47	1.4
2	$2fG_2 \times mG_1$	37.5	37	1.0
	$fG_2 \times mG_1$	25	17	1.5
	$fG_2 \times mG_2$	68	74	0.9

f, female; m, male.

<sup>a</sup>See Figure 1 A, B.

<sup>b</sup>Assumes 50 loss-of-function mutations carried by each  $G_1$  mouse.

<sup>c</sup>For  $P = 0.95$  of finding at least indicated number of homozygotes.

<sup>d</sup>The calculated screening efficiency quotients (SEQs) required to detect a mutant allele are based on the assumption that each  $G_1$  male contains 50 independent mutations that will have a phenotypic effect when brought to homozygosity.

mutations in common between the  $G_2$  females and the  $G_1$  male changes slightly, but the ranking of SEQs is the same (data not shown).

Sometimes a third type of ENU mating scheme is used, in which  $G_1$  male mice are crossed with wild-type or  $G_1$  females, and then  $G_2$  brother  $\times$  sister intercrosses (known as ‘micropedigrees’) are set up<sup>25</sup> (Figure 1 C). A typical number of  $G_2 \times G_2$  micropedigrees is 4, although larger numbers of intercrosses can be set up<sup>1</sup> but with diminishing returns. The advantage of the  $G_2 \times G_2$  system is that more of the mutations present in an individual  $G_1$  mouse are brought to homozygosity. Again assuming that each  $G_1$  mouse carries 50 loss-of-function mutations, a  $G_1$  male and  $G_1$  female have, on average, 100 different loss-of-function mutations. Each  $G_2$  mouse from a  $G_1 \times G_1$  cross has about 50 different mutations, of which 25 are shared between any 2 sister and brother  $G_2$  mice and thus potentially can be brought to homozygosity. Because of some redundancy in the genes shared between the  $G_2$  brothers and sisters, we calculated that only 68 of the 100 mutations are likely to be brought to homozygosity among the 4  $G_2 \times G_2$  micropedigrees. For mutations that can be brought to homozygosity only in a single micropedigree, only 1 in 16  $G_3$  mice, on average, will be homozygous for those mutations. Because at  $P = 0.95$ , 47 mice must be screened to obtain at least 1 homozygote for a mutation, the SEQ(1) is approximately 1.4 (that is, 68/47) and the SEQ(2) is 0.9 (that is, 68/74; Table 1). These SEQs are lower than the equivalent SEQs for the  $\mathbb{X}G_2 \times \mathbb{X}G_1$  and  $2\mathbb{X}G_2 \times \mathbb{X}G_1$  mating schemes (SEQ(1) = 2.3 and 1.6, SEQ(2) = 1.5 and 1 for the  $\mathbb{X}G_2 \times \mathbb{X}G_1$  and  $2\mathbb{X}G_2 \times \mathbb{X}G_1$  mating schemes respectively). Therefore, in terms of the efficiency of mutation detection alone, the  $G_2$  micropedigree approach is the least efficient.

**Minimizing animal husbandry costs of screening.** In the presented examples, we aimed to minimize the number of  $G_3$  mice that needed to be screened. This strategy is appropriate when the screening costs are high (for example, when  $G_3$  mice must be housed for long periods of time to detect late-onset phenotypes) or when the phenotyping assay itself is expensive per mouse. In an often-encountered alternative situation, the cost of generating the mice is the primary expense, and the screening of  $G_3$  animals is relatively inexpensive; for example, many dysmorphology screens are noninvasive and require only brief observation of the mice. In such cases, the overriding criterion for efficiency is the production of the  $G_3$  mice themselves, rather than the number to be screened.

We explored 2 different strategies: (i) producing  $G_3$  mice by crossing either 1 or 2  $G_2$  females back to the  $G_1$  male parent and screening either a single  $G_3$  litter from each  $G_2$  female, with the

aim of finding at least 1 homozygous mutant or (ii) screening the number of  $G_3$  mice required to achieve a 95% probability of producing 1 homozygote for any mutation carried by the  $G_1$  parent. The ‘single litter’ approach likely is useful because it reflects the minimal time required to screen  $G_3$  mice—there is no need to wait for subsequent litters to achieve the necessary numbers of animals to screen. The unit cost is defined as per ‘cage-week’, that is, the cost to house 1 cage of mice for 1 wk. This rate is a standard accounting measure for many mouse facilities.

When 1  $G_3$  homozygote for any  $\mathbb{X}G_1$  mutation is sufficient to detect a mutant phenotype reliably, the minimal cost (in terms of cost per mutation brought to homozygosity) is achieved by mating 2  $G_2$  sisters with the  $\mathbb{X}G_1$  parent and screening a single litter from each. When at least 2 homozygous mutants are required for an individual mutation, the costs obviously are increased, but again using the  $2\mathbb{X}G_2 \times \mathbb{X}G_1$  mating strategy is more cost efficient than is the  $\mathbb{X}G_2 \times \mathbb{X}G_1$  scheme (Table 2). Therefore, whereas  $\mathbb{X}G_2 \times \mathbb{X}G_1$  matings are more efficient when screening cost is the primary cost factor,  $2\mathbb{X}G_2 \times \mathbb{X}G_1$  matings are more efficient when mouse housing and husbandry costs are primary factors. An overall summary of the optimal strategies is shown in Table 3.

## Discussion

The aim of this work was to provide some guidelines and rationale for breeding schemes and animal numbers when planning recessive ENU mutagenesis screens. In line with the 3Rs (reduction, refinement, replacement), animal breeding and use needs to be minimized while the benefit from experimental work is maximized. Our results reveal that when the  $G_3$  screening costs are a key cost factor, then backcrossing a single  $G_2$  female to the  $G_1$  parent male is the optimal strategy. When animal housing costs are a primary consideration, backcrossing 2  $G_2$  females to the  $G_1$  parent male is the optimal strategy.

These suggestions provide guidelines for developing mating and screening protocols, but there are caveats. The exact strategy and animal numbers used will depend on the number and type of phenotypes under investigation in any single pedigree. Time constraints often mean that mice must be generated as quickly as possible, and more mutations can be covered in less time by using a  $2\mathbb{X}G_2 \times \mathbb{X}G_1$  mating scheme than when backcrossing a single  $G_2$  female ( $\mathbb{X}G_2 \times \mathbb{X}G_1$ ). For example, in some inbred mouse lines, mating a single  $G_2$  with a  $G_1$  is inefficient due to the poor breeding performance of the female mice. However, in the case of mating strategies that generate hybrids between 2 inbred

**Table 2.** Cost-effectiveness of using various mating strategies and screening different numbers of  $G_3$  mice to achieve the described number of homozygous  $G_3$  animals

	At least 1 homozygous $G_3$				At least 2 homozygous $G_3$	
	Screen single litter <sup>a</sup>		Screen to $P = 0.95$		Screen to $P = 0.95$	
	$fG_2 \times mG_1$	$2fG_2 \times mG_1$	$fG_2 \times mG_1$	$2fG_2 \times mG_1$	$fG_2 \times mG_1$	$2fG_2 \times mG_1$
	1 litter	1 litter from each $fG_2$	11 offspring (2 litters)	23 offspring (5 litters)	17 offspring (4 litters)	37 offspring (8 litters)
No. of cage-weeks	78	91	93	119	107	148
Average no. of mutations homozygous <sup>b</sup>	19.1	30.3	23.8	35.6	23.8	35.6
Cost per mutation <sup>c</sup>	4.1	3.0	3.9	3.3	4.5	4.2

f, female; m, male.

<sup>a</sup>A single litter is assumed to consist of 5 mice, a typical number for C57BL/6J in our facility.

<sup>b</sup>See Materials and Methods for an explanation about estimating the average number of mutations per homozygous pedigree.

<sup>c</sup>Relative unit costs for generating at least 1 homozygous mouse for any mutation by using the mating strategies shown and by screening the number of animals indicated. A unit cost of 1 refers to the cost of maintaining a cage of mice (maximum, 5 mice) for 1 wk (that is, 1 cage-week). For this example, the cost accounting is based on keeping the  $G_3$  mice until 12 wk of age.

**Table 3.** Summary of optimal strategies for phenotype-driven recessive ENU mutagenesis screens in mice

Primary cost consideration <sup>a</sup>	Minimum no. of homozygous $G_3$ mice required <sup>b</sup>	Mating scheme	No. of $G_3$ mice screened per pedigree
Screening of $G_3$ mice	1	$\text{f}G_2 \times \text{m}G_1$	11
	2	$2\text{f}G_2 \times \text{m}G_1$	17
Housing of $G_3$ mice	1	$2\text{f}G_2 \times \text{m}G_1$	Approximately 10 (a single litter from each $\text{f}G_2$ )

<sup>a</sup>Both considerations assume that minimizing breeding and use of mice is an inherent requirement.

<sup>b</sup>Per mutation.

strains, inefficiency of breeding may not be a problem because the mice should be fairly fecund. When multiple phenotypes are being analyzed in single pedigrees (as often happens), then the mating and screening strategy required for the less robust phenotypes takes precedent when calculating efficiency.

The  $G_2 \times G_2$  micropedigree approach is suboptimal in terms of overall recovery of different mutations. Although  $G_2$  brother-sister matings typically maximize the number of mutations brought to homozygosity in any  $G_1$  mouse, there is no advantage in detecting more mutations in a single  $G_1$  mouse compared with detecting the same total number of mutations in multiple  $G_1$  mice. However, other advantages to the  $G_2 \times G_2$  approach may take precedence. Because it optimizes the total number of mutations recovered from any  $G_1$  animal, the  $G_2 \times G_2$  approach minimizes the number of  $G_1$  mice that must be produced for a large screen. A consequence of this feature is that fewer mice must be injected with ENU to generate  $G_1$  mice; because ENU treatment has adverse consequences on the health and fecundity of mice, its use should be minimized. In addition,  $G_2 \times G_2$  intercrosses reduce the reproductive pressure on the  $G_1$  male.<sup>22</sup>

We also calculated the optimal number of  $G_3$  mice to screen for various breeding strategies. In many situations the numbers we obtained will be suitable, but situations may arise in which the confidence of having additional littermates with the phenotype warrants screening more animals than we suggest here. In conclusion, the calculations we present likely will assist scientists in planning and designing phenotype-driven recessive screens. Although these screening strategies have been devised by using the mouse as a model system, they will be equally applicable to ENU recessive screens in other organisms, such as zebrafish.<sup>11,36</sup>

## Acknowledgments

Thanks to Andreas Russ (Department of Biochemistry, University of Oxford) for critical reading of the manuscript and to Adam Steinberg and the Media Lab of the University of Wisconsin for providing the clip art drawings of mice.

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