

## MARKER-ASSISTED ACCELERATED BACKCROSSING (MAX-BAX<sup>SM</sup>)

Time is of the essence when creating research models, and the use of marker-assisted accelerated backcrossing as a congenic breeding strategy has proven valuable in expediting the process. Charles River Laboratories, the industry leader in rodent research models, offers MAX-BAX<sup>SM</sup> for the directed selection of individuals/breeders with preferred genetic backgrounds. By backcrossing selectively rather than randomly, an investigator can significantly decrease the number of backcross generations needed to produce a 95-99% congenic strain, thereby producing a research model faster, while simultaneously reducing facility, equipment and personnel costs.

### MAX-BAX<sup>SM</sup> Technology

Congenic strains are widely used in biomedical research; they provide insight into the contribution of genetic background onto model phenotype. Congenic strains are identical at all genetic loci except for one and usually, for our purposes, that differing locus is the transgene or knockout region of interest. Utilizing traditional, random backcrossing methods it may take upwards of 3 years to produce a congenic strain containing 99% of the recipient genome. Selectively breeding individuals containing more of the recipient genome from each generation allows for accelerated congenic strain production (Table 1). Those animals carrying the loci of interest with the highest percentage of recipient vs. donor strain DNA are preferentially bred.

Nucleotide repeats (microsatellites) mapped to specific locations on each chromosome are used to evaluate strain-specific genomic polymorphism. PCR primer pairs are used to identify chromosomal loci as belonging to a specific strain because, in many cases, different strains produce PCR products that vary in size. Our microsatellite-based primer panels scan all autosomes of the mouse or rat genome at approximately 15 centimorgan intervals. Results yield a defined analysis of the genome in question, and a preferred breeding rank is determined for all test individuals.

As the animals are backcrossed, individual loci and eventually entire chromosomes will become fixed for the recipient allele and no longer require monitoring. This allows us to custom design a panel for every subsequent backcross generation, saving both time and money in the process.

**TABLE 1. Congenic Strain Production Strategies**

| Traditional Backcross |                  | vs. | Speed Backcross |                  |
|-----------------------|------------------|-----|-----------------|------------------|
| Generation            | Recipient Genome |     | Generation      | Recipient Genome |
| F1                    | 50.00%           |     | F1              | 50.00%           |
| N2                    | 75.00%           |     | N2              | ~80.00%          |
| N3                    | 87.50%           |     | N3              | ~94.00%          |
| N4                    | 93.75%           |     | N4              | ~99.00%          |
| N5                    | 96.88%           |     | N5              | ~100.00%         |
| N6                    | 98.44%           |     |                 |                  |
| N7                    | 99.22%           |     |                 |                  |
| N8                    | 99.61%           |     |                 |                  |
| N9                    | 99.81%           |     |                 |                  |
| N10                   | 99.90%           |     |                 |                  |

**FIGURE 1. Mouse chromosome 8 data subset analysis**

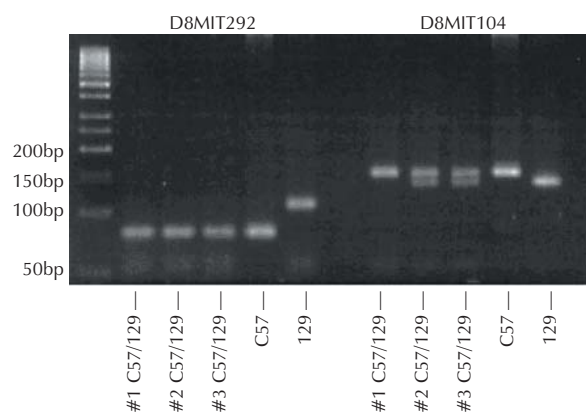


Figure 1. shows results produced by microsatellite analysis of two loci on mouse chromosome 8. In this study, a C57/129 hybrid mouse strain was backcrossed onto a pure C57BL/6 background. The three individuals represented were potential breeders from an N2 generation. A total of five loci on chromosome 8 (approximately 15 centimorgan intervals) were examined for donor vs. recipient strain DNA (data for two of the five loci, D8MIT292 and D8MIT104, are shown at left). Microsatellite data from three N2 generation littermates indicate a mixture of C57/129 loci. Analysis suggests that individual #1 has the highest percentage of C57BL/6 at the two loci examined in this dataset. If we were selecting breeders to produce the N3 generation based solely on this information, then individual #1 is the best candidate. In actuality, breeder determinations are made from an analysis carried out on multiple loci over the 19 mouse (or 20 rat) autosomes. The process of selected breeding results in the characteristic time savings of a speed congenic vs. a traditional backcross program.

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### **MAX-BAX<sup>SM</sup> General Principles**

While the absolute parameters of every MAX-BAX<sup>SM</sup> project are model dependent, we have listed some general principles that can be used as a road map to plan a MAX-BAX<sup>SM</sup> program.

1. If the initial animals are already on a defined background, the F1 progeny (of the founder animal and a mate of the recipient strain) will all be 50% recipient strain, so there is no advantage in performing a microsatellite analysis at this point. If the founder animal is homozygous for the gene of interest, the progeny are obligate heterozygotes, and genotyping for the gene of interest is also not necessary for this generation. If the initial animals are on a hybrid background, and the desired recipient strain is one of the strains contributing to this background, MAX-BAX<sup>SM</sup> genotyping of the F1 generation can identify animals with a higher-than-average percentage of the recipient strain.
2. Two to four animals having the highest percentage of recipient strain contribution, as determined by microsatellite analysis, will be mated to recipient strain animals. The aim is to produce about 10 heterozygotes for subsequent background strain assessment. These animals will be designated the N2 generation.
3. This process of selected breeding will be repeated at each generation out to N5, at which point individuals should contain greater than 99% of the recipient background.
4. The time frame, assuming no problems with breeding or health, etc. will be approximately 98 days per generation or 490 days to complete the project (1.3 years). Conventional backcrossing takes upwards of 2.5 years to produce the desired congenic strain.

### **MAX-BAX<sup>SM</sup> Program**

Each MAX-BAX<sup>SM</sup> program can be divided into two components: genetic testing and colony management. Charles River Laboratories can fulfill either or both needs giving you the flexibility to choose your level of service. Animals can be isolator-housed and bred through Charles River's Transgenic Services division, and tissue samples will be analyzed by our genetic testing laboratory. We will prescribe a breeding program for colonies housed in our facility or provide selected breeder information to those customers housing animals in their own facilities. A project specific quote will be generated at the customer's request.

### **Test Results and Sample Submission**

Results from microsatellite analysis are typically reported in two weeks. Tail snips should be collected and immersed in 70% ethanol and refrigerated (4°C) prior to shipment. Other tissue such as toe clips and ear punches may also be used. Samples should be shipped overnight on ice packs. Please contact Charles River Laboratories prior to shipment.

In addition to MAX-BAX<sup>SM</sup>, Charles River offers other services to help you genetically evaluate your animal models. These DNA-based testing services include:

- Genetic Monitoring (PCR and SNP analysis)
- Zygosity and Expression Testing
- Background Strain Characterization

**Please contact Charles River when planning your MAX-BAX<sup>SM</sup> or along with other questions regarding our genetic testing services of knockout and transgenic rodents.**

  
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1.877.CRIVER.1 • [www.criver.com](http://www.criver.com)

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