

# Accelerate your Backcross Breeding Program with MAX-BAX<sup>SM</sup>

## Marker-Assisted Accelerated Backcrossing

# THE SENTINEL

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 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 an 95-99% congenic strain, thereby producing a research model faster, while simultaneously reducing facility, equipment and personnel costs.

### MAX-BAX Technology

Congenic strains are widely used in biomedical research; they provide insight into the contribution of background strain onto model phenotype. Congenic strains are identical at all genetic loci except for one and usually, for our purposes, that differing loci is the transgene or knockout region of interest. Utilizing traditional, random backcrossing methods it may take upwards of 2.5 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.

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%			

TABLE 1. Congenic Strain Production Strategies

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 panel scans 19 chromosomes of the mouse genome at approximately 20 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 genotype 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.

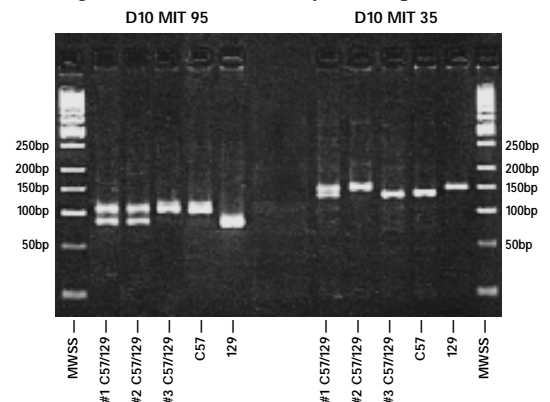
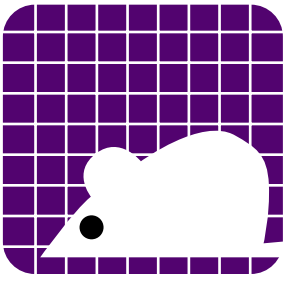


Figure 1. Chromosome #10 data subset analysis

Figure 1 shows results produced by microsatellite analysis of two loci on mouse chromosome #10. In this study, a C57/129 hybrid mouse strain was backcrossed onto a pure C57 background. The three individuals represented were potential breeders from an N2 generation. A total of four loci on chromosome 10 (approximately 20 centimorgan intervals) were examined for donor vs. recipient strain DNA (data for two of the four loci, D10 MIT 95 and D10 MIT 35, are shown above).

Microsatellite data from three N2 generation littermates indicate a mixture of C57/129 loci.

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Analysis suggests that individual number three has the highest percentage of C57 at the two loci examined in this dataset. If we were selecting N3 generation breeders based solely on this information, individual number three is the best candidate. In actuality, breeder determinations are made from analysis carried out on multiple loci over the 19 mouse autosomes. This process of selected breeding results in the hallmark timesavings of a speed congenic vs. a traditional backcross program.

### *MAX-BAX General Principles*

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

1. When beginning a project, the background strain composition of a representative sample of male heterozygotes should be assessed. The total number examined will depend on availability and the size of the population. Female heterozygotes may also be used if males are in short supply.

*Please note that preliminary background strain characterization is useful only if prior breeding could have produced animals with the potential to contain a higher percentage of the strain the investigator wishes to move onto (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 at least 10 heterozygotes for subsequent background strain assessment. These animals will be designated the N1 generation.
3. This process of selected breeding will be repeated at each generation out to N5, at which point individuals should contain greater than 98% 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 Program*

Each MAX-BAX 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.

*If you have any questions about our genetic testing services of knockout or transgenic rodents, please contact Charles River.*



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