charles river

Summary

Marker-assisted accelerated backcrossing has proven valuable in expediting the process of creating congenic strains. Charles River offers MAX-BAX for the directed selection of individuals/ breeders with a preferred genetic background.

Marker-Assisted Accelerated Backcrossing (MAX-BAX®)

MAX-BAX® Technology

Congenic strains are widely used in biomedical research because they reduce genetic variability and provide insight into the contribution of genetic background to phenotype. Congenic strains are identical at all genetic loci except for one; that differing locus is usually the transgene or knock-out region of interest. Utilizing traditional, random backcrossing methods, it takes 10 generations (upwards of 2.5 years) to produce a congenic strain. Selectively breeding individuals containing more of the recipient genome from each generation allows for accelerated congenic strain production (Table 1). Those animals carrying the locus of interest with the highest percentage of recipient versus donor strain DNA are preferentially bred.

Genetic markers mapped to specific locations on each chromosome are used to evaluate strain-specific genomic polymorphism. Our mouse single nucleotide polymorphism (SNP) panel, composed of 384 carefully selected SNP markers, was designed to maximize polymorphism between common inbred strains and provide even coverage of the mouse genome. Markers are spaced at approximately 7 Mbp intervals, and about half of the markers will be polymorphic between any inbred or outbred strains, allowing the same panel to be used for any donor and recipient strains.

The testing for the 384 SNP marker panel is performed on a microarray platform using robust fluorescence-based SNP genotyping assays (Figure 1). Results yield a defined analysis of the genome in question, and a preferred breeding rank is determined for all test individuals. The entire genome is analyzed at each generation, which may make unexpected genetic variation or breeding errors easier to detect.

The 384 SNP panel complements our other mouse and rat genetic background panels for inbred and outbred lines. These include our 32-marker SNP panels for routine genetic quality control and custom microsatellite panels for speed congenics, mapping, fine mapping, and substrain or background strain characterization.

Table 1. Congenic Strain Production Strategies

Traditional Backcross		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. SNP Genotyping Data for 2 Markers

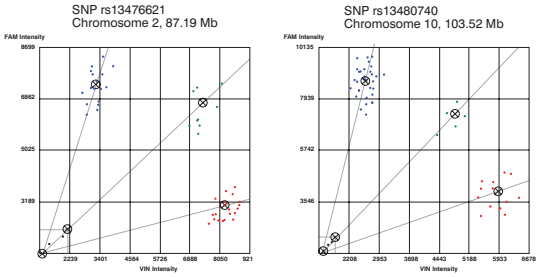

Figure 1. shows the fluorescence data for 2 SNP markers. Each point represents the genotype of one animal for that marker. Blue and red points are animals that are homozygous for one of the two possible alleles, while green points are animals that are heterozygous for both possible alleles. Each animal is assayed for 384 markers, and its genetic profile is compared to that of the recipient strain to identify animals with the highest amount of the desired background.

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.

MAX-BAX® General Principles

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

Principle 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 genetic 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 strain contributing to this background, MAX-BAX genotyping of the F1 generation can identify animals with a higher-than-average percentage of the recipient strain.

Principle 2

Two to three animals having the highest percentage of recipient strain contribution, as determined by MAX-BAX analysis, should 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.

Principle 3

This process of selected breeding will be repeated at each generation, typically to N5, at which point congenic individuals should be obtained.

Principle 4

The time frame, assuming no problems with breeding or health, 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.

Test Results and Sample Submission

Results from MAX-BAX analysis are typically reported in one week. Tail snips should be collected and frozen (-20 °C), dry (i.e., no liquid at all), and shipped overnight on dry ice. Other tissues such as ear punches or toe clips may also be used. Please contact Charles River Laboratories prior to shipment.