GVG Genetic Monitoring GmbH · Deutscher Platz 5 · 04103 Leipzig

Implementation of Speed Congenics projects

General notes

Speed Congenics, also known as "Marker assisted selection protocol" (MASP), is a method for accelerated generation of congenic mouse lines. A defined DNA locus with a modified gene (hereinafter referred to as target, e.g. knock-out, knock-in or a transgene) is transferred from a donor strain to another strain (recipient). This is done by means of a stepwise backcrossing process in which the offspring of a backcrossing generation are mated repeatedly with animals of the recipient strain. From generation to generation, the genomic share of donor DNA decreases continuously.
Within each backcrossing generation, first of all those individuals are identified which possess the modified gene. Subsequently, the chromosomes of these animals are genotyped to check for homozygous regions of recipient DNA. Thus, one or more individuals with the highest genomic proportion of the recipient strain are identified and recommended for further breeding.
The differentiation between heterozygous and homozygous (carrying recipient DNA only) status of particular chromosomal DNA regions is done with the help of 200 - 250 informative markers, which are distributed at regular intervals over all chromosomes and which distinguish between donor and recipient. Such markers are STRs (short tandem repeats, microsatellites). By selecting the respective "best" among the offspring for the generation of the next backcross generation, the total duration of backcrossing can be reduced from 10 generations with the classical method doen to 5 or6 generations. This leads to a time saving of 1-1.5 years.

In the following some essential aspects are described, which should enable an optimal implementation of Speed Congenics:

The optimal number of target-positive animals for genotyping is between 7 and 10 per generation which should be male. This number guarantees that sufficient candidates available for the selection. If less than 5 animals per generation are used, the acceleration effect achievable by Speed Congenics is reduced. The benefit of genotyping male animals only is that several female animals can be mated with one "particularly suitable" animal, thus producing a larger number of offspring. As normally every other animal is male and, also, every other animal contains the target, statistically speaking, between 28 and 40 offspring are ideal in each backcrossing generation.

A particular challenge for successful backcrossing is the chromosome on which the target is located (linked chromosome). At least two independent crossing over events in the DNA region around the target are required, one upstream and one downstream. Each crossing over should be as close as possible to the target. As practical experience shows such events occur relatively rarely. Since the concept of Speed Congenics envisages 5-6 backcrossing generations, it is quite realistic that without particular focus onto the linked chromosome, considerable amounts of donor DNA are still retained around the specific gene of interest.

For this reason, we perform a targeted selection for individuals where crossing over at the target has taken place in each backcrossing generation. For example, if in the N2 generation an animal with a crossing over is found on one side of the target, there is a chance for the detection of a second crossing over on the opposite side of the target for three subsequent backcrossing generations.
Din this case, deviating from the regular recommendation for further breeding (based on the analysis for the whole genome), we consider the individual with the closest crossing to the target. If there are several such animals, the one with the best result for the entire genome is selected.

In order to implement such a screening strategy, it is necessary that the client provides all required information: This is

- Name/number of the chromosome
- approximate position of the target on the chromosome (in MBp is usually sufficient).

In addition, information on the exact recipient strain is required. When using C57BL/6, for example, it is not sufficient to simply refer to the addition "J" or "N". Both "J" and "N" have several substrains, which in turn are genetically different such as JBomTac, JOlaHsd, JCrl, JRj, JRccHsd, NTac, NCrl, NHsd, NRj.

In some cases, the integration site of the target is not known. Using GVG's technology platform, it may be possible to identify the affected chromosome and determine the approximate position of the target on the chromosome from the N2 generation, based on the analysis of about 10 target-positive individuals. Once determined the above strategy can be employed also.

The F1 generation (N1)

The first step in generating a congenic line is the mating of a target-positive donor with an animal of the recipient strain. All animals of the resulting F1 generation contain 50% donor DNA and 50% recipient DNA. If the donor was homozygous with respect to the target to be transferred, all animals of the F1 generation contain the target and therefore do not need to be analysed. If, on the other hand, the target is present heterozygously in the donor, the animals possessing the target mutation must be determined from the F1 offspring first. Genome-wide genotyping is not yet necessary in either case.
(Note: If the aim of Speed Congenics is to backcross a mixed genetic background to a defined substrain, genotyping of those parent animals of the F1 generation that were used to produce the N2 generation is recommended. This allows to determine the actual status at the beginning of backcrossing process. The samples from the F1 parent animals can be submitted together with the samples from the N2 generation).

The N2 generation

During backcrossing, the fixation of the two sex chromosomes of the recipient strain must take place. If the donor animal is female, it will be mated with a male of the recipient strain and thus will directly produce F1 offspring with the correct Y chromosome.
Breeding mistakes often occur with respect to the Y chromosome if the first donor animal is male. For the fixation of the Y chromosome, while maintaining the optimal backcrossing speed, a mating strategy is recommended as follows and described in the figure on page 3: Ideally, several female animals of the F1 generation are mated with one or more male animals of the recipient strain for this purpose. Thus, all male individuals of the resulting N2-generation possess the correct Y-chromosome. Subsequently, male animals will be used for the backcrossing from the N2-generation onwards. Thus, the Y chromosome of the recipient strain is retained in all offspring.

Before sending samples of male animals for genotyping, it must be ensured that they carry the desired target. This pre-screening is carried out with suitable methods in the research institution (e.g. using a Snooplex kit for genotyping without biopsies of the tail tips). Sample material from target-positive animals is then sent to GVG Genetic Monitoring (biopsies of the tail tip, ear punches, blood or readily extracted DNA).
As a result of the genotyping, those 1-2 animals are identified which are best suited for further backcrossing. These are subsequently mated with female animals of the recipient strain.

The N3 generation (and following)

In the N3 generation, again male target-positive offspring are selected. The Y-chromosome comes from the parent animal of the N2-generation. In all male offspring, the X chromosome inevitably comes from the female parent of the recipient strain. From the N4 generation onwards, all animals, male and female, have the sex chromosomes of the recipient strain.

	Male donor animal			Female donor animal		
	DNA-Donor		DNA-Recipient	DNA-Donor		DNA-Recipient
F0	X Y	x	X X	X X	x	X Y
F1	X Y, X X	x	X Y	X X, X Y	x	X X
N2	X X, X X, X Y	x	X X	X X, X Y	x	X X
N3	X X, X Y	x	X X	X X, X Y	x	X X
N4	X X, X Y	x	X X	X X, X Y	x	X X

Figure: Optimal breeding scheme for fixation of the sex chromosomes of the recipient strain depending on the sex of the donor animal.

Generation N6 and subsequent

After successful completion of backcrossing, it is occasionally desired to further reduce the amount of donor DNA around the target. GVG Genetic Monitoring offers to search for additional informative markers not included in the standard panel on a project-specific basis. These allows a more detailed statement regarding the amount of remaining donor DNA around the mutation of interest on the linked chromosome. In this case, the backcrossing can be continued for 1-2 generations and the amount of donor DNA can be further reduced by crossing overs closer to the target mutation. Since all other chromosomes already contain recipient DNA only, just the linked chromosome needs to be analysed.

For the search for informative markers in the target area, it is helpful to utilize DNA samples of the F1 or N2 generation. Therefore, as a precautionary measure, some suitable retention samples should be preserved for the project.

If no further improvement of the situation in the target region is foreseen, target positive siblings are mated together and from the offspring those are selected which are homozygous with respect to the target. Statistically, this is every 4th animal.

Creation of double mutants

After successful backcrossing of the target, the animals can also be mated with other genetically modified mouse lines to create double mutants. Care should be taken to ensure that a line of the identical substrain is chosen. Only in this case, the resulting offspring have a uniform and defined genetic background. Otherwise, animals with a mixed genetic background will be generated and the previous effort in the speed congenics project will be useless.
It is again pointed out that when employing C57BL/6, for example, it is not sufficient to pay attention only to the suffix "J" or "N". Both "J" and "N" strains are produced by several commercial breeders which leads by definition to specific substrains which differ significantly from each other (JBomTac, JOlaHsd, JCrl, JRj, JRccHsd, NTac, NCrl, NHsd, NRj).