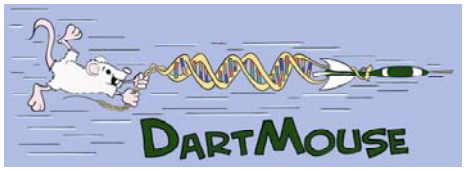

The Mouse Speed Congenic Core Facility at the Geisel School of Medicine at Dartmouth

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CREATING A SPEED CONGENIC LINE USING DARTMOUSE™

Speed Congenic mouse lines are created by introgressing genomic intervals (typically containing a modified gene such as a knockout, knockin, or transgene) from one strain (donor strain) to another (recipient strain) using a *marker assisted breeding strategy*. This is achieved by sequentially back-crossing donor strain mice containing your genetic region of interest to mice of the recipient strain. At each generation, multiple offspring mice are analyzed at the genomic DNA level, using single nucleotide polymorphisms (SNPs) spread throughout the genome, so as to select the "best" mouse (i.e. most similar to the recipient strain) to breed for the next generation, accelerating the development of the congenic mouse line. This is the basis for the ***SPEED*** aspect of the Speed Congenic approach.

To maximize the speed and efficiency with which you can develop your Congenic strain, DartMouse recommends sending us samples from **male** mice that are **carriers** (i.e. heterozygotes) of your gene/region of interest.[1] Since you can cycle-breed males (or place multiple females with each male) selecting males to breed will allow you to generate the desired number of pups at each generation, since the optimal number for us to screen at each generation is **10 male carriers,** as we explain in more detail below. This means you should plan to generate at least **40 pups at each generation** after the N1 generation (see below), a number arrived at by keeping in mind the following considerations: approximately 50% of pups born will be males, and approximately 50% will be carriers of your gene of interest. Therefore, about 25% of mice raised in your colony will be both **males** as well as **carriers** (heterozygotes) of the modified allele of your gene locus of interest. This is a theoretical calculation, of course, and real-world numbers will vary. Therefore, sometimes you will generate fewer than 10 carriers and sometimes greater. Keep in mind that the fewer mice that DartMouse screens per generation, the lower the benefit of the Speed Congenic approach. Screening four or fewer mice per generation is generally not recommended. Conversely, the cost-benefit ratio of screening greater than 11 male carriers begins to increase significantly, markedly reducing the **cost-effectiveness** of the Speed Congenic approach, so we do not advise this for our clients. Thus, we recommend a "sweet spot" target of 10 male carriers.

The N1 generation

The first step in creating a speed congenic line is to generate a first filial (F1) generation (also termed the first or "N1" generation) by crossing a donor strain carrier mouse (the *founder* mouse) to pure inbred mice of the recipient strain. This F1 generation usually does not need to be further analyzed by DartMouse™, quite simply because each offspring that is a carrier of your gene/allele of interest is otherwise genetically identical, with 50% donor strain genetic material and 50% recipient strain genetic material. If your founder mouse is heterozygous for your

[1] This assumes that your gene/region is located on one of the autosomes (chr. 1 through 19) and not on the X or Y chromosome; introgression of sex-linked genes requires a slightly different strategy than outlines above – call us if this is the case.

DartMouse™ is a not-for-profit core facility funded by the NIH's National Center for Research Resources. The mission of DartMouse is to facilitate the development of congenic mice in support of pre-clinical projects across the United States. Support for DartMouse is from: NIH - NCRR, COBRE, the Geisel School of Medicine at Dartmouth, Norris Cotton Cancer Center, ARRA.

gene/allele of interest, your lab will need to screen males from one or two litters (typically via a PCR assay done in your lab) so as to identify at least two F1 generation males that carry (i.e. are heterozygous for) your gene/allele of interest. If your founder mouse is homozygous for your gene/allele of interest, you don't need to screen any F1 mice, since by definition each one will be heterozygous.

Keep in mind, however, that you will need to have a working assay in your lab so as to be able to identify carrier (heterozygous) mice at subsequent (N2 and higher) generations.

Also, it is recommended that you snip and store (at -20° C) a ~1 cm piece of mouse tail from all founder mice, recipient mice, and F1 mice, as DartMouse may later request that you send them to us for analysis, to allow us to most accurately interpret SNP data from subsequent generations.

Select two F1 male carriers and breed each with three females of the recipient strain, to create the N2 generation. Each F1 male carrier mouse should be placed into a cage with three females of the recipient strain (two cages total: each F1 breeder male with three females in one cage), and then the male is removed after 2 weeks. It is also recommended that at that time you place each pregnant female into her own separate cage, to allow for maximum pup yield and to avoid overcrowding of cages. You should be able to produce ~40 or more N2 pups using this strategy.

The N2 generation

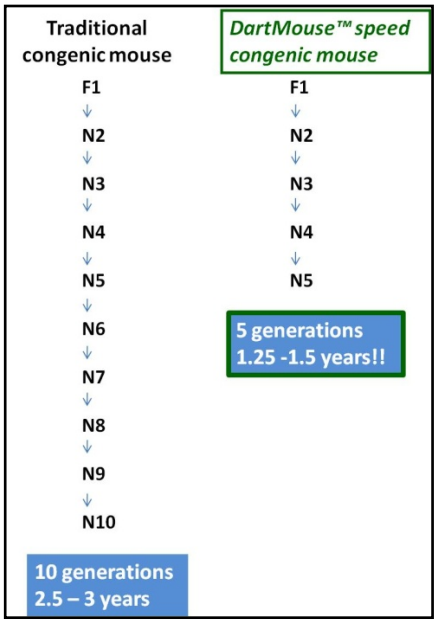
From the N2 generation litters, screen males in your own lab to determine which ones are carriers (heterozygotes) of your gene/allele of interest. Typically, up to 11 of these carrier males will then be screened by DartMouse, in order to select the very "best" two to breed at the next generation. For our purposes, we request ~1 cm of tail clipping from each mouse, placed in a well-labeled container (such as a microcentrifuge tube), and sent to the lab overnight on either wet or dry ice. If you have a larger number of carrier males (e.g 12-16), please send samples from all. We will ordinarily screen no more than 11 of these (we will select 11 at random, unless you specifically tell us otherwise). Our chip has an 11-genome capacity, so we generally do not screen additional samples, unless we happen to have a few open "spots" on another chip. If this is the case, we will screen as many as possible, but typically no more than 16. When beginning a project, please keep in mind that we may wish to analyze additional control mice (e.g. founder mice, F1 mice, etc.) in order to allow us interpret your SNP data as accurately as possible. (This would be the case if, for example, your founder mouse is not on a complete inbred background). This may mean that at the N2 generation, we may analyze slightly fewer than 11 N2 samples.

DartMouse's analysis and report are typically completed within **two weeks** of receipt of your samples. Our report comes complete with easily interpreted graphical data display, as well as our expert analysis of the raw data. For most clients, the most important features of our report are the **rank-order** of the male carrier mice analyzed, and our **recommendation** for which mice to breed for the next generation. We typically recommend using the "top two" mice from the N2 generation as breeder mice for the next (N3) generation. **Two** mice are selected to breed, in case one proves to be a poor breeder or falls ill. Each N2 male carrier mouse thus selected

should be placed into a cage with three females of the recipient strain (two cages total: each N2 breeder male with three females in one cage), then removed after 2 weeks. It is also recommended that at that time you place each pregnant female into her own separate cage, to allow for maximum pup yield and to avoid overcrowding of cages.

The N3 (and higher) generations

- Upon birth of the N3 generation litters, again screen male pups for your gene/allele of interest. Again, select 10 males that are carriers of your gene of interest, and send tail clippings to DartMouse for screening. Follow the same strategy as outlined for the N2 generation, above.
- Repeat this procedure at each generation, until your recipient strain is present at greater than 99%, typically achieved at either the N5 generation or the N6 generation.



After you have generated a Speed Congenic mouse line

At this stage, DartMouse screening is no longer needed, and you can proceed with your specific experiments, or with additional breeding, such as interbreeding heterozygotes to generate homozygotes, or crossing with other genetically-modified mice. Keep in mind that these "other" mice should also be on the desired recipient genetic background, so as not to undermine all the back-crossing work you have just completed! DartMouse can easily perform genetic "back-ground" checks to help reassure you that these "other" mice are indeed on the desired genetic background.

Following these recommendations should allow you to produce a congenic line in approximately half the time of traditional backcrossing (see figure).

We request that you fill out our accession form at each generation being screened – please email the lab if you need an additional copy of this form.

Please let us know if you have any questions about any of the information in this document. Thank you for working with us!

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