Outcrossing and phenotypic analysis of a hlh-25 mutant

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Abstract

HLH proteins are transcription factors: proteins responsible for regulating the expression of other genes. In C. Elegans, HLH-25 is one of six members in a subfamily of HLH proteins known at the ref-1 family. This particular family is made up of transcription factors that are regulated by Notch signaling. Notch signaling consists of a Notch protein which regulates the expressing of the ref-1 family. Little is known about the biological role of this family and so our lab is working to determine the phenotypic roles of this gene. My project works specifically with the phenotype of a reduced brood size in hlh-25 mutant C. Elegans. The purpose of this project is to outcross homozygous hlh-25 mutants and then to use the outcrossed worms in egg laying assays to determine the phenotypic effects of the hlh-25 deletion. Outcrossing means simply to mate mutants with wild type animals and to select offspring that have only the mutation of interest. This selection is made through PCR. The purpose of PCR is to screen for the mutation in the progeny from the outcrosses. The hlh-25 mutants originally obtained from CGC were created through a random mutagenesis so there is no way to tell if they have mutations other than the hlh-25 deletion. So to make sure they only have the mutation of interest I will outcross them by mating homozygous hlh-25 mutant hermaphrodites with wild type males until any other mutations have been bred out. Then the outcrossed strain will be used in phenotypic egg laying assays. To do this, I propose to count eggs laid by 12 worms from the outcrossed strain and then the averages compared with hlh-25 mutants that had not been outcrossed and with wild type animals. I expect to see that the outcrossed hlh-25 mutants will have a higher embryonic lethality rate than the wild type or the unoutcrossed mutants.