

An Attempt to Develop Homologous Gene Targeting in *C. elegans* Using Positive/Negative Selection and a Strain Deficient in Nonhomologous End-joining

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We are developing a method to carry out gene targeting by homologous recombination in *C. elegans* by using the worm's endogenous DNA repair and recombination machinery to replace a selected region of genomic DNA with any desired exogenous DNA. Double-strand breaks are repaired by two main recombination pathways in eukaryotes, homologous recombination (HR) which requires DNA sequence homology and non-homologous end-joining (NHEJ) which does not. Genomic integration occurs predominately by the HR pathway in *Saccharomyces cerevisiae*, but by NHEJ in other organisms, including *C. elegans*. A new approach to obtain homologous integrants has been recently reported for the filamentous fungus *Neurospora crassa*. In strains with deletions in KU70 or KU80 (components of the *Neurospora* NHEJ pathway) homologous integration events increased from 10% to 100% and nonhomologous integrations were eliminated (Y. Ninomiya *et al.*, PNAS **101**, 12248-53, 2004).

Our method for homologous gene targeting will use a *C. elegans* strain containing a deletion in the KU80 homologue, *cku-80(ok861)*, together with an extrachromosomal array containing a single transgenic donor construct comprised of two regions of homology to the targeted genomic locus separated by a positive selectable marker and containing a negative selectable marker distal to one of the homologous regions. For positive selection of genomic integration, we are using the *hsp16-2* promoter driving expression of the neomycin resistance ORF (kindly provided by Jesse Slone and Helen Chamberlin). To select against retention of the extrachromosomal array, we are using a transgene with the *unc-54* promoter driving expression of *avr-15* (kindly provided by Joe Dent) that confers sensitivity to ivermectin (paralysis) in a strain containing *avr-14(ad1302); avr-15(ad1051) glc-1(pk54)*.

We are testing the efficiency of this homologous recombination system by targeting *ced-3* for deletion in a strain containing an integrated *pkd-2::gfp* reporter, which expresses in the CEM neurons. Deletion of *ced-3* results in the failure of the male-specific CEM neurons to undergo programmed cell death in hermaphrodites. Following ivermectin treatment to select for loss of the extrachromosomal array and neomycin treatment to select for genomic integration, the *pkd-2::gfp* reporter will enable us to efficiently screen for homologous integration at the *ced-3* locus by scoring for GFP-positive CEM neurons in hermaphrodites. In addition to generating gene deletions, we hope to use this system of homologous recombination for the precise insertion of exogenous DNA into the *C. elegans* genome.

Poster

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