SUPPLEMENTAL MATERIALS

Molecular identification and analysis of the *spe-38* gene

The *eb44* mutation was mapped to linkage group I between the markers *dpy-5* and *unc-75*. The same two markers were used for two and three factor mapping and this localized *eb44* within a one map unit interval on the right arm of LG I (Table 1S, Fig. 6A). Three genes associated with fertility defects (*stu-10, sqv-5*, and *spe-9*) had been previously mapped to this region, but complementation analysis indicated that the *eb44* was not an allele of any of these genes (Table 1S). *eb44* was thus considered to define the *spe-38* gene. *eb44* also complemented two deficiencies in the region, *qDf7* and *hDf1.* This *qDf7* result was surprising since *qDf7* was thought to span the *spe-38* region, and it suggests that *qDf7*, like many other *C. elegans* deficiencies, is molecularly complex (Kadandale and Singson unpublished observations). Single nucleotide polymorphisms that generated restriction fragment length polymorphisms (SNIP-SNPs) between N2 and Hawaiian (H) strains of worms were used to further position *spe-38* on the physical map. N2/H hybrids were generated by crossing *spe-38(eb44)*; *dpy-5(e61)* and *spe-38(eb44)*; *unc-75(e950)* homozygous hermaphrodites to wild type Hawaiian males. Recombinant offspring from the hybrid worms (i.e. Dpy Non-Spe or Unc Non-Spe) were isolated and lines were established. Worm lysates were prepared for 41 such individual lines and SNP analysis was done by PCR amplification using specific primers in the region of the SNP followed by restriction digestion using specific enzymes. Data from five SNIP-SNPs (see Table 1S) effectively positioned *spe-38* between the two cosmids, F49D11 and W02B9 (Fig. 6A). This region of approximately 125 kb contains at least 14 predicted genes. After sequencing PCR products from this sub-region, we identified three new SNPs (Y52B11 SNP1-3) that can only be detected by sequencing. Using these new SNPs, analysis of several Dpy Non-Spe and Unc Non-Spe recombinants localized *spe-38* to a small region on the Yeast Artificial Chromosome (YAC) Y52B11A that contains only three predicted genes, Y52B11A.1, Y52B11A.2 and Y52B11A.3.

For the transgenic studies, PCR products were co-injected with the *myo-3::gfp* selectable marker (pPD118.20 Fire Lab Vector Kit). PCR products corresponding to the Y52B11A.1 and Y52B11A.2 genes were generated using the following primers:

3’A.1 (5’-CGATTATTGCCGTATTGCGTGTTCT-3’)

5’A.1 (5’-ACTTTCTGACTCCACGTGCGACTAC-3’)

5’A.1+ (5’-GCTATTACCATCACATTATCCGCTTTC-3’)

3’A.2 (5’-GCACACGTAGGGAGTTTAAAATTGA-3’)

5’A.2 (5’-CGCTAGGTGAGGCTCAGAGACTAC-3’)

To sequence the Y52B11A.1 gene from *spe-38(eb44)* the following primers were used to generate appropriate PCR products:

P1 (5’- TGTGTACATTATCACAATCACGATTTGG-3’)

P2 (5’-GGCGGAAAATTTGAGAAAATCTGA-3’)

Sequencing confirmed that the eb44 mutation was a deletion of 270 base pairs from nucleotide position 1:10975258 to nucleotide position 1:10975528. In place of the missing sequence were 17 bases with the sequence GCCCTTTCAACCCATTT.