Analysis of *mig-10*, a Gene Involved in Nervous System Development in *Caenorhabditis elegans*

By

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Abstract

The *mig-10* gene in *C. elegans* is required for proper axon guidance and/or cell migration of certain neurons during development. In *mig-10 (ct41)* mutant worms, there is incomplete migration of the anterior lateral microtubule cells (ALMs), hermaphrodite specific neuron (HSN), left coelomocyte cells (ccL), and canal associated neuron (CAN) (Manser and Wood, 1990). The *mig-10 (ct41)* mutation also causes axon guidance defects in the IL2 neurons, and it enhances *unc-6* defects in the axon guidance of the anterior ventral microtubule cell (AVM) (Rusiecki, 1999; C. Quinn, personal communication).

mig-10's function in axon guidance and neuronal migration is unknown, but is believed to be involved in a signal transduction pathway that uses a G-protein, such as ras. The two *mig-10* transcripts discussed in this thesis, *mig-10 A* and *mig-10 B,* encode proteins that are similar to Grb-7 and Grb-10 proteins, which are also believed to function in a signal transduction pathway (Manser *et al.,* 1997). One of these similarities is the presence of a proline-rich region, which may be used to bind another protein (Manser *et al.,* 1997). The MIG-10 A protein has an additional proline region, compared to MIG-10 B, which may indicate that the MIG-10 A and B proteins are utilized in different cells, or at different developmental stages.

As a first step in learning where MIG-10 is expressed, *mig-10 (ct41)* mutant worms containing a wild-type *mig-10 B::*GFP fusion were constructed. Rescue of the mutant phenotype would indicate that the expression pattern of the transgene was similar to that of the endogenous gene. As this experiment did not allow for rescue, even after integration of the construct, a strain of worms containing a *mig-10* promoter::GFP transgene was used. Preliminary observations of this strain indicated that *mig-10* is expressed in neuronal tissue.

The AIY neurons were observed in wild-type and *mig-10 (ct41)* worms to determine if they are affected by the *mig-10* mutation as previously reported (O. Hobert, personal communication). As no difference was detected, the AIYs were not used in any further experiments.

In order to determine which cells require functional MIG-10 protein for the proper development/migration of neurons to occur, *mig-10 (ct41)* worms containing *mec-3* promoter::*mig-10 A* or *B* cDNA transgenes were constructed. The *mec-3* promoter drives expression of the *mig-10* cDNA in the ALM neurons and other touch cells early in the development of the embryo. If these transgenes rescued the ALM migration defect, then *mig-10* would be acting cell autonomously in ALM. Partial rescue was obtained, which may be due to the need for both of the *mig-10* transcripts to be expressed in the same cell; alternatively, one or both transcripts may need to be expressed in a cell nonautonomous fashion in addition to being expressed cell autonomously. Low production of the rescuing protein, or expression of the protein at a later developmental stage than is needed for rescue to occur, may also have been the cause of the partial rescue.

Future work in this area includes putting *mig-10* promoter::*mig-10 A* or *B* cDNA in *mig-10 (ct41)* background to investigate if the different transcripts rescue different aspects of the *mig-10* phenotype. The *mig-10 A* and *mig-10 B* cDNA constructs could also be expressed in the same worm in an attempt to correct for partial rescue that may be due to the lack of both MIG-10 proteins.

"It is better to trust in the LORD than to put confidence in man." – Psalms 118: 8

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Introduction

During the development of an organism, the formation of neuronal connections is made possible, in part, by various molecular cues within the environment of the developing neurons. This process, in which axons are guided along a specific pathway to a final destination through their response to a molecule in the environment, is known as axon guidance. At the developing end of the axon is the growth cone, the part of the axon that receives ligands (molecular cues), and responds appropriately, guiding the axon in a specific direction (fig. 1). While some molecules are responsible for attracting neurons, others repel neurons. Netrins, for example, may repel or attract neurons. Ephrins, semaphorins, and slits may serve as adhesive or attractive molecules, but are often repellants. In addition to axon guidance, these molecules, or ligands, aid various cells in morphogenesis and cell migration (Yu and Bargmann, 2001).

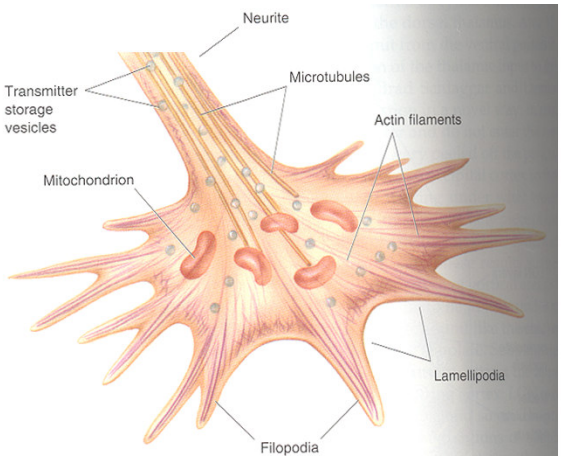


Figure 1: **Illustration of a Neuronal Growth Cone.** Bear, Connors, and Paradiso (2001)

When ligands interact with their receptors, the growth cone will respond as a result of a signaling cascade. Receptor signaling alters the growth cone's stability and initiates its change into an axon shaft through the conversion of the growth cone's actin cytoskeleton (Yu and Bargmann, 2001).

Mechanisms of Axon Guidance

The goal of this project was to elucidate the role of the *mig-10* gene in cell migration and axon guidance during the development of the nervous system in *C. elegans*. *mig-10* is thought to be involved in a signal transduction pathway with a member of the G-protein superfamily that includes both ras and rho-like GTPases. The Rho family of GTPases such as Cdc42, Rac, and RhoA are part of the signaling cascade for some guidance receptors (Patel and Van Vactor, 2002). Rho GTPases are activated when guanine nucleotide exchange factors (GEFs) support the exchange of GDP for GTP. These GTPases are inhibited when GTPase activating proteins (GAPs) promote GTP hydrolysis. The extension of the growth cone's filopodia and lamellipodia are made possible through the activation of Cdc42 and Rac, respectively (Patel and Van Vactor, 2002). The creation of stress fibres, as well as the contractibility of actinomysin occurs through RhoA, allowing for the growth cone to collapse and be repelled (Patel and Van Vactor, 2002).

An example of how GTPases are involved in signal transduction can be seen in the interaction between RhoA and the Eph receptor. When an ephrin ligand binds to the extracellular portion of an EphA4 receptor, the intracellular portion of the receptor - which contains a tyrosine kinase domain - activates ephexin. Ephexin then activates RhoA. Ephexin is an Eph-interacting exchange factor; more specifically, it is a GEF that interacts between the cytoplasmic domain of the EphA4 receptor and Rho GTPases. As ephexin activates RhoA by exchanging GDP for GTP, it also reduces the stimulation of Cdc42 and Rac1. Thus, ephexin is responsible for the collapse

of the growth cone. Without any stimulation, ephexin is bound to EphA4 and is able to allow for the growth cone's extension by activating Cdc42 and Rac1 (Patel and Van Vactor, 2002).

Alternative splicing can also help to regulate a response to a guidance cue. Eph receptor signaling typically results in a repelling response; however, alternative splicing can result in an EphA7 without a tyrosine kinase domain; this truncated protein prevents the full-length EphA7 from being phosphorylated. The lack of this catalytic activity allows for adhesion, rather than repulsion (Yu and Bargmann, 2001).

Axon guidance can be affected by the presence of more than one receptor. For example, the combination of various receptors can allow for more specific ligand-binding and alter the growth cone's response. A receptor can also suppress another receptor. Another possible silencing mechanism is for a signaling molecule downstream of the receptors to silence another downstream molecule. As an example, the chemokine SDF-1 ordinarilly activates the CXCR4 receptor. When the transmembrane protein ephrin B binds to the receptor CXCR4, a G-protein, which is bound to CXCR4, is activated. If ephrin B, which is downstream of CXCR4, receives a signal from its receptor, eph-B, ephrin B will bind to a regulator of G-protein signaling (RGS) instead of the CXCR4 receptor. This inactivates the G-protein. This inactivation will keep the granular cell, in which these receptors are found, from being guided towards SDF-1 (Yu and Bargmann, 2001).

As the location of molecular cues depends partially on the location of the cells from which they originate, the guidance or migration of some cells depend on the location of other cells. For example, the hermaphrodite specific neuron (HSN) migrates from the tail of the *C. elegans* embryo to a more anterior location near the canal associated neuron (CAN) (fig. 2A). If the CAN does not migrate posteriorly from its original location in the head of the embryo, HSN

will be found further anterior. Thus, the migration of HSN depends on some molecular cue from CAN (Forrester and Garriga, 1997).

mig-10 **Gene**

In order to gain a better understanding of the nervous system's development, the genes that regulate this development must be studied. One such gene is the *mig-10* gene in *C. elegans*, which is required for the proper migration and/or axon guidance of certain neurons (Manser and Wood, 1990).

Some of the neurons in *mig-10 (ct41)* mutant animals are unable to complete their normal migrations (fig. 2B). Ninety-four percent of the anterior lateral microtubule cells (ALMs) and 80% of the hermaphrodite specific neurons (HSN) display an incomplete migration in *mig-10 (ct41)* mutant worms. Other affected neurons displaying partial penetrance include left coelomocyte cells (ccLs) and canal-associated neurons (CANs); only 73% of the ccLs and 61% of the CANs experience an incomplete migration (Manser and Wood, 1990). As the mutation affects neuronal migration in more than one direction (anteriorward and posteriorward), the *mig-10 (ct41)* mutation probably affects some mechanism in cell migration rather than a directional signal to which the migrating cells react (Manser *et al*., 1997).

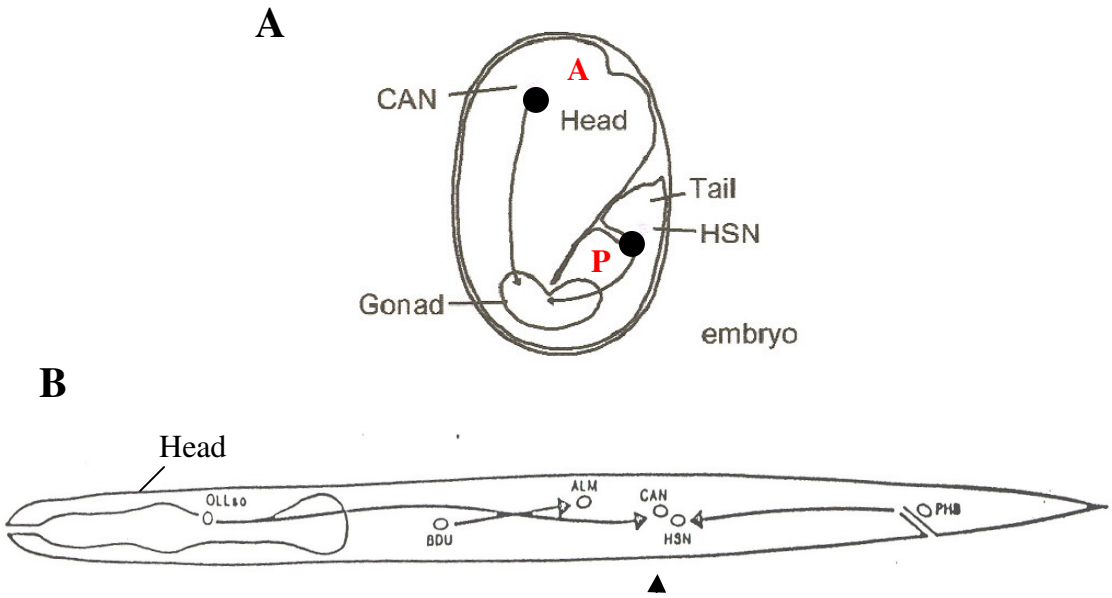


Figure 2: **Schematic of Embryonic Neuronal Migrations in *C. elegans*.** A) CAN neuron migrates posterior, HSN anterior. A, anterior region of embryo; P, posterior region. From Forrester and Garriga (1997). B) ALM, CAN, and HSN migrations projected onto L1 larvae. ▲ , area where vulva will develop. From Manser and Wood (1990).

In addition to neuronal migration, the *mig-10* mutant gene affects axons. The IL2 axons in *mig-10 (ct41)* animals don't always form a loop at the nerve ring, as they should (Rusiecki, 1999). The *mig-10* mutation also appears to enhance an axon guidance defect of AVM neurons in *unc-6* animals (C. Quinn, personal communication).

The only phenotype known to be completely penetrant within the *mig-10 (ct41)* mutant is a foreshortening of the processes of the excretory cells. Rather than extending almost to the posterior end of the worm, the cells in mutant worms come to an end near or anterior to the vulva (Manser *et al*., 1997).

Though the excretory cells are affected by the *mig-10 (ct41)* mutation, the wild-type phenotype of these cells cannot be rescued by expressing the wild-type gene only in these cells. In addition, the wild-type phenotype can be rescued by expression only in other cells (Manser *et al*., 1997). This indicates that, with regard to the excretory cells, the *mig-10* gene is cell

nonautonomous. As cells affected by the *mig-10 (ct41)* mutation tend to be located in the vicinity of the epidermal basal lamina, the *mig-10* gene may be expressed in epidermal tissue (Manser *et al*., 1997).

Two protein isoforms, MIG-10 A and MIG-10 B, can be produced by the *mig-10* gene (fig. 3). Protein predictions from the cDNA indicate that six hundred twenty-nine amino acids from the carboxy-terminal of the MIG-10 B protein are also found in the MIG-10 A protein (Manser *et al*., 1997). MIG-10 B consists of 650 amino acids, encoded in exons 1b through 9. MIG-10 A consists of 667 amino acids, encoded in exons 1a through 9 (Manser *et al*., 1997).

In the *mig-10 (ct41)* allele, a base pair change, resulting in an amber stop codon, occurs within exon 3 of the *mig-10* gene (fig. 3). This termination in the reading frame should result in the severe truncation of the MIG-10 proteins and is very likely a null mutation (Manser *et al*., 1997). Genetic support for *mig-10 (ct41)* being a null comes from experiments comparing homozygous and hemizygous *mig-10 (ct41)* mutant worms for certain phenotypes, including egg-laying defective (Egl) and withered tail phenotype, locations of migratory nuclei in L1s, and the ability of adult worms to live. No major differences were observed between the two genotypes; thus, the *mig-10 (ct41)* mutation likely results in a completely, or mostly, nonfunctional *mig-10* gene (Manser and Wood, 1990).

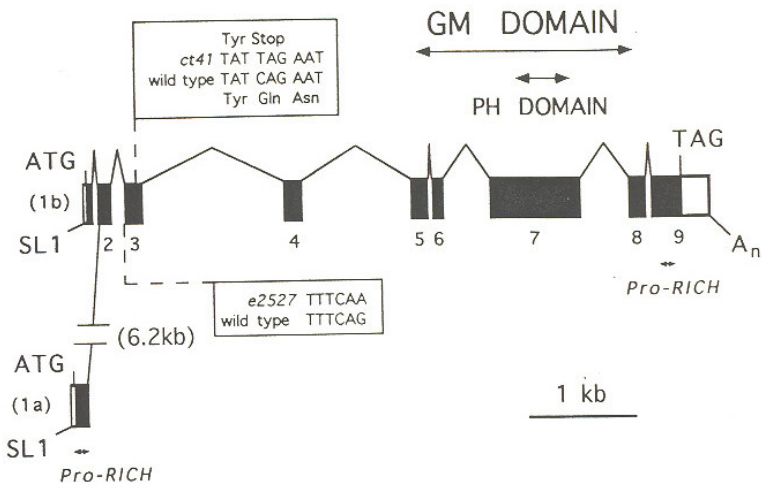


Figure 3: **Map of *mig-10 A* & *mig-10 B*.** *mig-10 B* (top) has an alternative first exon, which is downstream of *mig-10 A*'s first exon (bottom). Also displayed is the Grb-Mig (GM) domain, and the Pleckstrin Homology (PH) domain. The *mig-10 (ct41)* allele is the result of a nonsense mutation in the third exon, as shown here. Proline-rich regions are also found at both ends of MIG-10, with an additional proline region in MIG-10 A. Schematic taken from Manser *et al.,* (1997).

The predicted MIG-10 protein shares similarities with Grb 7 and Grb 10, members of SH2 domain proteins that are believed to function in signal transduction pathways (fig. 4) (Manser *et al*., 1997). The GM (Grb and Mig) regions of these proteins contain the most similarities. The Pleckstrin Homology (PH) domains, found within the GM region, are about 33% identical between Grb 7 and *mig-10*. Between *mig-10* and Grb 10, the PH domains are about 36% identical. These domains are involved in interactions between molecules such as protein-lipid and protein-protein. It has been suggested that the PH domains might aid in steering the proteins to the membranes (Manser *et al*., 1997). While the function of the PH domain is uncertain, it has been found in several proteins involved in cytoskeletal activity or cell-signaling. These domains contain a structure comparable to certain phosphotyrosine binding (PTB) domains (Manser *et al.*, 1997).

In addition to the PH region, the GM domains of the Grb and MIG-10 proteins contain a ras-associating domain (fig. 4) (Wojcik *et al.,* 1999). Ras is a member of the G protein family that is found in many signal transduction pathways known to moderate axon guidance and cell migration (Forrester and Garriga, 1997).

The MIG-10 and Grb proteins both contain regions rich in proline (fig. 4, black stars). These regions are located in the amino termini within Grb 7 and Grb 10. In the MIG-10 proteins, they are located in both termini. These regions may be involved in protein-protein interactions (fig. 4) (Manser *et al*., 1997).

The SH2 region in Grb 7 and Grb 10 proteins interacts with a phosphotyrosine that is part of a tyrosine kinase receptor (De Vet *et al.*, 2003; Giovannone *et al.*, 2003). The ras-associating domain in the Grb proteins allows Grb to link the tyrosine kinase receptor to ras.

MIG-10 proteins don't have an SH2 domain, and thus, cannot directly bind phosphotyrosine (fig. 4) (Manser *et al*., 1997). The proline-rich regions of MIG-10 contain a PXXP consensus sequence, which is necessary for the binding of a protein to an SH3 protein domain; SH3-containing proteins are found in signal transduction pathways (Manser *et al*., 1997). An example of such a protein is Grb-2, which contains two SH3 domains and an SH2 region (Gomperts, 2003).

A potential model of MIG-10's function is seen in figure 5. A protein, like Grb-2, may act as an adaptor, linking a phosphotyrosine (through an SH2 domain) to MIG-10 (through an SH3-proline interaction), which can then interact with ras.

The MIG-10 A isoform may be expressed at another developmental stage, used in different cells, or utilized in a different manner from the MIG-10 B isoform, as MIG-10 A has an extra proline-rich region. This additional proline region not found in MIG-10 B may allow for the binding of more, or different, SH3 domain proteins.

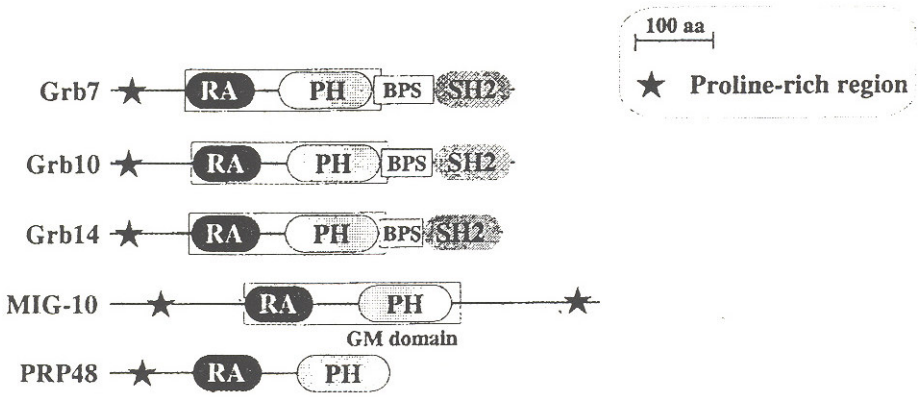


Figure 4: **Comparison of MIG-10 to Grb 7 & Grb 10.** In addition to the PH domain (white in diagram), a considerable amount of similarity can be found within the Ras associating domains (RA, black in diagram). This region, which is part of the GM domain of the Grb and MIG-10 proteins, is amino-terminal to the PH domain. Between MIG-10 and Grb-7, these domains are about 29% identical. These domains are about 34% identical between MIG-10 and Grb-10 (Manser *et al*., 1997). BPS, Between Pleckstrin Homology; PH, plextrin homology domain; GM, Grb and MIG; SH2, src homology domain-2. Illustration from Wojcik *et al.* (1999).

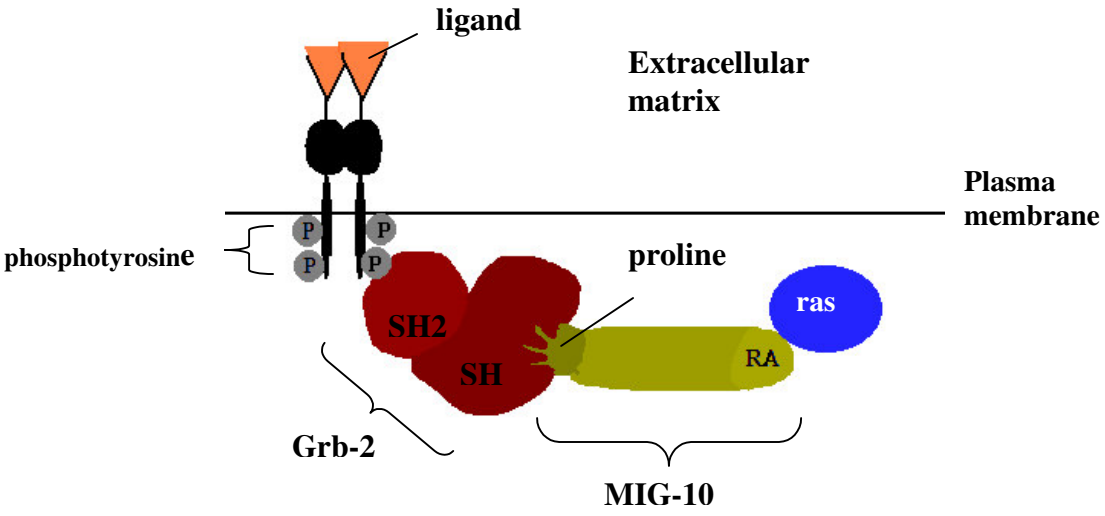


Figure 5: **Proposed Model of Signal Transduction Pathway with MIG-10 and Grb-2.** The Grb-2 protein, consisting of SH2 and SH3 domains, links the proline region of MIG-10 with the phosphotyrosine of an activated tyrosine kinase receptor. MIG-10's ras -associating domain (RA) interacts with ras.

Project Goals

MIG-10 is predicted to be a cytoplasmic protein with domains involved in signal transduction. In the simplest model, such a molecule would be expected to be cell autonomous. As previously mentioned, however, *mig-10* is cell nonautonomous to the excretory canal (Manser *et al*., 1997). One part of this project was to determine how *mig-10* works with regard to neurons – if, for example, *mig-10* is also cell nonautonomous to neurons. Observing the expression pattern of *mig-10* in *C. elegans* might help to resolve this matter. Thus, one goal of this project was to construct a strain containing a *mig-10* promoter followed by *mig-10* genomic DNA and GFP, in order to determine what cell types express *mig-10.*

While *mig-10* is cell nonautonomous to the excretory canal, it's possible that it could be cell autonomous to the neurons that it affects. To investigate this possibility for the ALM neuronal migration, constructs were designed to express wild-type *mig-10* early in the ALMs of *mig-10* mutant worms. Transgenic strains containing these constructs were examined for rescue.

Methods

DiO Staining of IL2 Neurons

Fifty millimolar calcium acetate (CA) was used to pipette a growing worm culture from a plate into a 1.5 mL microfuge tube. The supernatant was pipetted from the tube after the worms settled to the bottom, which took about five minutes. The worms were rinsed with 50 mM calcium acetate. After the worms settled in the tube, and the supernatant was taken off a second time, one milliliter of 10 µg/ml DiO in 50 mM CA was added. The tubes were rotated for two hours on a Labquake® Shaker; after which, the worms were allowed to settle. Distilled water was used to rinse the worms twice. The worms were transferred to a plate with *E. coli* for anywhere between 30 minutes and overnight, and were stored in a dark area. Worms were then washed off the plate with M9 solution, and transferred to a microfuge tube, where they settled to the bottom. The M9 solution consisted of 22 mM KH_2PO_4, 42 mM Na_2HPO_4, 86 mM NaCl, and 1 mM $MgSO_4$.

As the worms were settling, microscope slides with agarose pads were prepared. A Pasteur pipette was used to place a drop of melted 2% agarose containing 10 mM azide onto a 24 x 60 mm microscopic slide, which had been placed parallel between two other slides. Another slide was dropped on top of the agarose so that it was resting on all of the other slides. This flattened the agarose, but kept it from being too thin.

The top slide was pulled along the bottom slide until the two were separated, leaving the agarose pad on one slide. Three microliters of the worms from the tube were transferred to the pad, and a 24 x 24 coverslip was placed on top of the pad. The worms were observed using a fluorescein filter on an Axioskip compound microscope equipped with epifluorescence.

Genetic Crosses

In order to construct *C. elegans* strains carrying various integrated marker GFP arrays in a *mig-10 (ct41)* background, the strategy in figure 6 was used.

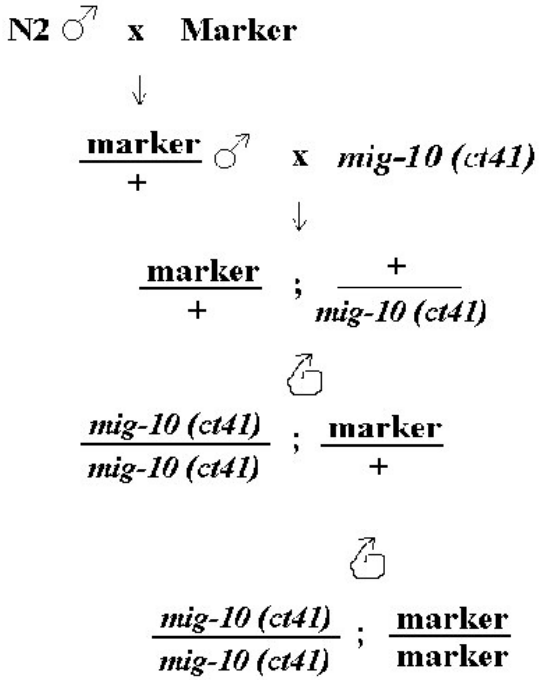


Figure 6: **Strategy for Integrating Marker GFP Array into *mig-10 (ct41)* Background.** In order to put a GFP array, which acts as a marker, into the *mig-10 (ct41)* background, a hermaphroditic worm containing the marker had to first be mated with a male. Mating one strain to a male allows the genes of the first strain to be transferred to another strain. Progeny males containing the marker were then mated to *mig-10 (ct41)* mutant hermaphrodites, yielding progeny that were heterozygous for both the marker and the mutant allele of *mig-10.* The self-fertilization ( ) of this last generation of worms resulted in progeny that were homozygous for the *mig-10 (ct41)* gene, but probably still heterozygous for the marker. The self-fertilization of this generation produces worms that are homozygous for both the *mig-10 (ct41)* gene, and the marker.

In some of the crosses, worms containing two extrachromasomal arrays were desired. For example, worms containing fluorescent excretory cells (due to the transgene *bgIs312*) were mated with *mig-10 (ct41); mpEx16* worms (table 1 and 2) in order to obtain *bgIs312; mig-10 (ct41);*

mpEx16, using the same strategy as in figure 6, but retaining both marker arrays. The *bgIs312* strain was also mated with *mig-10* (*ct41); mpEx15* and *mig-10* (*ct41); mpEx17* (table 1 and 2). The fluorescent excretory cells were observed by placing the worms under a Zeiss dissection microscope equipped with epifluorescence.

Confirming that Worms are Homozygous for *mig-10 (ct41)* Mutation

After the final step of a cross in which one genotype was put into a *mig-10 (ct41)* background, the gene was confirmed to be homozygous for the *mig-10 (ct41)* mutation through 10 worm PCR, in which the region of the mutation was amplified.

Before PCR could be performed, the worms were lysed in order to make their DNA available for PCR. Several worms (ranging from about 5 worms to 20 or more) were taken from a plate with very little or no *E. coli* remaining, and were put into the cap of a PCR tube containing 2.5 µl of a digest buffer, which consisted of the following ingredients: 50 mM KCl, 10 mM Tris (pH 8.2), 2.5 mM $MgCl_2$, 0.45% NP-40, 0.45% Tween 20, 0.01% gelatin, and 0.15 mg/ml Proteinase K. The tube was closed and spun at top speed in a microcentrifuge for about 10 seconds to pellet the worms. Fifty to one hundred microliters of mineral oil was pipetted on top of the pelleted worms. The tube was then placed at -80°C for at least 30 minutes, but could be stored indefinitely at -80°C. The frozen tubes were transferred to the thermocycler, which ran for an hour at 65°C (lysis), 15 minutes at 95°C (inactivation of Proteinase K), and then at 4°C until the next step.

Shortly before/during the holding stage (4°C) of the lysis reaction, a PCR master solution was prepared, which consisted of the following for each sample in the thermocycler: 11 µl sterile water, 2.5 µl 10X Long PCR Buffer 3, 2.5 µl 2.5 mM dNTP mix, 2.5 µl 3 µM top primer, 2.5 µl bottom primer, and 1.5 µl Taq polymerase (5 U/µl). The 10X Long PCR Buffer 3 was taken

from the Expand Long Template PCR System, manufactured by Roche Diagnostics. Twenty-two and a half microliters of the master mix was added to each tube in the thermocycler. The tube's contents were mixed by flicking the tube, which was then microcentrifuged for a few seconds. The tube was returned to the thermocycler, which ran for 10 minutes at 94°C (initial denaturing). The thermocycler then went through 30 cycles of the following: 30 seconds at 94°C (denature), 1 minute at 60°C (anneal), and 2 minutes at 72°C (primer extension). The final step (final extension) was at 72°C for 10 minutes; after which, the samples were held at 4°C until they were either used or transferred to a refrigerator or freezer.

The replicated DNA was digested with Hpy188I restriction enzyme and run on a 0.8% agarose gel at 70 volts for 1.5 to 2 hours. As the *mig-10 (ct41)* mutation causes a RFLP, Hpy188I-digested wild-type *mig-10* DNA will yield 79 bp, 190 bp, 192 bp, and 366 bp fragments (Appendix A). A *mig-10 (ct41)* mutant gene digested with Hpy188I will also yield the 79 bp and 190 bp fragments, but, instead of a 192 and 366 bp fragment, will produce a 558 bp fragment (Appendix A).

Integrating Plasmid DNA into the Genome of *C. elegans*

To integrate the wild-type *mig-10 B* genomic DNA (*mpEx15*) into the genome of the *mig-10* mutant worms, a protocol from Melissa Hunter-Ensor of Massachusetts Institute of Technology was used. About twenty 60 mm^2 plates of mixed stage cultures *mig-10* (*ct41); mpEx15* worms were treated with 3600-4800 Rads of gamma radiation (done at UMass Medical School). The irradiated transgenic L4 hermaphrodites were transferred to 20 agar plates containing *E. coli*, five worms per plate. About ten days later, a chunk from each of the 20 plates (which were starved) was transferred onto a new *E. coli*-spotted agar plate. After the worms grew up on these new plates, 20 of the transgenic worms from each plate were individually plated,

resulting in 400 singled worms. Worms were picked only if most of their intestinal cells expressed the co-transformation marker *elt-2::*GFP. These worms were allowed to grow up and reproduce; after which, each of the 400 plates were checked for worms in which at least half of the population had the majority of their intestinal cells expressing *elt-2::*GFP. Eight transgenic worms were isolated from each plate that fit this description. If a newly isolated worm was observed to have progeny in which 100% of them were transgenic, this line of worms was considered to have the *mpEx15* (*mig-10 B* DNA) integrated into the worms' genomic DNA. Only one line, *mpIs5; mig-10 (ct41)*, was isolated using this integration procedure.

Constructing *mig-10 A* & *mig-10 B* cDNA with *mec-3* Promoter

The *mig-10* cDNA needed to be inserted into the second multiple cloning site (MCS II) of pPD57.56 between NheI and KpnI. In order to isolate the *mig-10 A* and *mig-10 B* cDNAs from their original plasmid vectors ((pCRII-TOPO) – a generous gift from Chris Quinn), the restriction enzymes KpnI and NheI were used (Appendix B). The spliced coding regions for *mig-10 A* and *B* (found through http://www.wormbase.org/db/gene/gene?name = mig-10) were analyzed in Clone Manager to ensure that the *mig-10 A* and *B* cDNAs are not cut by either NheI or KpnI. The digested DNA was run on a TAE agarose gel. The DNA bands at 2.0 kb were extracted from the gel and purified using the Eppendorf® Perfectprep® Gel Cleanup kit. The *mec-3* vector contained two KpnI sites. Thus, a partial digest, using KpnI, was performed on pPD57.56 (containing the *mec-3* promoter region); this digest was run on a TAE agarose gel. A 5.0 kb DNA band, resulting from either one of the KpnI sites being cut, was extracted from the gel and purified, also with the use of the Eppendorf® Perfectprep® Gel Cleanup kit. This 5.0 kb DNA was digested with NheI, yielding (among other things) a 5.0 kb band, which was also purified from its TAE agarose gel. T4 DNA Ligase was used to ligate the cut pPD57.56 with the *mig-10 A* cDNA, and (in a separate

ligation) pPD57.56 with *mig-10 B* cDNA. These ligation products were used in transforming DH5α *E. coli* cells.

The plasmid DNA obtained from the resulting colonies (Appendixes C and D) (after the miniprep was performed) was digested with the restriction enzyme SspI, which was predicted to produce the following DNA fragments:

pPD57.56 digested with SspI yields:	pPD57.56 + *mig-10* A digested with SspI yields:	pPD57.56 + *mig-10* B digested with SspI yields:
2455 (bp)	901 (bp)	~2180 (bp)
2833	~1335	~2180
	~2220	2833
	2833	

The two appropriately sized bands for the pPD57.56 vector with *mig-10 B* cDNA were achieved (Results, lane 4 of figure 13S), as well as the four bands for pPD57.56 + *mig-10 A* (Results, lanes 5-8 of figure 13S).

As there was some uncertainty - due to partial digests - in regards to whether or not the *mig-10 A* (or *mig-10 B*) DNA fragments were contained within the pPD57.56 vector DNA, the HindIII restriction enzyme was used to confirm the presence of the *mig-10* cDNA.

Clone Manager was used to predict the following fragments that would be produced from a HindIII digest:

pPD57.56 (digested with HindIII)	pPD57.56 + *mig-10* A (digested with HindIII)	pPD57.56 + *mig-10* B (digested with HindIII)
~1.3 (kb)	1.15 (kb)	1.15 (kb)
~4.0	1.31	1.31
	1.57	1.53
	3.32	3.32

Figure 13H (Results, lanes 4, 6-9) confirms that the *mig-10* insert was present in these DNA samples.

Transformation and Minipreps

Using sterile technique throughout this protocol, five microliters of plasmid prep were added to 100 µL of DH5α competent cells thawed on ice. This solution was incubated on ice for 30 minutes. The cells were resuspended and heat-shocked at 37°C for 2 minutes. Half a milliliter of LB media, which had been pre-warmed to 37°C, was added to the tube containing the cells. After gently mixing the cells, they were incubated for 1 hour at 37°C, and then resuspended. Ten microliters of the resuspended cells were spread over a plate containing 50 µg/mL of ampicillin or kanamycin; 100 µL of resuspended cells were placed onto another ampicillin (or kanamycin) plate. The remaining cells were spun down, and supernatant was removed. A drop of LB media was used to resuspend the cells. These suspended cells were spread over a third ampicillin (or kanamycin) plate. The plates were incubated overnight at 37°C.

Five tubes, each containing five milliliters of LB media and 50 µg/ml ampicillin or kanamycin, were inoculated with bacteria from five individual colonies from one of the plates grown overnight. These tubes were incubated overnight with shaking at 37°C. In order to freeze a sample of the transformed bacteria, one milliliter of the bacterial broth was added to one milliliter of 50% glycerol; this mixture was stored at -80°C.

The following day, a QIAprep® Spin Miniprep Kit (250) was used to isolate plasmid DNA from the bacterial broth. A restriction digest was performed on the DNA obtained from the miniprep, which was then run on an agarose gel to confirm that the appropriate DNA had been cloned.

Preparation of DNA for Injection

Before DNA injections could be made, five tubes (each containing five milliliters of LB media plus 50 µg/mL of ampicillin) were inoculated with DH5α *E. coli* cells containing the cloned DNA of interest. After incubating at 37°C overnight, plasmid isolation was performed on these cultures. A restriction digest was performed on the combined samples, which was then run on an agarose gel to confirm the DNA of interest had been obtained. The concentration of the DNA from the combined samples was checked.

To precipitate the DNA, one-tenth volume of 1 M potassium acetate (pH 7.4) was added to the sample. Two volumes of 100% ice-cold ethanol were added to the solution, which was then placed at -80°C overnight. The next day, the solution was spun down at the highest setting in a microcentrifuge at 4°C for 30 minutes. The supernatant was decanted, and the pellet was gently washed with 70% ethanol. The tube containing the washed pellet was inverted, and allowed to dry on the bench top overnight. The following day, the pellet (which may/may not be visible) was resuspended in 50 µl of 1X injection buffer and placed at 4°C overnight. The 10X

injection buffer contained 20% polyethylene glycol, 200 mM potassium phosphate (pH 7.5), and 30 mM potassium citrate (pH 7.5). The DNA concentration was checked by UV spectrophotometry, and the sample diluted to an approximate concentration of 80 ng/µl, or 160 ng/µl if it was to be combined with other DNA. When this DNA was not being used, it was stored at -80°C. Co-transformation markers were typically injected at a concentration of 80 ng/µl. Rescuing DNA constructs were also injected at 80 ng/µl, except in the case of the *mec-3::mig-10 A* cDNA construct, where embryos containing the extrachromasomal arrays failed to hatch. The concentration of the co-transformation marker (pflp-20) was kept at 80 ng/µl, but the *mec-3::mig-10 A* cDNA construct was reduced to 40 ng/µl.

Preparation of Microinjection Needles

In order to make the needles used to inject DNA into *C. elegans*, 1.0 mm glass capillary tubes were placed in a micropipette puller (World Precision Instruments), which pulled a single capillary tube into two capillary tubes – each one having a fine, pointed end. The settings used to pull the tubes were typically 8.5 for heat, and a delay of 4. As these pointed ends had no opening, the tip of the pointed end was placed in concentrated hydrofluoric acid (HF); the other end of the capillary tube was connected to tubing from a nitrogen gas tank. With the pressure of the gas at about 60 psi, nitrogen was pumped through the needle until bubbles were formed in the hydrofluoric acid. The needle was then transferred to reagent-grade distilled water where the acid was diluted as nitrogen was pumped through the needle. Needles were placed on top of two rows of clay in a plastic box for storage. In some cases, needles were not etched using HF, but had the tip broken off simply by inserting the needle into the worm.

Injection of DNA into *C. elegans*

The end of a plastic one milliliter syringe was passed over a Bunsen burner until it could be drawn out into fine thread-like tubing. This fine tubing was cut and used to draw up the final DNA solution – which had been centrifuged at 14,000 rpm for five minutes to bring any debris out of solution. The DNA was transferred to a microinjection needle, which was carefully tapped to eliminate any tiny air bubbles. A tube was attached to the blunt end of the needle, which allowed nitrogen gas (60 psi) to push the DNA solution through the needle with the use of a foot pedal. The needle was attached to the mobile arm of the Zeiss Axiovert S100 microinjection microscope. A microinjection manipulator (type MO-102L) from Narishige Scientific Instrument Lab allowed for the positioning of the mobile arm, and thus the needle.

A *C. elegans* worm was mounted onto a 24 x 60 mm coverslip containing a circle of dried 2% agarose. Worms were chosen to be newly gravid, containing only a few eggs or one row of eggs in the uterus. Halocarbon oil (manufactured by Halocarbon Products Corp.) was used to pick the worm from the plate to the agarose pad. As soon as the worm was made immobile in its attachment to the agarose (within a drop of hydrocarbon oil), the coverslip was transferred to the microinjection microscope, where the DNA solution was injected into the worm's gonad. The injected worm was carefully transferred with a pick to an agar plate with *E. coli*, where it could produce progeny.

C. elegans Strains and Arrays

Several strains and arrays were used throughout this project. A description of each strain and array is seen in tables 1 and 2.

Strain Name	Genotype	Comments
N2	**Wild-type**	
BW0315	***mig-10 (ct41)***	**Embryonic cell migration defects in ALM, HSN, & CAN; withered tail & egg-laying defective (Egl); foreshortened excretory canal; defective IL2 axons**
OH0099	***mgIs18***	**Integrated GFP construct. GFP expressed in AIYs.**
RY0044	***mig-10 (ct41); mgIs18***	***mgIs18* in *mig-10 (ct41)* background. Constructed by Elizabeth Stovall.**
NN0001	***bgIs312***	**Integrated GFP construct. GFP expressed in processes of excretory cells.**
RY0024	***dpy-20 (e1282ts); mpIs1***	**Contains integrated *mig-10* promoter::GFP construct. Backcrossed 4X.**
RY0056	***mig-10 (ct41); mpEx15***	***mig-10 B* genomic array. GFP in intestinal cells.**
RY0057	***mig-10 (ct41); mpEx16***	***mig-10 B* genomic array. GFP in intestinal cells.**
RY0058	***mig-10 (ct41); mpEx17***	***mig-10 B* genomic array. GFP in intestinal cells.**
RY0079	***mig-10 (ct41); mpIs5***	**Integration of mpEx15. Constructed by Elizabeth Stovall & Liz Ryder.**
NY2053	***ynIs53 IV; him-5***	***Integrated flp-20::GFP.* GFP expressed in ALMs, AVM, & a few other touch cells.**
RY0096	***mig-10 (ct41); ynIs53 IV***	***ynIs53 IV* in *mig-10 (ct41)* background. Constructed by E. Stovall.**
RY0086	***mpEx12***	**"A1" (*mig-10 A*) Constructed by E. Stovall.**

RY0087	***mig-10 (ct41); mpEx12***	***mpEx12* in *mig-10 (ct41)* background. "A1*; mig-10*" Constructed by E. Stovall.**
RY0088	***mpEx13***	**"B1" (*mig-10 B*) Constructed by E. Stovall.**
RY0089	***mig-10 (ct41); mpEx13***	***mpEx13* in *mig-10 (ct41)* background. "B1*; mig-10*" Constructed by E. Stovall.**
RY0090	***mpEx14***	**"B8" (*mig-10 B*) Constructed by E. Stovall.**
RY0091	***mig-10 (ct41); mpEx14***	***mpEx14* in *mig-10 (ct41)* background. "B8; *mig-10*" Constructed by E. Stovall.**

Table 1: **Strain Name, Genotype, and Description of Various Worms Strains**

mp	Arrays	Comments
mpIs1	***mig-10 B* promoter::GFP; pdpy-20 cotransformation marker**	**GFP in many neurons. Constructed by M. Rivard.**
mpEx15	***elt-2::*GFP*; mig-10* promoter::GFP*; GFP::mig-10 B* genomic**	***mig-10 B* genomic array**
mpEx16	***elt-2::*GFP*; mig-10* promoter::GFP*; GFP::mig-10 B* genomic**	***mig-10 B* genomic array**
mpEx17	***elt-2::*GFP*; mig-10* promoter::GFP*; GFP::mig-10 B* genomic**	***mig-10 B* genomic array**
mpIs5	**Integrated *mpEx15***	***mig-10 B* genomic integrated**

mpEx12	***flp-20::*GFP*;* *mec-3::mig-10 A* cDNA**	**GFP in ALMs, AVM, & a few other touch cells. Constructed by E. Stovall.**
mpEx13	***flp-20::*GFP*;* *mec-3::mig-10 B* cDNA**	**GFP in ALMs, AVM, & a few other touch cells. Constructed by E. Stovall.**
mpEx14	***flp-20::*GFP*;* *mec-3::mig-10 B* cDNA**	**GFP in ALMs, AVM, & a few other touch cells. Constructed by E. Stovall.**

Table 2: **Name and Description of Arrays Used in *C. elegans***

Results

Marker strains were constructed to easily identify several aspects of the *mig-10* phenotype. To determine the expression pattern of *mig-10,* a transgenic strain containing genomic *mig-10* fused to GFP was constructed. To examine cell autonomy in the ALM neurons, transgenic strains expressing *mig-10* cDNA in the ALM neurons were made.

Analysis of *mig-10* Phenotype

ALM migration

One partially penetrant phenotype in *mig-10* mutants is the incomplete migration of anterior lateral microtubule cells (ALMs). The ALM cell bodies in wild-type animals are typically posterior to the AVM cell bodies and anterior to the vulva, while ALM neurons in *mig-10* animals often fail to complete their posterior migration past AVM (fig. 7). Worms containing the *ynIs53* transgene, received from Chris Li's lab, express GFP in the ALM as well as other touch cells. A strain was constructed to visualize the *mig-10 (ct41)* ALM migration defects by crossing *mig-10 (ct41)* into *ynIs53;him-5.*

The relative positions of the ALM and AVM cell bodies were observed in 50 *ynIs53; him-5* worms. Worms younger than the L3 stage were not used in these observations because the AVM doesn't migra te to its final location until the L2 stage. Twelve percent of these wild-type worms had at least one misplaced ALM, in which the ALM cell body was either equidistant with the AVM cell body from the worm's nose or anterior to the AVM cell body (fig. 7). The relative positions of ALM and AVM cell bodies were scored in 50 worms that were confirmed to be homozygous for the *mig-10* mutant gene in the *ynIs53* background. Eighty percent of these worms contained at least one misplaced ALM.

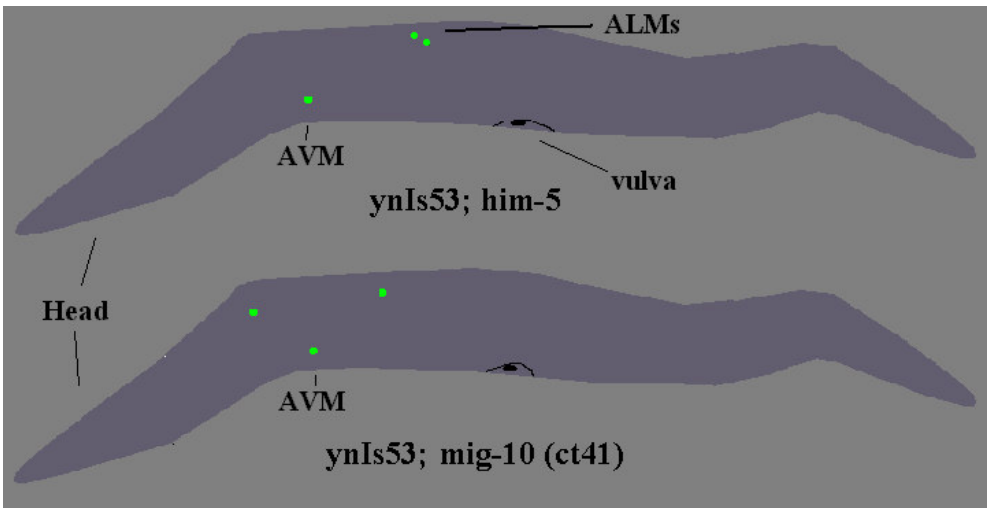


Figure 7: **Location of ALMs and AVM in Wild-type and *mig-10* Mutant Worms.** Schematic of *ynIs53; him-5* worm in wild-type (top) and *mig-10* mutant (bottom) background.

Excretory canal growth

One *mig-10* mutant phenotype that is 100% penetrant is the foreshortened processes of the excretory cells. In the wild-type worm, these processes extend through the length of the worm, stopping almost at the end of the worm's tail. The *bgIs312* array expresses GFP in the excretory cell processes. A strain was constructed to visualize the outgrowth defects of the *mig-10 (ct41)* excretory cell processes by crossing *mig-10 (ct41)* into *bgIs312*.

The excretory cell processes of 30 *bgIs312* worms were all observed to run the length of the worm. The processes observed in 30 worms confirmed to be homozygous for the *mig-10* mutant gene in the *bgIs312* background all contained excretory cell processes that came to an end near or anterior to the vulva.

Observation of AIYs

The AIY neurons in the head were observed for any effects of *mig-10* because another lab had previously reported that they were affected by *mig-10* (O. Hobert, personal

communication). To determine if the AIY neurons are affected, *mig-10 (ct41)* mutant worms were crossed with *mgIs18* worms, in which GFP is expressed in the AIYs. PCR (followed by an Hpy188I digest) was performed to confirm that the *mig-10* gene was homozygous.

The AIY neurons in 50 *mgIS18* worms were observed and compared to those in 50 *mgIs18; mig-10 (ct41)* mutant worms. There did not appear to be any difference between the two strains; it was concluded that the *mig-10* mutant gene does not affect the AIYs.

Observation of IL2s

Previous results showed that IL2 neurons are defective in *mig-10* mutant animals; this aspect of the phenotype is partially penetrant (Rusiecki, 1999). To confirm these results, the IL2 neurons were compared between the wild-type (N2) and *mig-10* mutant (BW0315) worms after they had been DiO stained (see Methods). A wild-type IL2 neuron has a dendrite that extends from the nose of the worm to a cell body (in the head); an axon extends from the cell body to the nerve ring (or brain) where it then loops back and branches to form connections with other neurons (fig. 8A-8C). In the *mig-10* mutant, the majority of an IL2 neuron may be present, but the loop is absent (fig. 8D). In observing 42 wild-type worms, 100% were observed to contain normal IL2 processes. In observing 47 *mig-10 (ct41)* worms, ~30% appeared to have at least one IL2 neuron that lacked the loop.

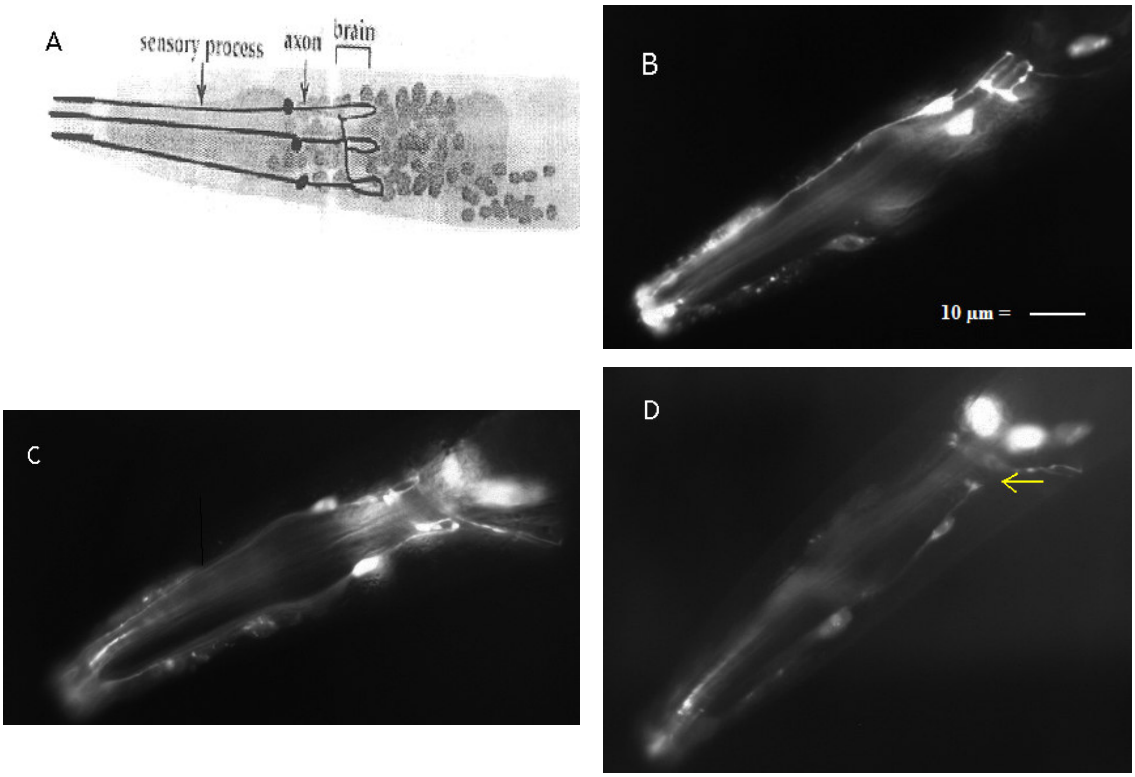


Figure 8: **IL2 Neurons in Wild-type and *mig-10* Mutant Worms.** A) Schematic of wild-type IL2 neurons. B-D) Photomicrographs of wild-type (B & C) and *mig-10* mutant (D) worms stained using DiO to visualize IL2 neurons. Note the absence of the loop in D (arrow). DiO-positive cells in the right upper corner of B-D – which are posterior to the IL2s – are the worm's amphid neurons; these were not of interest in observing the IL2s.

Construction of Strain Containing Genomic *mig-10*::GFP Fusion for Expression Analysis

One goal of this project was to determine the expression pattern of *mig-10*. In order to study this gene and how it works to allow for the proper migration of neurons and development of different cell types, it is important to learn in which cells this gene is expressed. The *mig-10* gene contains three transcripts, *mig-10 A, B,* and *C* (fig. 9). The *mig-10 C* gene was not used in any of the experiments mentioned in this paper as it has just recently been discovered (wormbase, 2004).

As indicated in Manser *et al.*, 1997, *mig-10 B* genomic DNA was believed to be sufficient to rescue the *mig-10* mutant phenotype back to the wild-type. Thus, DNA constructs were made (by Chris Burket) to contain *mig-10 B* genomic DNA and GFP, which would rescue at least some aspect of the mutant phenotype back to the wild-type. The expression pattern of a rescuing construct is desired as this suggests that the expression pattern is correct.

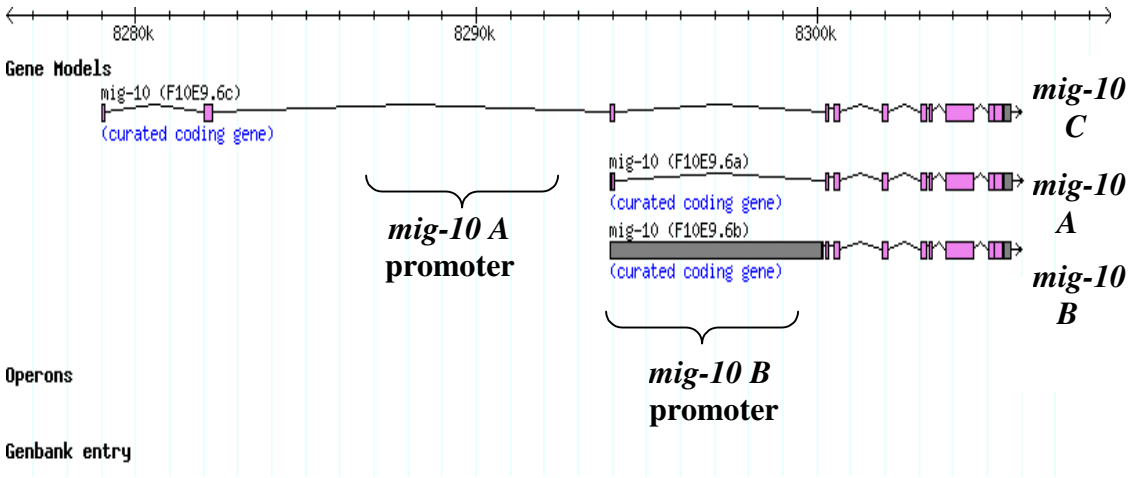


Figure 9: **Map of *mig-10* Genes.** Diagram of *mig-10 C* (F10E9.6c), *mig-10 A* (F10E9.6a), and *mig-10 B* (F10E9.6b) DNA taken from Worm Base (http://www.wormbase.org/db/seq/sequence?name=F10E9.6a;class=CDS). The *mig-10 A* gene contains an alternate first exon and promoter located further upstream than that of the *mig-10 B* gene. Regions used as *mig-10 A* and *B* promoters are shown above. The *mig-10 B* promoter region is mistakenly shown as an exon; this is a database error.

To determine if *mig-10 B* genomic DNA would rescue the *mig-10* mutant back to the wild-type phenotype, a plasmid containing the *mig-10* promoter and GFP was co-injected with a plasmid containing GFP and the *mig-10 B* genomic DNA into *mig-10* mutant worms (fig. 10). A third plasmid that was co-injected contained the *elt-2* promoter and GFP; this plasmid served as a

co-injection marker. The resulting extrachromasomal arrays were designated *mpEx15, mpEx16,* and *mpEx17*.

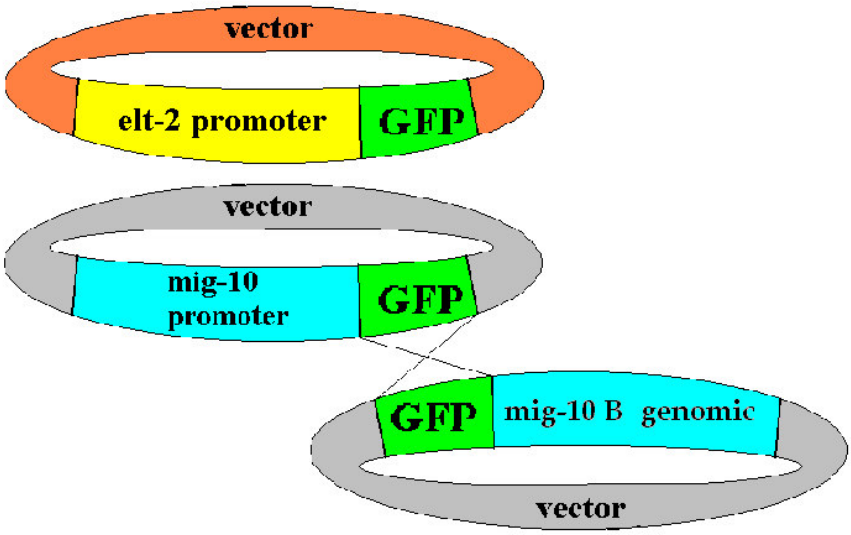


Figure 10: **DNA Constructs Used in Expressing Wild-type *mig-10 Gene* in *mig-10* Mutant Worms.** Schematic of DNA constructs used in an attempt to determine the expression pattern of *mig-10*. The *mig-10 B* promoter and genomic DNA were placed on two different plasmids because the DNA was too long for one plasmid. Recombination at the GFP site on the two plasmids allowed for the promoter to be positioned next to the genomic DNA (with GFP between them) (C. Burket, 2002). The co-injection marker (containing the *elt-2* promoter) allowed the worm's intestinal cells (where *elt-2* is expressed) to fluoresce green (via GFP), thereby revealing that the worm contains the extrachromasomal DNA. The GFP found on the other plasmids with *mig-10* is also expressed, but is very weak and difficult to observe in the larval and adult worms.

The RY56 strain carries the *mpEx15* array in the *mig-10 (ct41)* background. This strain was examined for rescue of neuron morphology. The results can be seen in table 3.

Because the transgenic array was not integrated into the chromosome, it was lost in some meioses. This allowed a direct comparison between worms carrying the array and those that had lost it. The percentage of worms with abnormal IL2 processes was 43% in nontransgenic worms

and 17% in transgenic worms (table 3, rows 1 and 2). To determine if this difference was statistically significant, a chi-square test was performed. Although the χ^2 value did not quite reach significance ($0.05 < p < 0.10$), the data looked promising, and as the p value was approaching the 0.05 level (in which the data would have been accepted as significantly different), the rescue experiments were continued.

	Defective	**Non-defective**	**Total**
mig-10 **Lost Transgene**	**15** **(43%)**	**20** **(57%)**	**35**
mig-10 (ct41); mpEx15 **Nonintegrated Transgenic**	**4** **(17%)**	**19** **(83%)**	**23**
mig-10 (ct41); mpIs5 **Integrated Transgenic**	**12** **(24%)**	**38** **(76%)**	**50**

Table 3: ***mig-10 B* Genomic DNA Did Not Rescue Defective IL2 Neuronal Processes.** Worms were DiO stained and the phenotype of the IL2s was determined without knowledge of the transgene's presence. Though there was a decrease in the percentage of worms with defective processes in the *mig-10; mpEx15* nonintegrated transgenic worms, the P value from the chi-square tests ($0.05 < P < 0.10$) indicated that this difference was not significant. The integration of the *mig-10 B* plasmid DNA into the genome of *C. elegans* to generate *mig-10 (ct41); mpIs5* did not rescue *mig-10* mutants back to the wild-type phenotype. The second chi square test, which was performed on all three rows of data from this table, confirmed that there was no significant difference in the percentage of defective worms between the two transgenic and the nontransgenic strains ($0.10 < P < 0.20$).

Observation of Excretory Canal

Three different nonintegrated transgenes containing *mig-10 B* genomic extrachromasomal DNA were constructed (*mpEx15, mpEx16,* and *mpEx17*). To determine whether any of these

transgenes rescue the excretory canal defect of *mig-10 (ct41),* they were crossed with the *bgIs312* strain, in which the excretory canal fluoresces green. PCR (followed by an Hpy188I restriction digest) was used to confirm that the *mig-10* mutant gene was homozygous in all three strains; the expected fragment sizes are seen in appendix A. Figure 11 shows the results of this digest of the PCR product from *mig-10; mpEx17; bgIs312*. The other lines were checked by the same assay.

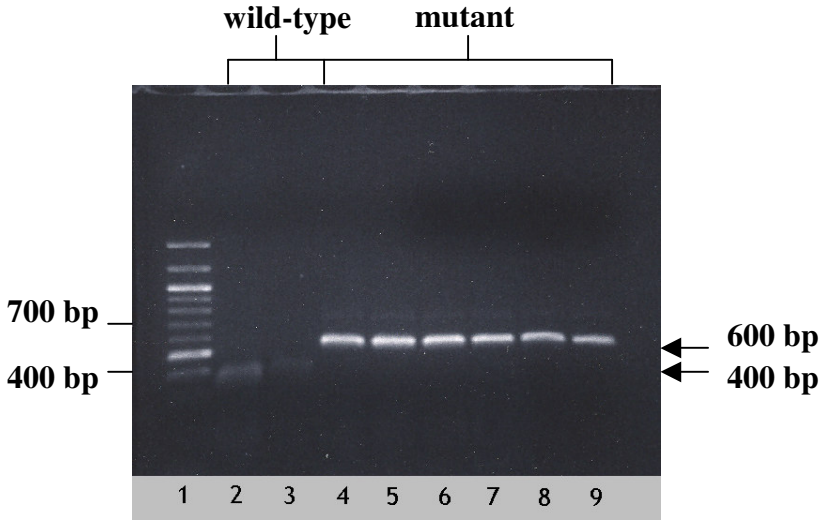


Figure 11: **Confirmation of Genotype of *mig-10 (ct41); mpEx17; bgIs312.*** PCR products from all strains were digested with Hpy188I. Lane 1, 100 bp Ladder. Digested PCR products show that a 400 bp band is only observed with the N2 (wild-type) samples (lanes 2 and 3) (Appendix A). The *mig-10* homozygous mutants, on the other hand, display a strong band at about 600 bp (lanes 4 and 5). Lanes 6 through 9 contain the Hpy188I-digested PCR product from *mig-10 (ct41); bgIs312* worms that had lost the nonintegrated array. PCR was performed with the worms that had "lost" the extrachromasomal DNA (the extrachromasomal DNA had not been inherited from the parental generation) so that the genomic *mig-10* DNA, and not the extrachromasomal DNA would be replicated.

The excretory canal in these strains containing *bgIs312* was observed in 30 worms for each strain. Thirty out of thirty *mig-10 (ct41); mpEx15*; *bgIs312* and *mig-10 (ct41); mpEx17;* bgIs312 worms were found to have a foreshortened excretory canal. Only one out of thirty *mig-10 (ct41); mpEx16*; *bgIs312* worms was observed to have a full-length excretory canal. This

result shows that the *mig-10 B* genomic DNA does not rescue the *mig-10* mutant excretory canal phenotype.

Results of Integrating *mig-10 B* DNA into *C. elegans'* Genome

The DiO staining of the IL2 neurons suggested that there might be partial rescue of this aspect of the *mig-10 (ct41)* phenotype by the *mig-10 B* genomic construct. Extrachromosomal arrays usually exhibit mosaicism due to loss of the array from some cells during mitosis. Thus, the array was integrated into the genome to determine whether this would result in complete rescue (see Methods). The integrated transgene is denoted *mpIs5.*

After the integration of the *mig-10 B* gene into the genome, the IL2s of 50 *mig-10 (ct41); mpIs5* worms were observed. As table 3 shows, there was a decrease in the percentage of worms containing defective IL2s in the *mig-10 (ct41)* nontransgenic worms as compared to the *mig-10 (ct41); mpIs5* strain. In comparing the *mig-10 (ct41); mpEx15* transgenic worms to *mig-10 (ct41); mpIs5*, however, it was observed that the integration of the *mig-10 B* DNA did not rescue mutant IL2s back to the wild-type phenotype. To confirm this, a chi square test was performed on all of the data in table 3. The chi square value was 4.04 with 2 degrees of freedom. The P value was greater than 0.10 ($0.10<P<0.20$). As a result, the null hypothesis - the percentage of worms with the mutant phenotype is not significantly different between the two transgenic and the nontransgenic animals - was accepted.

Construction of *mig-10 A* & *mig-10 B* cDNA with *mec-3* Promoter for Cell Autonomy Experiments

Preliminary analysis of both *mig-10 (ct41); mpIs5* and *dpy-20 (e1282ts); mpIs1* (containing the *mig-10 B* promoter driving GFP) suggested *mig-10* is expressed in neurons. This observation suggested that, unlike the case in the excretory canal, *mig-10* might act cell autonomously in neurons. To test this hypothesis for ALM migration, it was desired to specifically express *mig-10* cDNA in ALMs. Rescue of the mutant back to the wild-type phenotype would indicate that the MIG-10 protein functions within the ALMs. No rescue occurring would suggest that the protein needed to be in another cell for the proper placement of ALMs.

The *mec-3* promoter was used to drive expression of the *mig-10* A and *mig-10* B cDNA constructs because this promoter drives expression in the ALMs, AVM, and only a few other cells in the early development of *C. elegans.* Vectors containing *mig-10 A* and *B* were kindly provided by Chris Quinn. The presence of *mig-10 A* and *B* were verified by restriction digestion (fig. 12). pPD57.56, a vector containing the *mec-3* promoter, was kindly provided by Andy Fire. Both the vector pPD57.56 and *mig-10 A* and *B* cDNA-containing vectors were digested with KpnI and NheI (see Methods). The appropriate fragments were gel purified and ligated to create the desired *mec-3::mig-10* constructs (Appendix D).

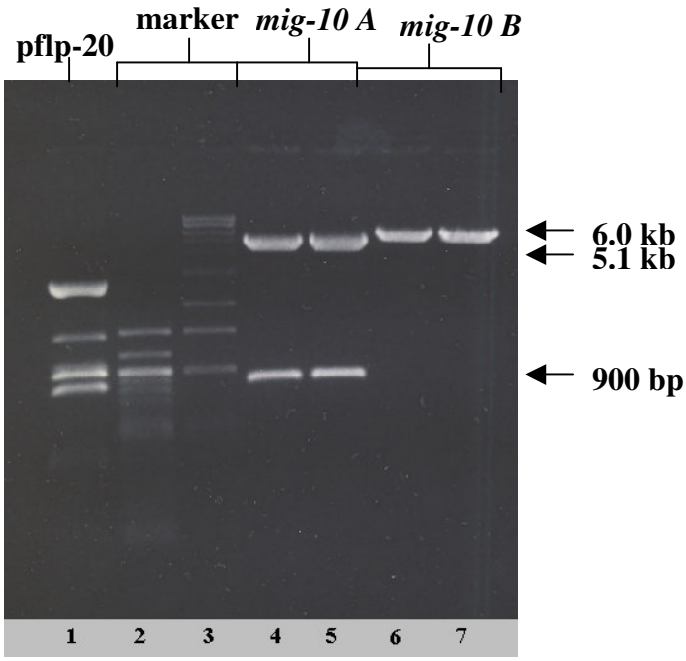


Figure 12: **Confirmation of *mig-10 A* and *mig-10 B* Initial Constructs.** SspI digest of *mig-10 A* (lanes 4 and 5) and *mig-10 B* (lanes 6 and 7) cDNA-containing pCRII-TOPO plasmids. The pCRII-TOPO vector does not contain an SspI restriction site, but the *mig-10 A* sequence contains two, yielding a 900 base pair (bp) and 5.1 kilobase (kb) fragment (Appendix B). There is only one SspI site in the *mig-10 B*-containing pCRII-TOPO plasmid, yielding a single band of about 6.0 kb (Appendix B). DNA bands of these approximate sizes were obtained, as seen in the figure. The 100 bp and 1.0 kb Ladders are seen in lanes 2 and 3, respectively. (Lane 1 contains EcoRI/SpeI-digested pflp-20 DNA.)

Following the confirmations that plasmids containing the *mec-3* promoter and the *mig-10* cDNA had been obtained (fig. 13), each type of plasmid (the one containing *mig-10 A*, and the other containing *mig-10 B*) was separately combined with the pflp-20 plasmid. The mixture containing pflp-20 and one of the *mec-3::mig-10* plasmids was injected into N2 worms, as described in the Methods section. As a result of these injections, one line containing the *mig-10 A* cDNA (*mpEx12*, referred to as the A1 line), and two lines containing the *mig-10 B* cDNA (*mpEx13* and *mpEx14*, referred to as the B1 and B8 lines, respectively) were obtained.

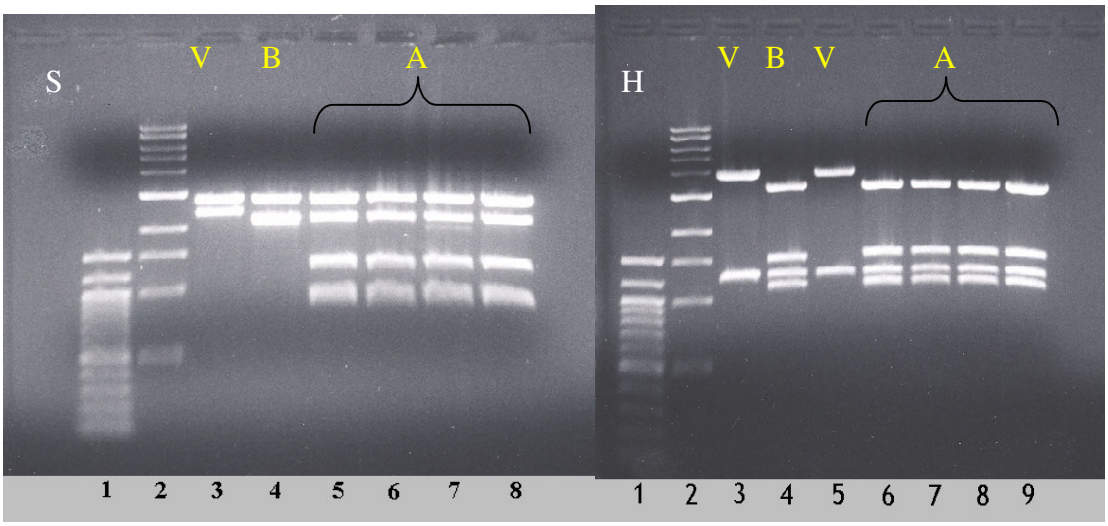


Figure 13: **pPD57.56 + *mig-10* Digested to Completion with SspI and HindIII.** Panel S, miniprep DNA digested with SspI. Panel H, miniprep DNA digested with HindIII. V, vector pPD57.56 alone; B, vector + *mig-10 B* cDNA insert; A, vector + *mig-10 A* cDNA insert. Lanes 1 and 2 (S and H) contain a 100 bp and 1.0 kb DNA Ladder, respectively. (See Appendixes C and D).

***mec-3::mig-10* Constructs Partially Rescue Defective ALM Migration in *mig-10* Mutants**

To determine whether the *mec-3::mig-10* cDNAs had any detrimental effect in a wild-type background, the relative positions of the ALMs and AVM were observed in 30 worms for each line (table 4). Thirty out of thirty worms from the B1 and A1 lines were observed to have ALMs that were posterior to the AVM (the wild-type phenotype). One out of thirty worms in the B8 line contained a misplaced ALM. This wild-type phenotype was important to demonstrate, as overexpressed wild-type DNA can result in a mutant phenotype.

The *mig-10* mutation was crossed into the A1, B1, and B8 strains to create strains *mig-10 (ct41); mpEx12*, *mig-10 (ct41); mpEx13,* and *mig-10 (ct41); mpEx14* respectively, which are referred to as A1; *mig-10,* B1; *mig-10,* and B8; *mig-10.* PCR (followed by an Hpy188I restriction digest) was used to confirm that this mutant gene was homozygous in each strain (data not

shown). Fifty worms from each of the three lines containing the *mig-10* mutation - at about the L4 stage - were observed for the relative positions of the ALMs and AVM (table 4). All three strains containing the wild-type cDNA in the *mig-10* mutant background appeared to show partial rescue of the ALMs when compared with the strain containing *mig-10 (ct41)* and the *flp-20* construct with no rescuing *mig-10* (table 4). The rescuing array strains were significantly different both from wild-type strains and from mutant strains (table 5). The strains with the rescuing DNA (lines B1; *mig-10*, B8; *mig-10*, and A1; *mig-10*) displayed varying levels of misplaced ALMs. The A1; *mig-10* strain had the highest number of misplaced ALMs (50%), which was significantly below the number of mutants observed in the *mig-10 (ct41); ynIs534* strain (80%).

Row #	Strain	Defective	Nondefective	Total
1	ynIs534; him-5	**6 (12%)**	**44 (88%)**	**50**
2	*mig-10* (ct41); ynIs534	**40 (80%)**	**10 (20%)**	**50**
3	B1	**0**	**30**	**30**
4	B1:*mig-10*	**21 (42%)**	**29 (58%)**	**50**
5	B8	**1**	**29**	**30**
6	B8:*mig-10*	**18 (36%)**	**32 (64%)**	**50**
7	A1	**0**	**30**	**30**
8	A1:*mig-10*	**25 (50%)**	**25 (50%)**	**50**

Table 4: **Partial Rescue of Defective ALM Migration.** Defective worms were scored as those in which at least one of the ALMs is either anterior to the AVM or equidistant from the worm's nose with the AVM. y*nIs534* is an integrated array containing pflp-20:: GFP, which is a marker for ALM and other touch neurons. *him-5* is a mutation that creates a higher incidence of males in the strain and does not affect ALM migration. A1, B1, and B8 are extrachromasomal arrays containing *mec-3::mig-10 A* or *B* cDNA and pflp-20::GFP constructs.

Test	Row #	X^2	P
1	1, 2	43.84	< 0.001
2	3, 4	14.98	< 0.001
3	5, 6	9.32	0.001<P<0.01
4	7, 8	19.54	< 0.001
5	2, 4	13.62	< 0.001
6	2, 6	18.1	< 0.001
7	2, 8	8.62	0.001<P<0.01

Table 5: **Chi Square Values/Significance of Data in Table 4.** This data corresponds to the data in table 4. In order to confirm that there was a significant difference in the percentage of misplaced ALMs between the wild-type *ynIs534; him-5* and the mutant *mig-10 (ct41); ynIs534*, a chi square test was performed (test 1). Other chi square tests were done to verify that the percentage of misplaced ALMs between each of the mutant strains containing a potential rescuing plasmid (B1:*mig-10*, B8:*mig-10*, and A1:mig-*10*) and the wild-type worms containing the appropriate *mec-3::mig-10* rescuing plasmid constructs (B1, B8, and A1) were significantly different (tests 2, 3, 4). Additional chi square tests were done to verify that there was a significant difference in the percentage of worms with misplaced ALMs between the mutant worm strain *mig-10 (ct41); ynIs534* and the mutant worms containing one of the *mec-3::mig-10* rescuing plasmid constructs (B1:*mig-10*, B8:*mig-10*, and A1:mig-*10*) (tests 5, 6, and 7). The chi square (X^2) and p values, and the rows (from table 4) for which the tests were performed are seen above.

Confirmation of *mig-10* cDNA Vectors with *mig-10* Promoters

As the pPD57.56 vectors with the inserted *mig-10* cDNA appeared to only result in partial rescue of the ALM position, these vectors were used to insert either a *mig-10 A* or *B* promoter in the place of the *mec-3* promoter region (Appendix E). These new vectors, constructed by Turkan Arca, allow the hypothesis that *mig-10 A* and *mig-10 B* may rescue different aspects of the *mig-10 (ct41)* phenotype to be tested. As the *mig-10* promoter should

allow the *mig-10* cDNAs to be expressed where necessary, nearly a 100% rescue of the ALMs would be expected in the worms in which these vectors would be injected.

To confirm that each vector contained the appropriate *mig-10* cDNA region, PCR was performed on the vector containing the *mig-10 A* promoter with *mig-10 A* cDNA, and the vector containing the *mig-10 B* promoter with *mig-10 B* cDNA (fig. 14). Each vector containing a *mig-10* promoter and *mig-10* cDNA was also digested with SpeI in order to confirm that they contained the appropriate promoter (fig. 15). Thus, the vectors were confirmed to contain the appropriate *mig-10* regions.

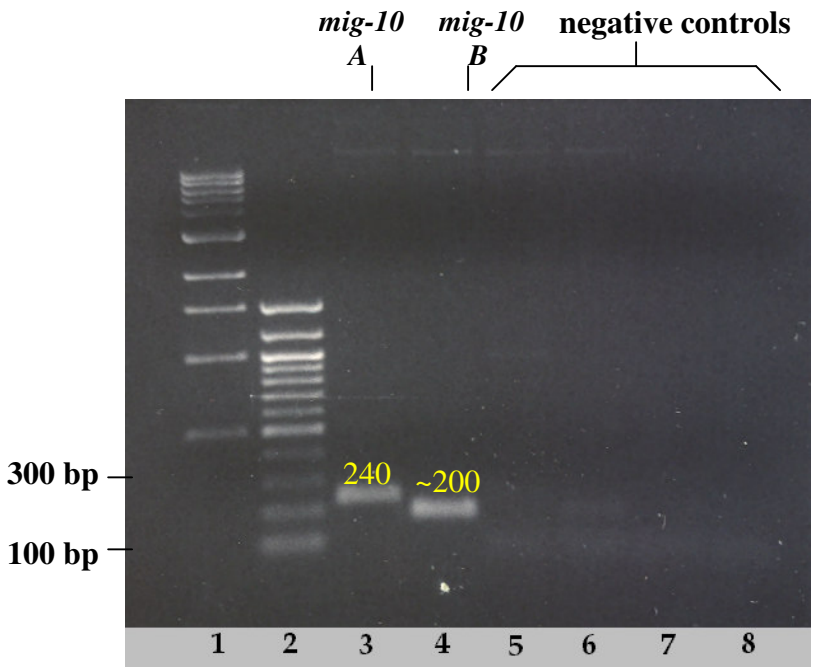


Figure 14: **PCR Products from *mig-10 A* and *B* cDNA.** The expected PCR product from the vector with the *mig-10* A cDNA was about 240 bp. Lane 3 contains PCR product (between 200 and 300 bp) using the *mig-10* tran B primer and *mig-10* exon A primer with the *mig-10 A* cDNA. The PCR product from the vector with the *mig-10* B cDNA was expected to be about 186 bp. A DNA band (~200 bp) is seen as the PCR product using the *mig-10* tran B primer and *mig-10* exon B primer with the *mig-10 B* cDNA (lane 4). As a control, each set of primers was used with the other *mig-10* cDNA, from which no PCR product was expected (lanes 5 and 6). Another control used was the primers without any template DNA (lanes 7 and 8). Faint bands observed in lanes 5-8 were believed to be either the result of the primers loosely annealing to a similar sequence as what is found in the original vector used with the primers, or primer dimer. Lanes 1 and 2, 1.0 kb and 100 bp Ladder, respectively. (Refer to the Appendix F for where the primers anneal to the *mig-10* cDNA regions.)

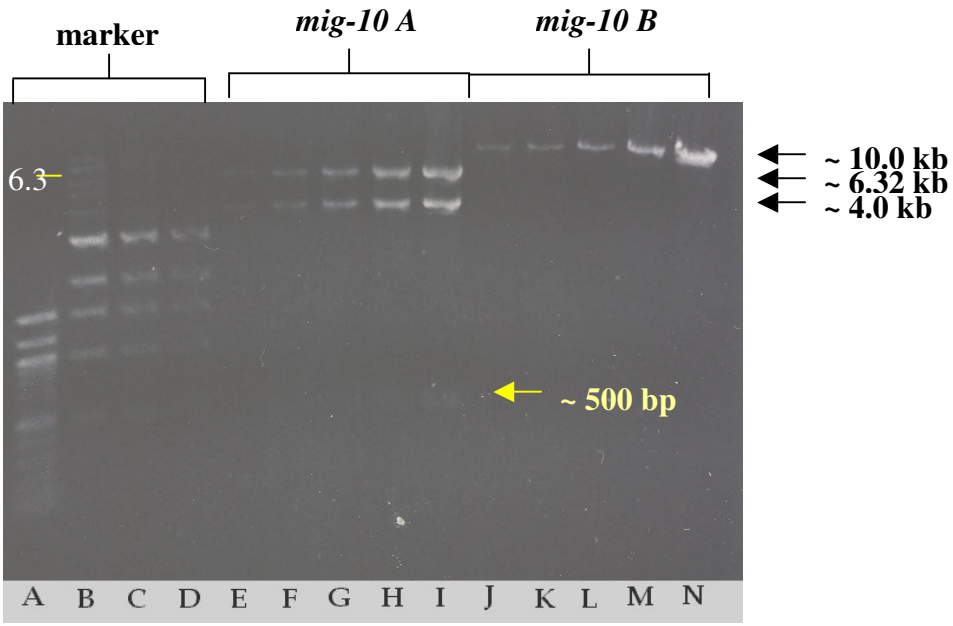


Figure 15: **Vector with *mig-10 A* Promoter and cDNA & Vector with *mig-10 B* Promoter and cDNA Digested with SpeI**. Lane A, 100 bp Ladder; Lanes B-D, 1.0 kb Ladder; Lanes E-I, SpeI digested *mig-10 A* promoter + cDNA vector, (Expected bands: 6,318 bp, 3,973 bp, 473 bp, and 13 bp; Actual bands: 6.0-7.0 kb, 4.0 kb, and 500 bp; 500 bp band shown by yellow arrow.) Lanes J-N, SpeI digested *mig-10* B promoter + cDNA vector, (Expected band: 10.5 kb; Actual band: ~10.0 kb).

Discussion

mig-10 is required for the migration of certain neurons including the ALMs, the outgrowth of the excretory canal, and axon outgrowth of the IL2s (Manser *et al*., 1997); (Rusiecki, 1999). In order to understand how *mig-10* functions, its phenotype, expression pattern, and cell autonomy were examined. As *mig-10 A* and *B* have different promoters, the MIG-10 proteins differ in their N-termini, and MIG-10 A has a second proline-rich region, it would not be unreasonable to suggest that *mig-10 A* and *B* would be expressed and function differently.

Expression Pattern

In order to determine the most accurate expression pattern, plasmid constructs containing a *mig-10* promoter, genomic wild-type *mig-10 B* DNA, and a GFP region were injected into *C. elegans*. The *mig-10 A* DNA was not used as previous experiments indicated that the *mig-10 B* DNA alone was sufficient for rescue of *mig-10 (ct41)* mutant phenotypes back to the wild-type phenotype (Manser *et al*., 1997). However, these constructs, even when integrated, appeared to give only partial rescue of IL2 mutant phenotypes, and no rescue of the excretory canal mutant phenotype.

This lack of rescue could be due to a lack of the necessary amount of expression of the *mig-10 B* genomic construct. Another possibility includes an incorrect sequence contained within the *mig-10 B* genomic construct, as its sequence has not been checked. Also, as there is more than one *mig-10* transcript, different transcripts may be needed to rescue the different aspects of the *mig-10* mutant phenotype. For example, complete rescue may actually require the *mig-10 A* transcript, in addition to *mig-10 B*. Despite the lack of complete rescue, the strain obtained from the integration of the DNA may be useful for expression pattern studies.

Cell Autonomy

Analysis of transgenic lines containing *mig-10* promoter::GFP or *mig-10 B* genomic::GFP suggested the expression of the gene is mostly in neurons. Thus, cell autonomy experiments focused on expressing *mig-10* specifically in affected neurons to see if this would rescue the *mig-10* mutant phenotype back to the wild-type phenotype. As the *mig-10* gene is believed to produce a protein involved in signal transduction, it seems that this gene should be cell autonomous. However, *mig-10* is known to be cell nonautonomous in excretory cell outgrowth (Manser *et al.,* 1997). Models showing how *mig-10* might function cell autonomously, cell nonautonomously, or both are shown in figure 16.

In determining which neurons would be ideal for experiments in cell autonomy, the AIY neurons were rejected as there was no significant difference between the wild-type and *mig-10 (ct41)* mutants. The ALMs, on the other hand, became a focal point for cell autonomy as only 12% of the wild-type worms appeared to have misplaced ALMs, and 80% of the *mig-10* mutant worms had misplaced ALMs.

A *mec-3* promoter was used to force the expression of *mig-10 A* or *B* in ALM neurons. After the *mec-3::mig-10 A* DNA or *mec-3::mig-10 B* DNA-injected worms (in which *mig-10* was expressed in the ALMs) were crossed with *mig-10 (ct41)* mutant worms, partial rescue of the ALM migration defect to the wild-type was seen in the two lines containing *mig-10 B* cDNA and the one line containing *mig-10 A* cDNA. This partial rescue could be due, again, to the need for both of the *mig-10* transcripts to be expressed in one cell, or for one or both transcripts to be expressed in a cell nonautonomous fashion in addition to being expressed cell autonomously. Other possibilities include a low production of the rescuing protein, or expression of the protein at a developmental stage that is not early enough for complete rescue of the *mig-10* mutant back to the wild-type.

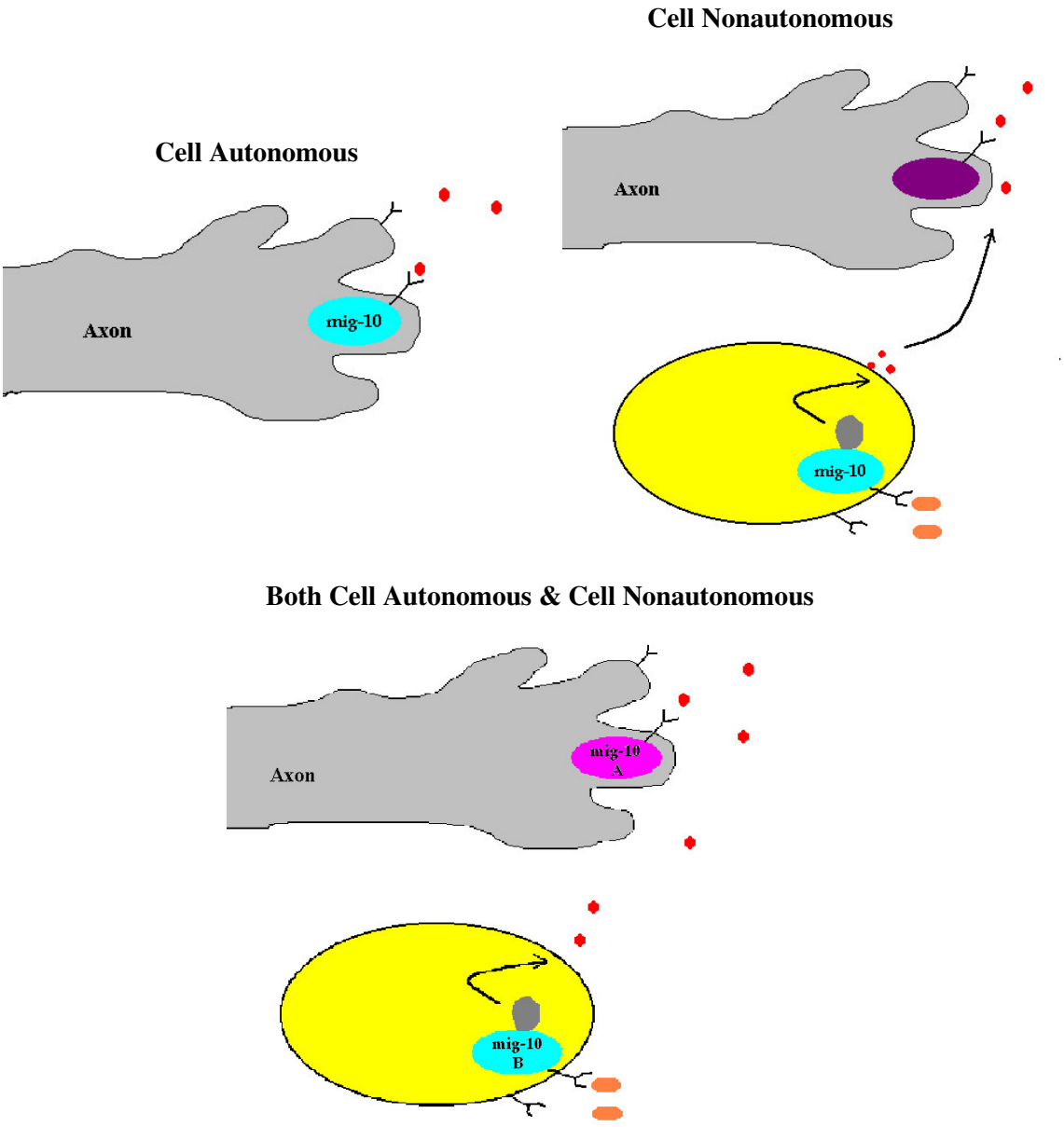


Figure 16: **Proposed Models of How MIG-10 May Function as a Signal Transduction Protein.** *mig-10* may act in a cell autonomous, or cell nonautonomous fashion, or may use a combination of cell autonomy and cell nonautonomy. If *mig-10* is cell autonomous, then the protein will function in the cell that requires it for proper function or development. For example, MIG-10 protein might be required in the growth cone of a neuron to transduce a guidance signal affecting that cell. A cell nonautonomous protein functions in a cell other than the one that requires the expression of that protein. For example, *mig-10* might facilitate the secretion of a guidance signal that acts on the neuron's growth cone. One other possibiltiy (not shown) is that MIG-10 A and B may both be needed in the same cell.

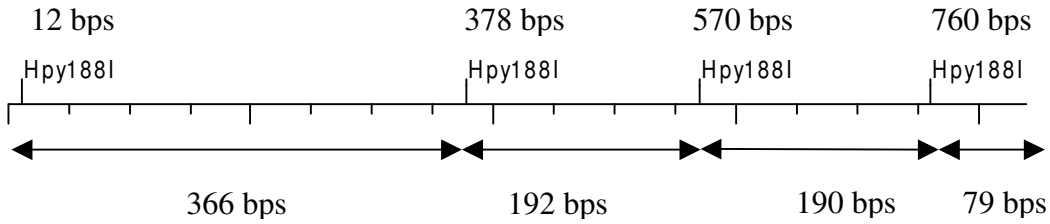
If the cDNA constructs are fully functional, and were expressed wherever they were needed, complete rescue of the *mig-10* mutant back to the wild-type phenotype would be expected. Results from another lab, in which the A1; *mig-10*, B1; *mig-10,* and B8; *mig-10* strains were observed for AVM axon defects, showed that either *mec-3::mig-10* construct results in complete rescue of these axon defects (C. Quinn, personal communication). This suggests that the rescuing constructs express fully functioning MIG-10 A and MIG-10 B proteins. To determine if the individual *mig-10* transcripts rescue different aspects of the *mig-10 (ct41)* phenotype, the *mec-3* promoter in the *mig-10 A* and *mig-10 B* cDNA constructs was replaced with *mig-10* promoters (work of Turkan Arca), though these constructs have not yet been used in any experiments. For example, *mig-10 A* might rescue defective neurons, while *mig-10 B* could rescue a mutant excretory canal. Another experiment in progress is the injection of *mig-10* promoter::*mig-10 A* and *mig-10* promoter::*mig-10 B* together. This should correct any partial rescue that may be due to the lack of both MIG-10 proteins.

Though few conclusions have been made about the cell autonomy of *mig-10 (ct41)*, and the expression pattern of this gene has yet to be fully determined, the groundwork for these projects has been laid. An integrated *mig-10 B* genomic line was obtained and can be used for expression studies. The partial rescue obtained in the various experiments is confusing and intriguing. Further experiments may lead to a better understanding of how *mig-10 A* differs from *mig-10 B* (and possibly *mig-10 C*), and *mig-10*'s role in the migration of neurons and axon guidance.

Appendix A

Map of *mig-10* (mutant & wild-type) gene with Hpy188I restriction sites

Map of mutant *mig-10* Hpy188I restriction sites

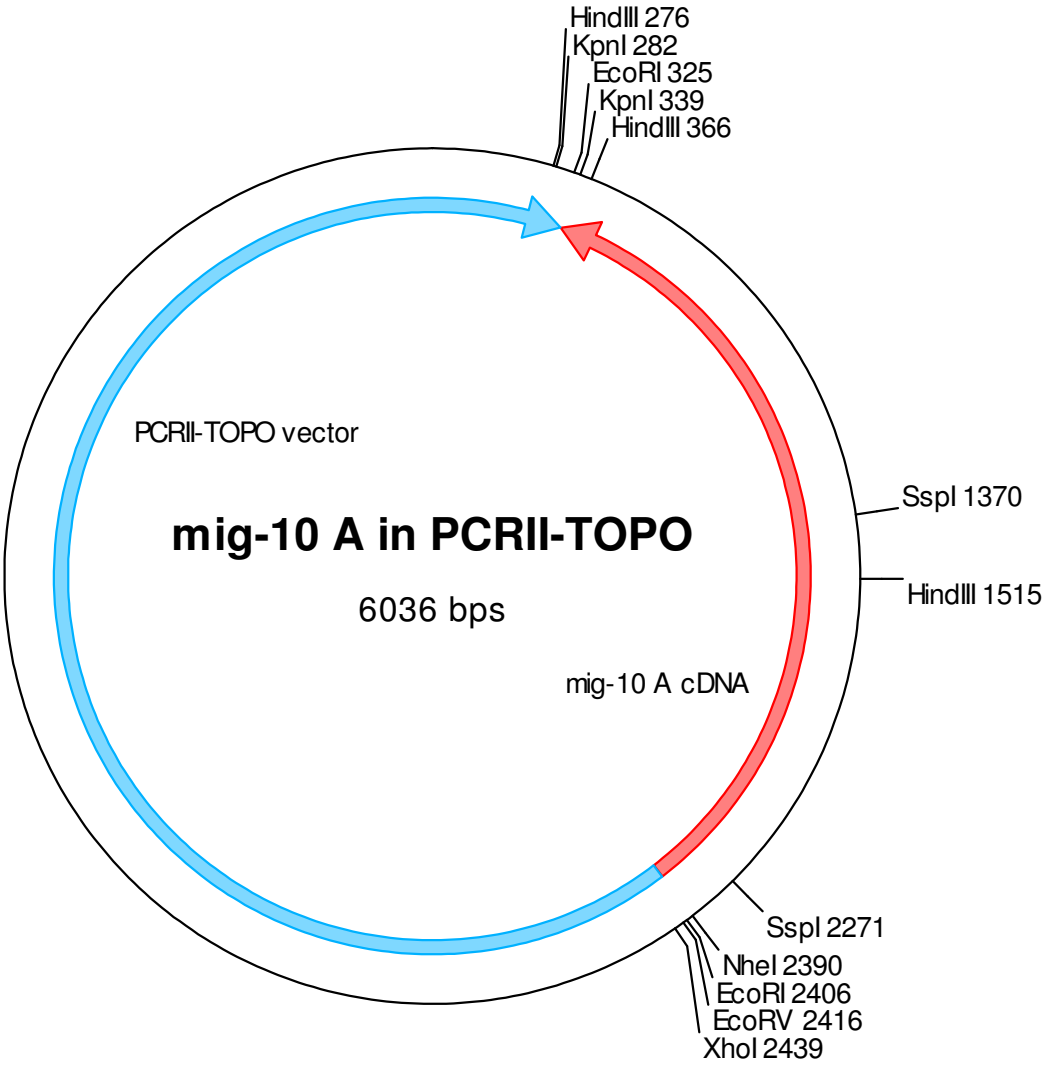


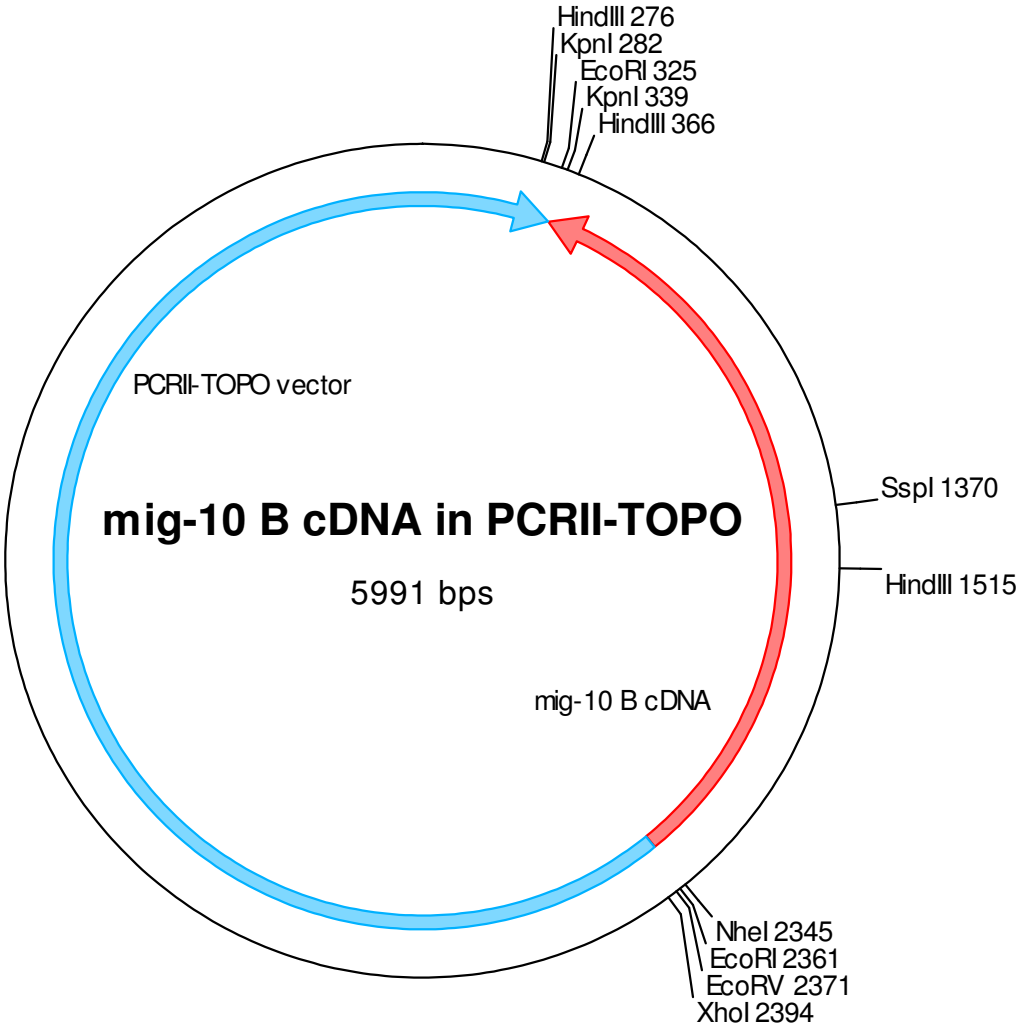
Map of *mig-10* wild-type Hpy188I restriction sites

The primers used in the PCR of the *mig-10* gene were MIG10-WT1 and MIG10-WT2. The genetic sequence of MIG10-WT1 is 5'-TGTTTGAATTTTCAGAATCCGC-3', and that of the MIG10-WT2 primer is 5'-TGTTTCTTCTCACAATCCAACC-3'. (These maps were taken from the MQP project of Romiya Glover and Stephanie Morin.)

Appendix B

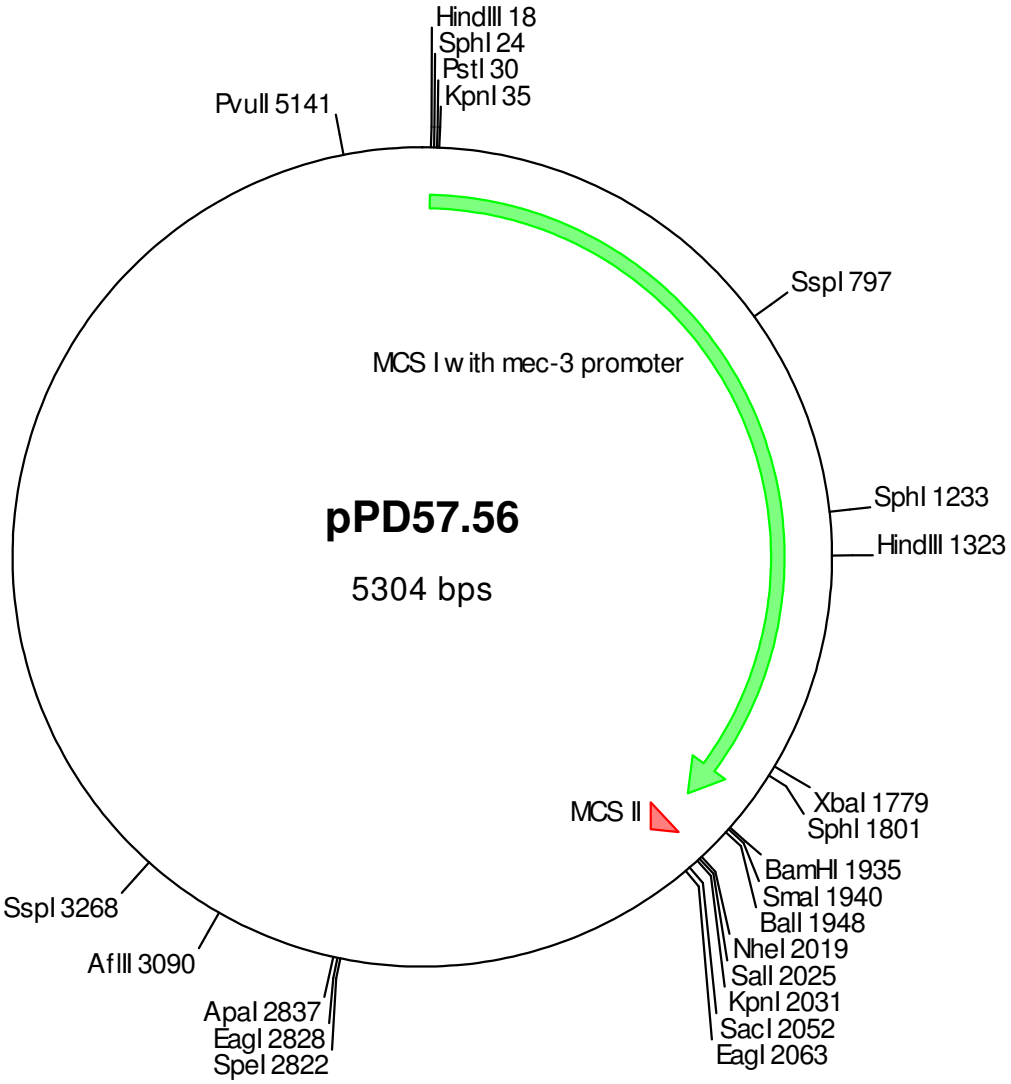
Maps of *mig-10 A* and *B* cDNA in PCRII-TOPO vector


HindIII 276
KpnI 282
EcoRI 325
KpnI 339
HindIII 366
PCRII-TOPO vector
mig-10 A in PCRII-TOPO
6036 bps
mig-10 A cDNA
SspI 1370
HindIII 1515
SspI 2271
NheI 2390
EcoRI 2406
EcoRV 2416
XhoI 2439


HindIII 276
KpnI 282
EcoRI 325
KpnI 339
HindIII 366
PCRII-TOPO vector
mig-10 B cDNA in PCRII-TOPO
5991 bps
mig-10 B cDNA
SspI 1370
HindIII 1515
NheI 2345
EcoRI 2361
EcoRV 2371
XhoI 2394

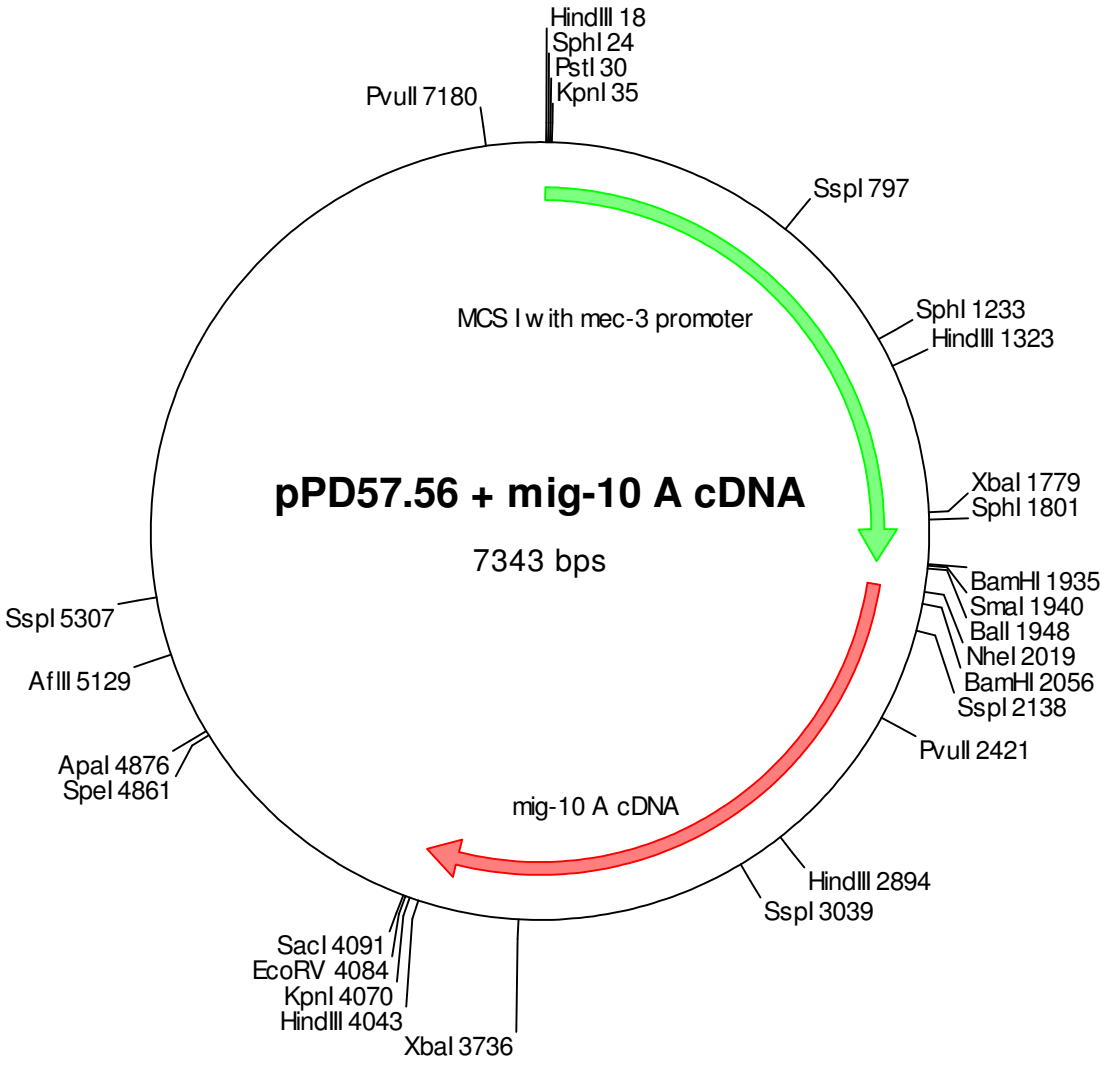
Appendix C

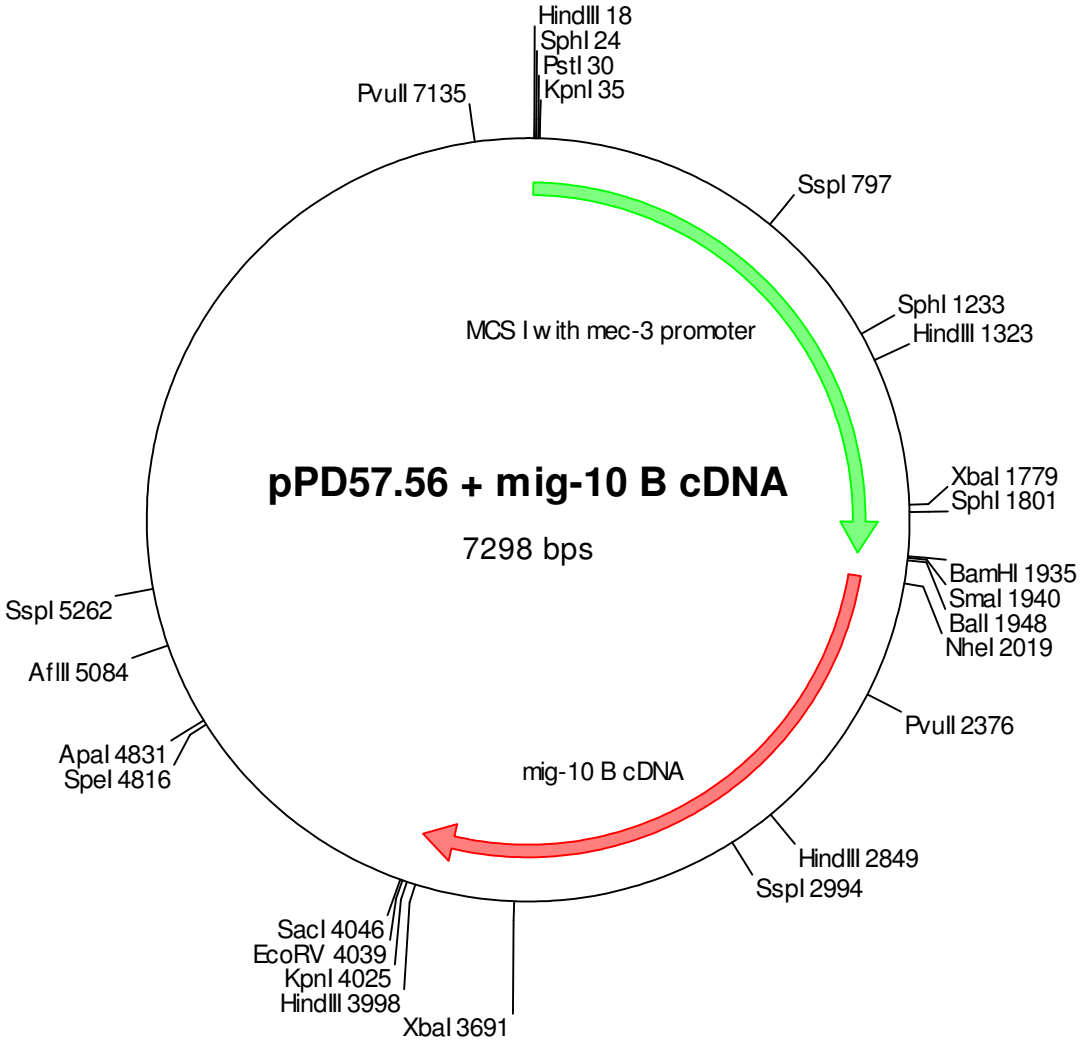
pPD57.56 vector


HindIII 18
SphI 24
PstI 30
KpnI 35
PvuII 5141
SspI 797
MCS I with mec-3 promoter
pPD57.56
5304 bps
SphI 1233
HindIII 1323
MCS II
XbaI 1779
SphI 1801
BamHI 1935
SmaI 1940
BalI 1948
NheI 2019
SalI 2025
KpnI 2031
SacI 2052
EagI 2063
SspI 3268
AflII 3090
ApaI 2837
EagI 2828
SpeI 2822

Appendix D

pPD57.56 vector with inserted *mig-10 A* and *B* cDNA

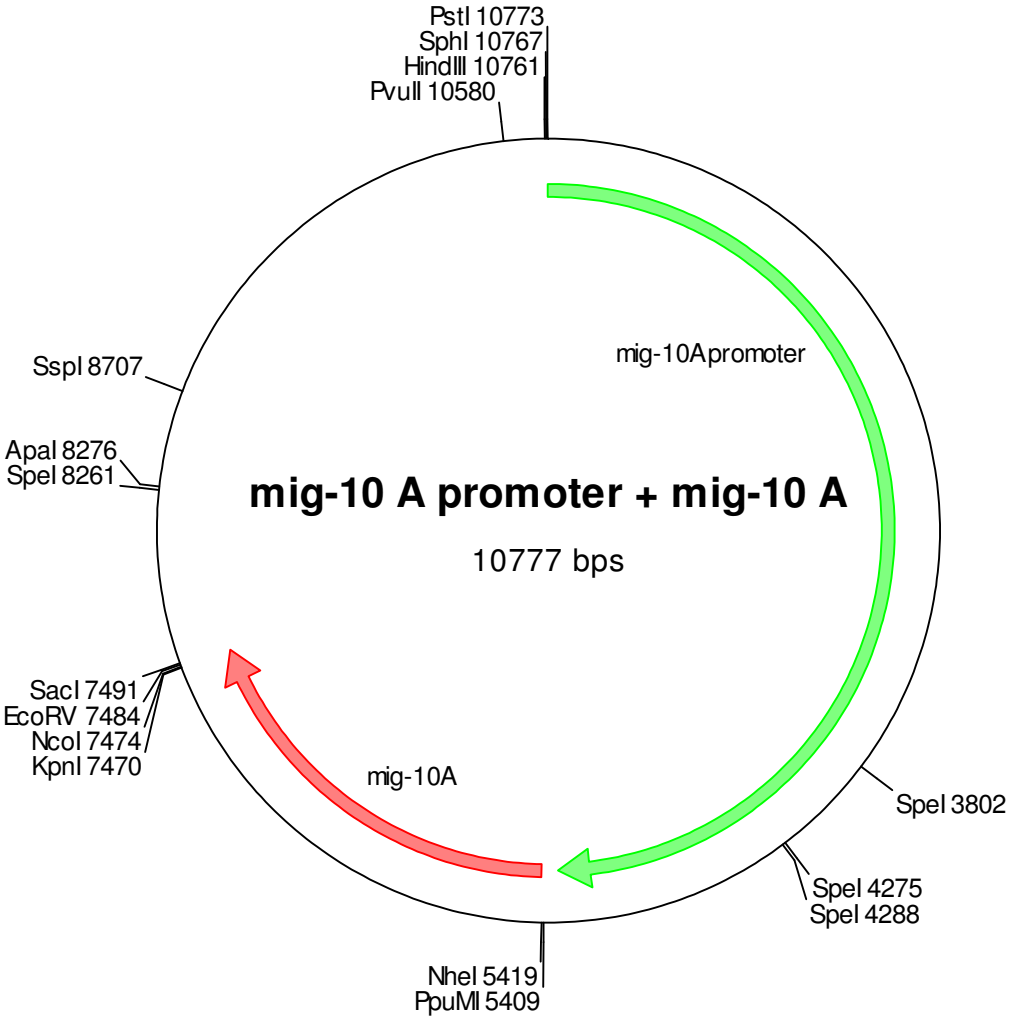

HindIII 18
SphI 24
PstI 30
KpnI 35
PvuII 7180
SspI 797
MCS I with mec-3 promoter
SphI 1233
HindIII 1323
pPD57.56 + mig-10 A cDNA
7343 bps
XbaI 1779
SphI 1801
BamHI 1935
SmaI 1940
BalI 1948
NheI 2019
BamHI 2056
SspI 2138
PvuII 2421
SspI 5307
AflII 5129
ApaI 4876
SpeI 4861
mig-10 A cDNA
HindIII 2894
SspI 3039
SacI 4091
EcoRV 4084
KpnI 4070
HindIII 4043
XbaI 3736

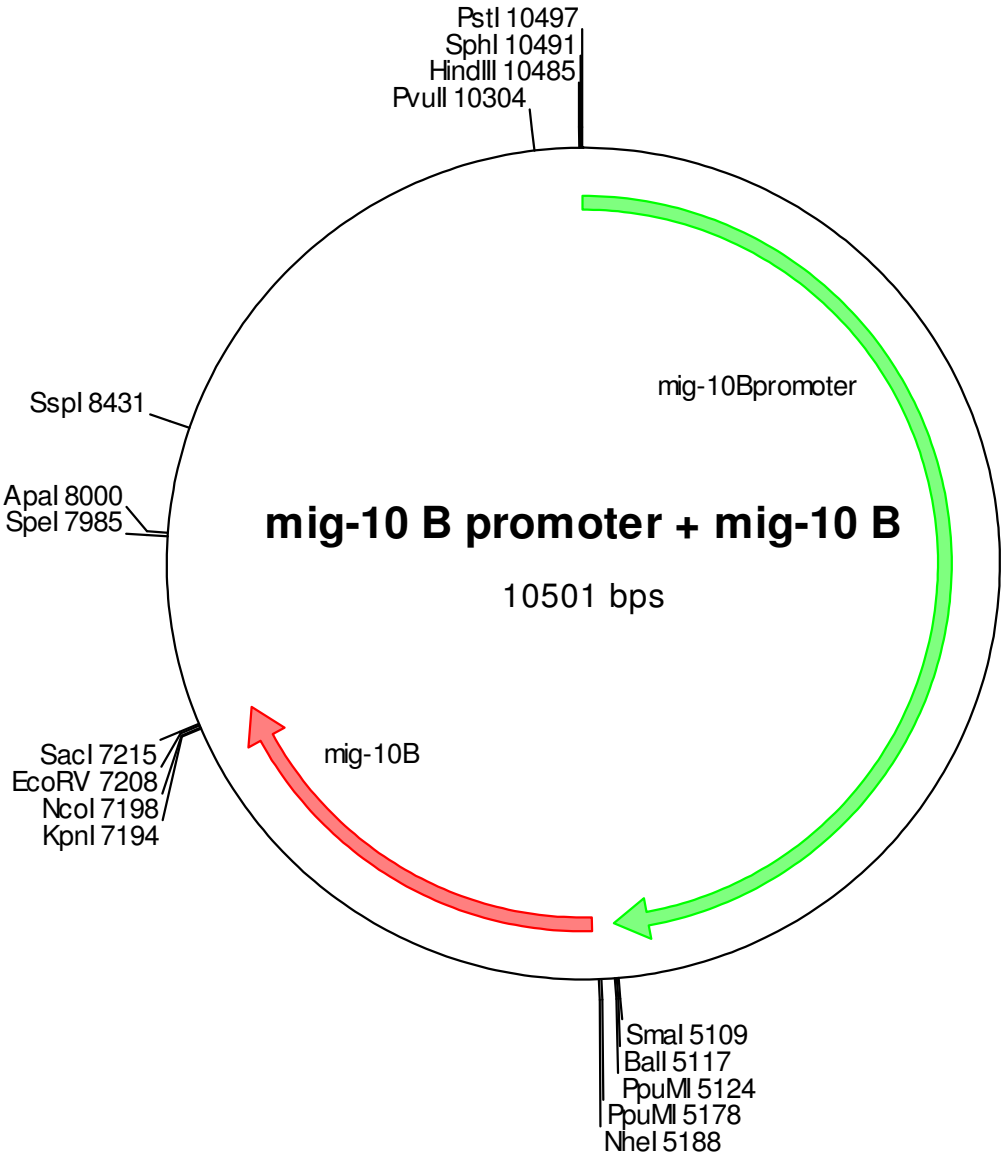

HindIII 18
SphI 24
PstI 30
KpnI 35
PvuII 7135
SspI 797
SphI 1233
HindIII 1323
MCS I with mec-3 promoter
pPD57.56 + mig-10 B cDNA
7298 bps
XbaI 1779
SphI 1801
BamHI 1935
SmaI 1940
BglI 1948
NheI 2019
PvuII 2376
mig-10 B cDNA
HindIII 2849
SspI 2994
SspI 5262
AflII 5084
ApaI 4831
SpeI 4816
SacI 4046
EcoRV 4039
KpnI 4025
HindIII 3998
XbaI 3691

Appendix E

Vectors containing *mig-10 A* promoter::*mig-10 A* cDNA

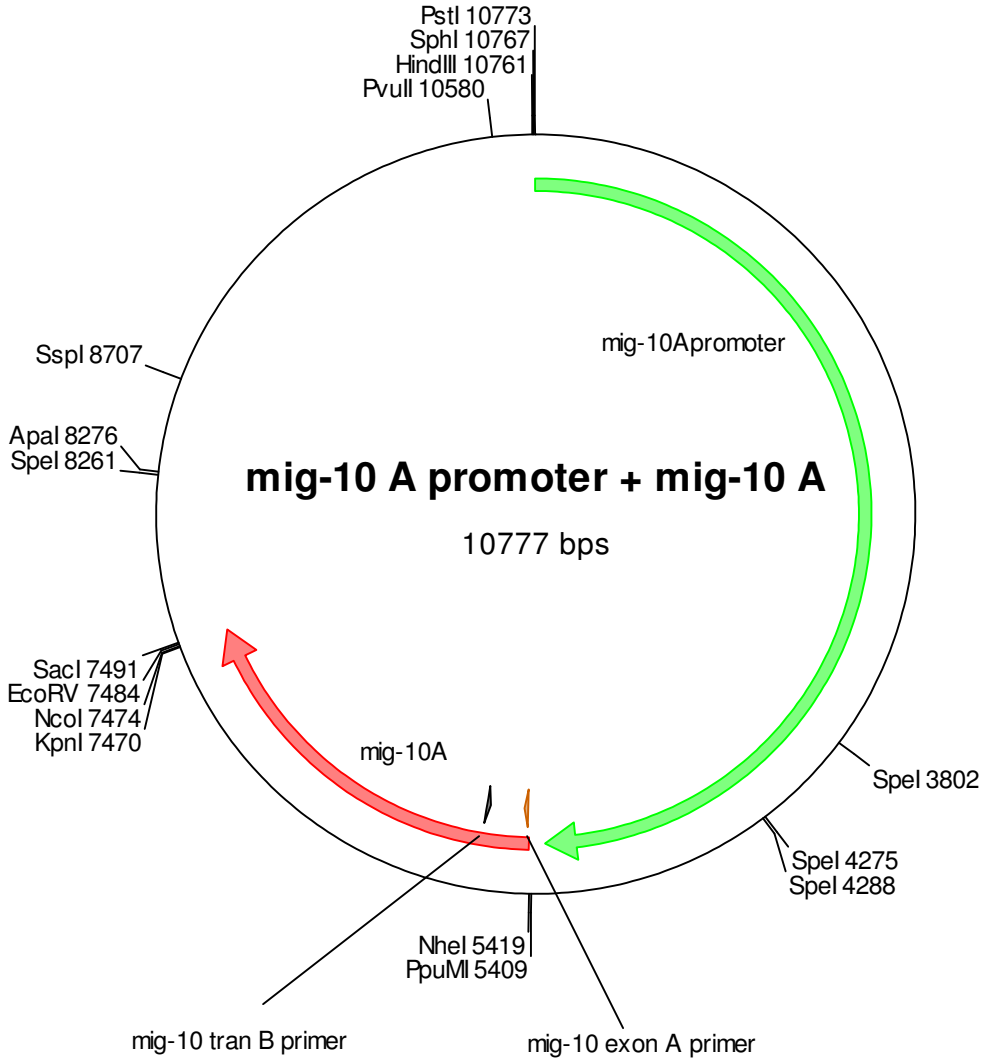
& *mig-10 B* promoter::*mig-10 B* cDNA


PstI 10773
SphI 10767
HindIII 10761
PvuII 10580
mig-10A promoter
SspI 8707
ApaI 8276
SpeI 8261
mig-10 A promoter + mig-10 A
10777 bps
SacI 7491
EcoRV 7484
NcoI 7474
KpnI 7470
mig-10A
SpeI 3802
SpeI 4275
SpeI 4288
NheI 5419
PpuMI 5409

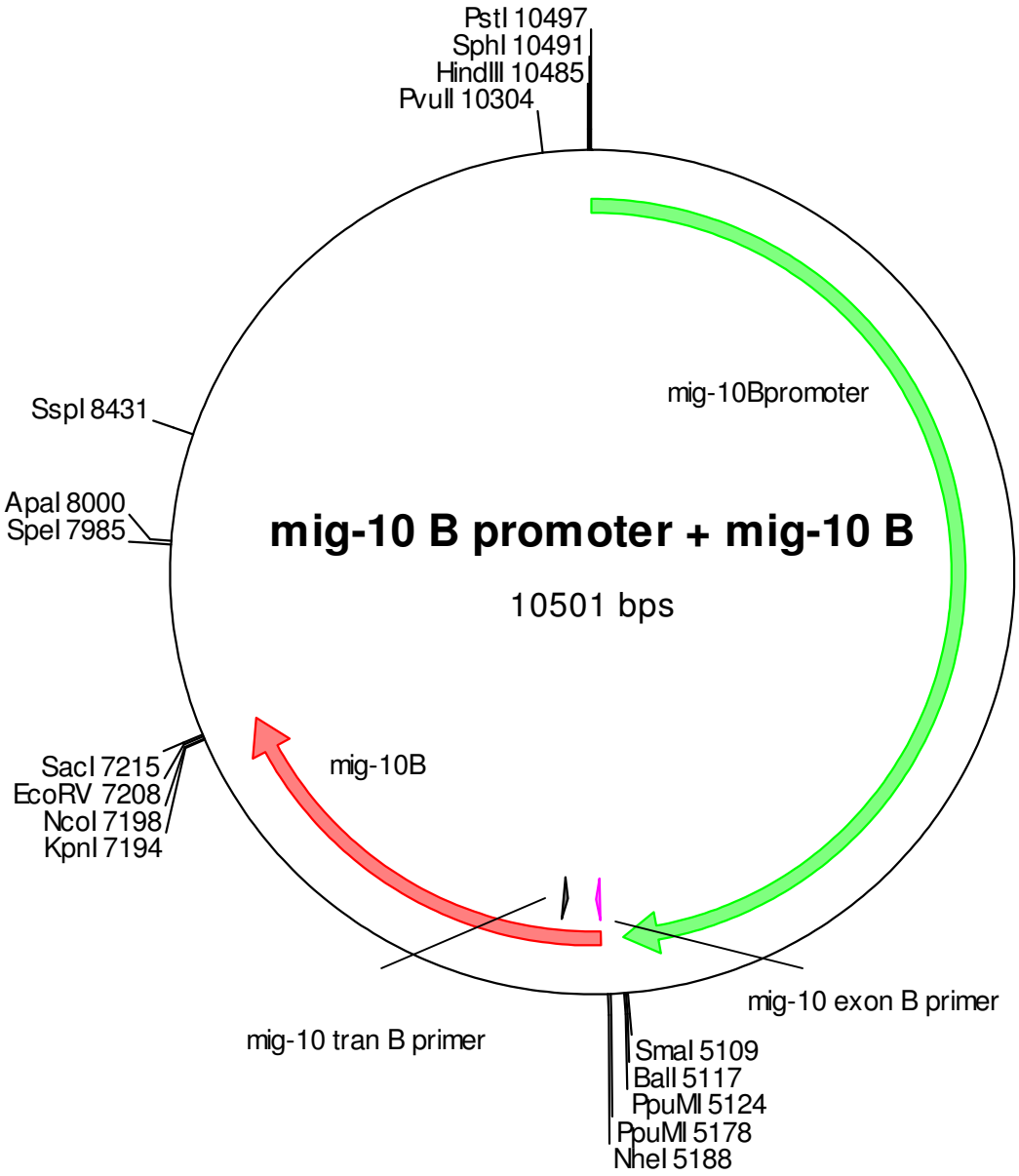

PstI 10497
SphI 10491
HindIII 10485
PvuII 10304
mig-10Bpromoter
SspI 8431
ApaI 8000
SpeI 7985
mig-10 B promoter + mig-10 B
10501 bps
mig-10B
SacI 7215
EcoRV 7208
NcoI 7198
KpnI 7194
SmaI 5109
BalI 5117
PpuMI 5124
PpuMI 5178
NheI 5188

Appendix F

Primers annealing to vector containing *mig-10 A* promoter::*mig-10 A* cDNA and *mig-10 B* promoter::*mig-10 B* cDNA



The *mig-10* tran B primer anneals to both the *mig-10 A* and *B* cDNA sequences. The primer *mig-10* exon A anneals only to *mig-10* A cDNA, as *mig-10* exon B primer anneals only to *mig-10* B cDNA. The *mig-10* exon A primer sequence is 5'-ACCATGGACACTTACGACTTCC-3'.



The sequence of the *mig-10* tran B primer is 5'-GGCTAGAAGAAGCTGGAATCCCG-3'. The *mig-10* exon B primer sequence is 5'-AATGTATCACGATCGACGGCG-3'.

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