Project Number: EFR-0504

Analysis of *mig-10* expression to determine cell autonomy or nonautonomy in *Caenorhabditis elegans*

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by

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1. *C. elegans*
2. *mig-10*
3. Neuron Migration

Abstract

Early in *C. elegans* development, several neurons must migrate to specific locations within the body. Mutations in *mig-10* result in incomplete migration of several neurons, as well as defects in egg laying and excretory cell development. We tested the ability of each of the two *mig-10* splice forms to rescue the egg laying defect; partial rescue was observed. These results indicate that perhaps more than one isoform of the MIG-10 protein must be expressed simultaneously in multiple cells in order to achieve total mutant rescue. To test the hypothesis that expression in specific neurons is required for rescue of the migration and excretory cell defects, constructs were made to express each *mig-10* splice form in a particular neuron.

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1.0 Introduction

1.1 *Caenorhabditis elegans* as a model

1.1.1 Comparing C. elegans to Humans

Neuronal migration in the nematode *Caenorhabditis elegans* has been extensively studied. Early in *C. elegans* development, extracellular cues signal neurons to migrate and guide them to their final destinations. Several neurons must be relocated to specific places within the worm's body in order to function properly. Due to present understanding of the nervous system, *C. elegans* serve as an excellent specimen for cell migration studies (Manser et al. 1997).

C. elegans are a much simpler system to study in comparison to humans. Their transparent bodies make them an ideal model for research, as their eggs and neurons can be easily viewed under the microscope. Compared to humans, they contain fewer genes, cells, and signal transduction pathways. Humans have a very complex genetic make up, with approximately 3000 Mbp spread throughout 23 pairs of chromosomes. However, *C. elegans* contains a 97 Mbp genome contained in six pairs of chromosomes (Wood 1988). The nematode also has a life cycle of 2-3 weeks, and a reproductive cycle that begins 40 hours after fertilization. This allows scientists to study a large number of worms over a short period of time.

1.1.2 Improving Understanding of Human Genetics

Studies of *C. elegans* have the potential to contribute a great deal toward the understanding of the human nervous system. The nematode may appear to be unrelated to the more complex human, but genetic, cellular, and molecular similarities have been identified between the two systems. These similarities have the ability to increase understanding of processes such as neuronal migration and signal transduction pathways in humans.

1.2 Comparison of Wild Type and Mutant *C. elegans* Development

1.2.1 Components of Axon Growth and Development

Neuronal axon development and formation occurs during differentiation where dendrites and axons grow out from the cell body of each neuron. The way each axon selects the correct pathway to grow, how it chooses a specific target end region, and how it recognizes distinctive cells with which it must synapse is primarily determined by growth cones.

Axons are guided throughout their growth and development by growth cones on their tips (Figure 1). Receptors on the growth cone plasma membranes identify extracellular guidance cues that initiate a signal transduction cascade (Lundquist 2003). This cascade adjusts lamelipodial and filipodial actin-cytoskeleton morphology. The cascade also controls steering, affecting further growth cone, and ultimately axon, outgrowth.

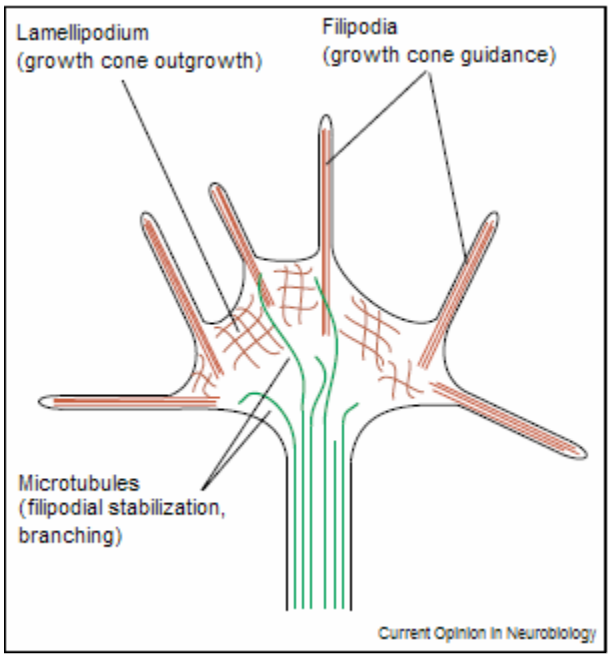

Figure 1: Factors affecting axon outgrowth. Lamellipodium for growth cone outgrowth and filopodia for growth cone guidance are both necessary for proper axon expansion. Racs have control over these cytoskeleton processes. Picture from Lundquist 2003.

MIG-10 is related to cytoplasmic factors, such as RIAM and Lamellipodin, which are in part responsible for proper neuronal outgrowth. According to Lafuente et al., MIG-10 is an ortholog of RIAM and Lamellipodin. All three proteins lack the SH2 domains characteristic of the Grb protein family, to which scholars initially thought MIG-10 was related. On the other hand, MIG-10, RIAM and Lamelliodin all share a C-terminal proline-rich region, an RA domain, and a conserved 27aa sequence in common with each other. Lafuente et al. have used these sequences to categorize RIAM,

Lamellipodin and MIG-10 separately from the Grb protein family; they proposed that a new family, MRL (MIG-10/RIAM/Lamellipodin) be used to distinguish these three proteins from the Grb proteins (Lafuente et al. 2004).

In addition to RIAM and Lamellipodin, Rac proteins play a key role in *C. elegans* axon development, including axon outgrowth, axon guidance, axon branching, and suppression of ectopic axon branching. Rac proteins have both upstream and downstream effectors, which imply that Rac control of axon development is tightly regulated and affects axon development in different stages and aspects (Lundquist 2003). The way Rac affects axon outgrowth reflects on differential regulation of the lamellipodial and filopodial growth-cone actin-cytoskeleton. In a similar manner, MIG-10 protein also appears to function in proper neuronal development, possibly involved in one of the Rac pathways. In mutant *mig-10 C. elegans*, several axons do not develop correctly and irregular neuronal migration is observed.

1.2.2 The mig-10 Gene and Cell Migration

The *mig-10* gene is involved in long-range neuron migration. When the gene is defective there is an incomplete migration of the following embryonic neurons: the canal associated neurons (CAN), anterior lateral microtubule cells (ALM), and hermaphrodite specific neurons (HSN). In mutant animals these cells do not migrate to their normal wild type position (Figure 2).

In normal worms, the CAN migrates from the head of the worm to approximately mid-body during embryogenesis. The ALM also migrates from anterior to posterior and is normally positioned in the midsection of the worm. In mutant worms these cells are more anterior within the body. The HSN cells migrate from posterior to anterior; in mutated worms these cells are positioned more posterior within the body. There are multiple phenotypes caused by the *mig-10*

mutations, including withered tail (Wit), shortened posterior excretory canal, egg laying defective (Egl), and axon outgrowth defects, that may be associated with cell migration. However, the exact role of the *mig-10* gene and its effects are unknown.

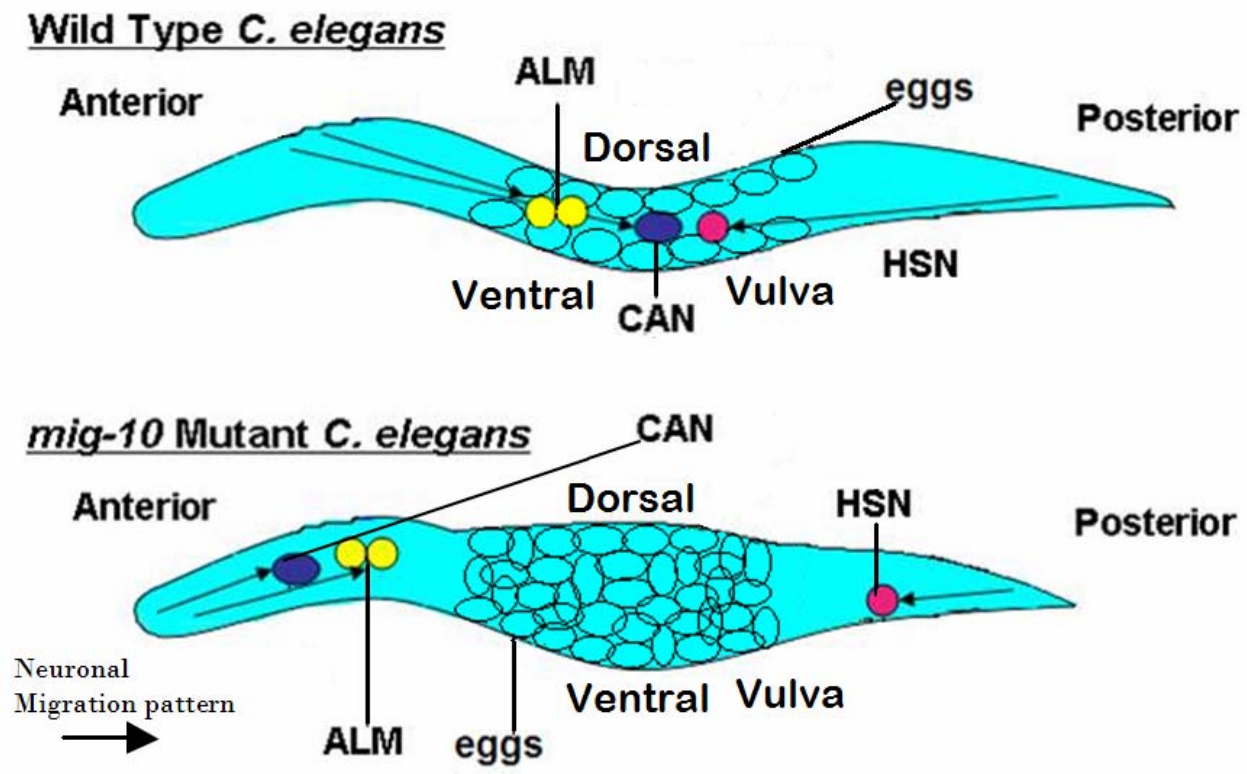

Figure 2: Comparison of neuron migration in wild type *C. elegans* and *mig-10* mutant *C. elegans.* The diagram depicts the positioning of the neurons after migration in wild type *C. elegans* as well as in *mig-10* mutant *C. elegans.*

1.2.3 Mutant Phenotypes

There are multiple phenotypes caused by neuronal misplacement. One is known as the withered tail (Wit). Nematodes with Wit appear to have a shorter tail with a blunter end than the tail of wild type worms (Figure 2). The mutant *C. elegans* typically moves more slowly and erratically because of its withered tail. The severity of the defect may be dependent on the extent of the migratory CAN misplacement (Manser and Wood 1990).

Another aspect of the phenotype is shortened posterior excretory canal. In wild type *C. elegans* the canal is a long process that extends the length of the body. However, in mutant nematodes the process does not extend the full length of the body; it ends at or anterior to the vulva. It is known that the misplacement of the CAN may be related to the defect, but cannot completely explain it due to the differences in the penetrance of the two defects. While the excretory canal defect is 100% penetrant, the penetrance of CAN misplacement is only 61%. Since the correlation between the two is not inclusive, the CAN defect is not wholly responsibe for the excretory canal mutation (Manser and Wood 1990).

A third phenotypic mutation of the *C. elegans* is the egg laying defect (Egl). In wild type worms the eggs are evenly placed in a row along the length of the body. In mutant *C. elegans* the eggs are laid at a much slower rate in comparison to the wild type nematode. The slow rate at which the eggs are laid causes eggs to build up in the body of the worm, making the body bulge and appear distorted. The degree of misplacement of the HSN may be correlated to how Egl the *C. elegans* will be (Manser and Wood 1990).

1.3 Transcript Variations

There are two different *mig-10* transcripts, which are *mig-10a* and *mig10b*. There are multiple characteristics that differentiate these and their respective protein isoforms. MIG-10A is 667aa in length and shares a carboxy-terminal end with the 650aa isoform of MIG-10B (Figure 3). It is not certain, which transcript is needed to accomplish the rescue of mutant *mig-10* defects.

Defects in migratory neurons are caused by mutations in MIG-10. It is hypothesized, however, that certain protein functions are associated with one or the other protein isoform. Consequently, different neuronal migration defects would need to be rescued by specific

promoter:gene expression in specific cells. These defects are caused by either of two recessive alleles of *mig-10, ct41* and *e2527*. *ct41*, one of the defective *mig-10* alleles, produces an amber stop codon in *mig-10* exon 3 that destroys protein function (Manser et al. 1997). The *e2527* allele carries a point mutation that alters one nucleotide, resulting in a splice acceptor mutation that is phenotypically less severe. Although there are two known *mig-10* mutations, only *ct41,* which results in a null protein, will be considered during this project because it causes more severe defects (Manser et al. 1997).

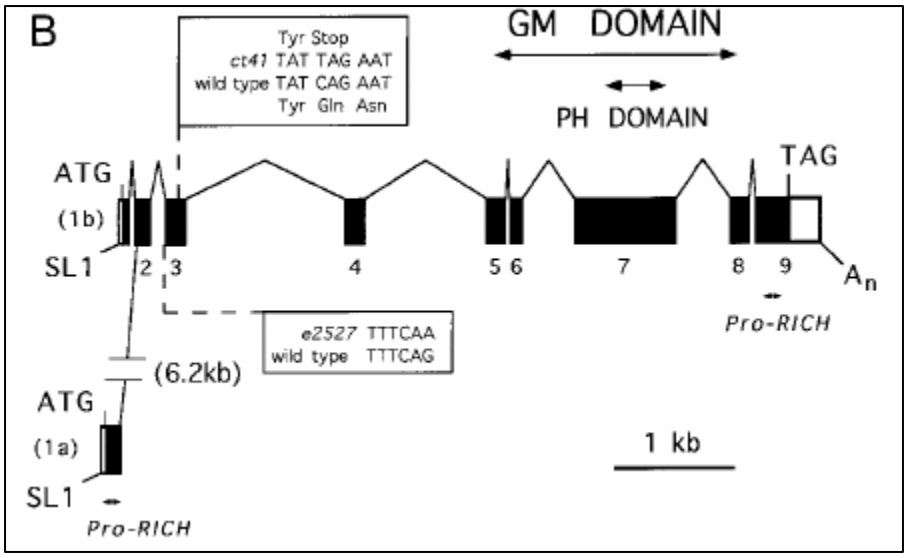

Figure 3: Map of MIG-10A and MIG-10B. MIG-10B and MIG-10A arise from transcripts which alternative first exons, MIG-10B being downstream of MIG-10A's first exon . The GM, PH, and proline-rich domains are shown; MIG-10A contains an additional proline-rich region on exon 1a. The *ct41* allele of *mig-10* is the result of a nonsense mutation in the third exon. Diagram taken from Manser et al., 1997.

1.4 Expression of Promoters

The promoter regions dictate where and when a gene will be expressed. The location of gene expression can influence whether cell autonomous or nonautonomous rescue is achieved. If rescue is cell autonomous, the expressed wild type proteins will rescue the mutant phenotypes associated with the cells in which the proteins are produced. Cell nonautonomous rescue indicates that proteins expressed in one cell type influence the surrounding cells. It is important to determine whether mutant rescue is cell autonomous or cell nonautonomous because it reveals where specific proteins should be actively expressed in order to obtain a wild typephenotype.

1.5 Previous Experimentation and Results

In past studies carried out by Rarus and Stovall, various promoters were used to determine the function and expression pattern of *mig-10*. Native promoters and others such as dpy-7 and mec-3 were used to test for rescue of the mutated neurons. The promoters used were paired with either *mig-10a* or *mig-10b* to determine if certain aspects of the defects were associated with a particular transcript.

Partial rescue of the ALM migration defect was achieved using several of these constructs, which were expressed both cell autonomously and cell nonautonomously. Rarus evaluated ALM migration rescue by expressing *mig-10a* cell autonomously using the mec-3 promoter, cell nonautonomously in the hypodermis using the dpy-7 promoter, and also using the native promoters coupled with their respective transcripts. Stovall had previously examined the ALM migration and shortened posterior excretory canal defects and evaluated the success of their rescue using various promoter:*mig-10* constructs. The expression of *mig-10a* or *mig-10b* yielded only partial rescue, which may indicate that the transcripts have different roles. These results led to the hypothesis that both cell autonomous and nonautonomous expression are necessary for proper migration as well as to obtain complete phenotype rescue. The possibility of achieving rescue by the simultaneous expression of transcripts *mig-10a* and *b*, as well as autonomous and nonautonomous expression, has also lead to ideas for future experiments.

1.6 Objectives

The determination of *mig-10* function and expression is the main objective of this project. While previous teams had studied the ALM migration phenotype, we focused on the Egl and Wit phenotypes. Attempts were made to rescue the *mig-10* mutation in *C. elegans* using the native *mig-10* promoters, which expressed the *mig-10* transcripts in different locations of the *C. elegans*.

The ability of the mig-10a:*mig10a* and mig10b:*mig-10b* constructs to rescue the Egl and Wit phenotypes in the mutant worm was observed. Comparison of known expression patterns of *mig-10a* and *b* with rescue patterns observed suggested whether rescue of Egl and Wit was occurring through expression in vulva (*mig-10b*) or HSN (*mig-10a*).

An additional promoter, ceh-23, was coupled with each of the *mig-10* transcripts to promote cell autonomous expression in the CAN as well as nonautonomous expression in the excretory canal. It is hypothesized that complete rescue of mutant phenotypes and migratory defects will be achieved only if both transcripts of *mig-10* are expressed simultaneously.

2.0 Materials and Methods

2.1 Transformation

DH5α-competent cells were thawed on ice and plasmid DNA was added to cells. The competent cells and DNA were left to incubate on ice for one half hour. After this incubation the cells were heat shocked at 42°C for 45 seconds and then returned to ice for two minutes. Competent cells were resuspended in 500 μL of liquid LB media and the solution was then placed in a 37°C shaker for approximately 45 minutes to an hour to confer ampicillin resistance. Cells were plated on LB + 50 μg/ml ampicillin plates in the following volumes: Plate 1, 10 μL; Plate 2, 100 μL; Plate 3, remainder of cells. The plates were then placed in a 37°C incubator overnight.

2.2 Miniprep

A miniprep was performed using the procedure published in the 2004 *QIAprep Miniprep Handbook.* Multiple colonies were chosen from the ampicillin-containing plates to use for cloning. Each colony was inoculated into separate 5 mL aliquots of Luria-Bertani (LB) broth containing 50 μg/mL ampicillin. The tubes were then placed in a 37°C shaker for 12-16 hours. After this duration the cultures were centrifuged in a microcentrifuge at 12,000 rpm for 2 minutes or until there was formation of a compact pellet.

Following pellet formation, 250 μL of Buffer P1 was added and the contents were vortexed until the entire pellet had been resuspended. Immediately following resuspension, 250 μL of Buffer P2 was added to cause a lysis reaction. The tube containing solution and cells was inverted to mix, forming a clear solution. In order to neutralize the mixture, 350 μL of Buffer N3 was added and the tube was inverted to mix. The solution was then microcentrifuged for 10 minutes.

The supernatant from the spin was pipetted into a spin column and centrifuged for 60 seconds. 0.5 mL of Buffer PB was added to the spin column and centrifuged for 60 seconds. The solution that flowed through the spin column during centrifugation was discarded. 0.75 mL of Buffer PE was added to the spin column. The spin column was centrifuged for 60 seconds. The flow-through was once again discarded, and then the spin column was centrifuged for another 60 seconds. Once this step was complete 50 uL of Buffer EB was added to the spin column. The spin column stood for 60 seconds and was then centrifuged for 60 seconds. The minipreps were stored at -20°C.

2.3 Restriction Enzyme Digest

3-5 uL of mini-prep DNA were digested in a 20uL reaction containing dH_2O, Buffer, BSA, and necessary restriction enzymes. The digest was carried out in a 37°C incubator overnight.

2.4 Agarose Gel Electrophoresis

An 0.8% agarose gel was prepared to run the cloned samples. 0.8 g of agarose was added to 100 mL of TBE buffer. This mixture was then microwaved for approximately 4 minutes, or until all of the agarose had thoroughly dissolved into the buffer, making a clear solution. The solution was left until it had cooled to slightly warmer than room temperature. 10 µL of 10 mg/mL ethidium bromide was added. The solution was poured into a gel box and sat at room temperature until it solidified.

5 µL of 0.25 % Xylene Cylanole/0.25% Bromophenol Blue (BPB) dye was added to each 8-10 µL DNA sample and loaded into separate wells of the gel. 10 µL of 1 kb marker mixed with 5 µL of BPB was also added to the gel. The gel was run at 50-60 Volts for 3.5- 4 hours.

2.5 Gel Isolation and Extraction

Both mec-3:mig-10a[b] and ceh-23:GFP samples were digested with SphI and XmaI. The double digested products were run on separate 0.8% agarose gels in a TAE buffer to obtain the necessary fragments for extraction. Once the bands had separated correctly, the ceh-23 vector backbone (from the ceh-23:GFP plasmid), and the *mig-10a*[*b*] insert (from the mec-3:mig-10a[b]) fragments were cut out of the TAE gel using a razor blade under low frequency UV light. These samples were then prepared using a QIAquick Gel Extraction Kit (QIAGEN Cat. No. 5028704) to extract and purify the DNA.

2.6 Ligation

The sample concentration of the purified ceh-23 vector DNA and *mig-10a*[*b*] DNA were calculated by comparing multiple sample dilutions to the New England Biolabs 500 µg/µL 1kb ladder. The *New England BioLabs Catalog* lists the sample weight of each band in the ladder. Two dilutions (3 µL, 6µL) of ceh-23 vector DNA and *mig-10a*[*b*] DNA were run on the electrophoresis gel for comparison to the ladder. The intensity of the fragments was compared to the varying intensities of each band composing the ladder. Once the band of the 1kb ladder that was identical to the sample (ceh-23 vector backbone, *mig-10a*[*b*]) was identified the sample weight could be determined. The weight of the fragment of the 1kb ladder was obtained from the *New England Biolabs Catalog*. The actual weight was then calculated by multiplying the known weight of the band by the concentration. The actual band weight was then divided by the concentration of the sample.

Once the sample weight had been determined for each sample the ratio between the two samples of DNA had to be calculated. The following formulas were used to calculate the proportional difference between the ceh-23 vector backbone and *mig-10a[b]*.

λ_V = #μL of vector to pipette
λ_I = #μL of insert to pipette
C_X = Concentration
$[(ng/mol_V)/(ng/mol_I)] \equiv [(kb/mol_V)/(kb/mol_I)]$

$\lambda_V * (mol/\mu L)_V = \lambda_I * (mol/\mu L)_I$

$\lambda_V * (mol/ng)_V * (ng/\mu L)_V = \lambda_I * (mol/ng)_I * (ng/\mu L)_I$

$\lambda_V/\lambda_I = (kb_V/kb_I) * (C_I/C_V)$

Based on these calculations a 20 μL ligation reaction was prepared using a 7.5:1 molar ratio for both ceh-23 to *mig-10a* and *mig-10b*. The reaction was also composed of New England Biolabs 10X T4 ligase buffer, and New England Biolabs T4 DNA ligase. The reaction was left at room temperature for 2 hours.

The Reactions Consisted of:

ceh-23 Vector Backbone and *mig-10a* Ligation Reaction
15 μL 7kb ceh-23 vector backbone
2 μL 5kb mig-10A insert
2 μL New England Biolabs 10X T4 ligase buffer
1 μL New England Biolabs T4 DNA ligase

ceh-23 Vector Backbone and *mig-10b* Ligation Reaction
15 μL 7kb ceh-23 vector backbone
2 μL 5kb mig-10B insert
2 μL New England Biolabs 10X T4 ligase buffer
1 μL New England Biolabs T4 DNA ligase

2.7 Polymerase Chain Reaction

Master Mix
13.5 µL rdH_2O
2.5 µL 10X PCR Buffer 3 (Boehringer Mannheim)
2.5 µL dNTP (2.5 mM dTTP, 2.5 mM dATP, 2.5 mM dCTP, 2.5 mM dGTP)
2.5 µL Trans primer
0.5 µL Taq polymerase (Fisher Scientific)
21.5 µL total master mix/sample

Master mix was prepared and 21.5 µL were pipetted into four small microfuge tubes. 1 µL of sample DNA was pipetted into each microfuge tube and then 2.5 µL of specified primer was added. Following DNA and primer addition, each tube contained 25 µL of sample solution. These tubes were then transferred to the thermal cycler to proceed through the following programmed cycle:

10 min @ 94º C (initial denaturing step)
Cycle 30 X:
    30 sec @ 94ºC (denature)
    1 min @ 60ºC (anneal)
    2 min @ 72ºC (primer extension)
10 min @ 72ºC (final extension)
Hold @ 12º C indefinitely

2.8 *C. elegans Egl* and *Wit* Assay

Wild type NY2053, mutant RY96, and rescued RY0135 L4's were picked and each placed separately onto individual agar plates that contained OP50 *E. coli* for food. Worms were kept at 20°C. Plates were observed every 8 hours over a 72 hour period. The phenotypes of the selected worms were examined, noting severity of the Egl and Wit phenotypes. Egg and L1 worm counts were recorded for each plate at each time interval. The gravid worms were picked separately onto new plates after every observation period.

2.9 Genetic Crosses

To analyze the effects of *mig-10* mutations on the HSN, a strain was constructed to include a marker for the particular neuron (Figure 4). In the first cross, eight wild type males were plated along with three hermaphrodites homozygous for the HSN marker (Figure 4A). Eight heterozygous male progeny from this cross were then plated with three RY0135 hermaphrodites (Figure 4B). In order to complete the third cross, hermaphrodite progeny containing both markers (i.e. GFP and HSN markers) were selected to self-fertilize on separate plates (Figure 4C). The progeny of the self-cross were later observed in order to determine whether all progeny contained the GFP marker. If the GFP marker was visible within all the progeny it would indicate that they were all homozygous for that marker. The aforementioned strategy was also repeated in order to create a similar strain derived from the RY0128 rescue strain. The progeny that were homozygous for *mig-10* were isolated due to their Egl phenotype.

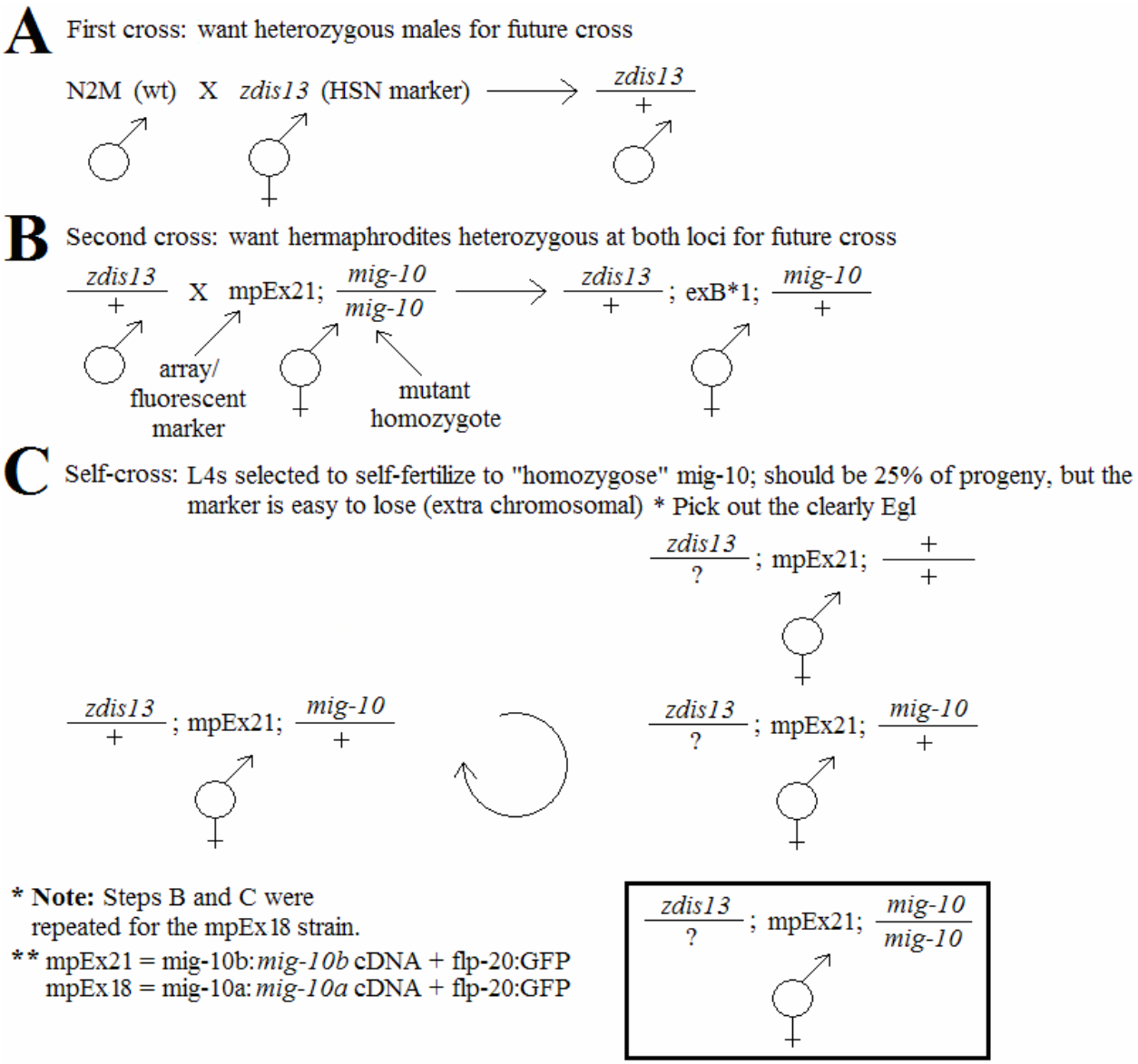

Figure 4: Genetic crosses to incorporate fluorescent HSN marker into *mig-10* mutant *C. elegans*

2.10 10 Worm PCR

Lysis Buffer and Enzyme Solution
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
0.15 mg/mL Proteinase K (lysis enzyme)

2.5 µL of pre-prepared lysis buffer and enzyme solution was pipetted into each of six PCR tube caps. Ten worms were transferred into each tube cap; two tubes contained wild type worms, two tubes contained homozygous mutant RY0128 worms, and two tubes contained RY0128 worms containing both markers from the genetic cross procedure. The tubes were microcentrifuged briefly before 50 µL of mineral oil was pipetted over the solution. The tubes were transferred into a -80ºC freezer for 30 minutes, and then immediately placed into the thermocycler to undergo the following lysis program:

1 hour @ 65ºC (lysis step)
15 min @ 95ºC (inactivation of Proteinase K)
Hold @ 12º C indefinitely

Master Mix
12 µL rdH_2O
2.5 µL 10X Long PCR Buffer 3 (contains $MgCl_2$)
2.5 µL 2.5 mM dNTP mix
2.5 µL 3 mM Top Primer
2.5 µL 3 mM Bottom Primer
0.5 µL Taq polymerase (Fisher Scientific)
22.5 µL total master mix/sample

Following the lysis step, a master mix was prepared and 22.5 µL was pipetted into each of the six tubes containing the lysed worms. These tubes were then transferred to the thermalcycler to proceed through the following programmed cycle:

10 min @ 94º C (initial denaturing step)
Cycle 30 X:
    30 sec @ 94ºC (denature)
    1 min @ 60ºC (anneal)
    2 min @ 72ºC (primer extension)
10 min @ 72ºC (final extension)
Hold @ 12º C indefinitely

3.0 Results

The purpose of this project was to determine in which cells MIG-10 needs to be expressed, as well as the transcripts that are needed in order to rescue mutant phenotypes. The mig-10a:*mig-10a* and the mig-10b:*mig-10b* constructs were used to observe whether the expression of either of these transcripts was sufficient to obtain complete rescue of the mutant phenotypes Egl and Wit. The ceh-23:*mig-10a* and ceh-23:*mig-10b* constructs were created to determine whether rescue of the foreshortened excretory canal could be achieved cell nonautonomously through expression of *mig-10* in the CAN as well as rescuing CAN migration autonomously. Further attempts were made to reveal the relationship between HSN neuron migration and the Egl phenotype through utilization of fluorescent HSN markers.

3.1 *C. elegans Egl* Assay

Previous assays have been conducted in attempts to understand which isoforms and expression patterns of *mig-10* are needed to rescue ALM migration and the excretory canal outgrowth mutation using the mec-3:*mig-10b* construct. In a new assay, the severity of Egl and Wit defects was examined between three different strains of *C. elegans*. The NY2053 strain was used to observe wild type behavior, while the RY96 mutant strain was assayed to examine mutant phenotypes (Table 1). To test the success of attempted rescue, the phenotypes and the egg laying rate of the RY0135 strain, which contains a transgene with *mig-10b* driven by its native mig-10b promoter, was compared to those of the wild type NY2053 and mutant RY96 worms. Egg and L1 counts were recorded for each strain at each time point to assess Egl severity (Figure 5). The assay was carried out two times. The difference between the two assays conducted was the first assay had an extra time point of observation and the second assay looked more qualitatively at the severity of the Egl and Wit phenotypes.

Name in paper	Strain name	Genotype	Array Description	Comments
N2	N2	wild type	-	-
***mig-10*(ct41)**	BW0315	*mig-10*	-	-
flp-20:GFP	NY2053	ynIs53	ynIs53- Integrated GFP construct, GFP expressed in ALM, AVM, PLM, and PVM	Used as ALM migration model From Chris Li lab
***mig-10*; flp-20:GFP**	RY0096	*mig-10*; ynIs53	ynIs53- Integrated GFP construct, GFP expressed in ALM, AVM, PLM, and PVM	Used as *mig-10* mutant model Created by Elizabeth Stovall
mig-10a:*mig-10a*	RY0128	*mig-10;* mpEx18	mpEx18- mig-10a promoter expressing *mig-10a* in ALM, AVM, PLM, and PVM with flp-20:GFP	Created by Elizabeth Stovall
mig-10b:*mig-10b*	RY0135	*mig-10;* mpEx21	mpEx21- mig-10b promoter expressing *mig-10b* in vulva with flp-20:GFP	Created by Elizabeth Stovall
ceh-23:*mig-10a*			Expresses *mig-10a* in the CAN	
ceh-23:*mig-10b*			Expresses *mig-10b* in the CAN	

Table 1: Strain names, arrays, and array descriptions.

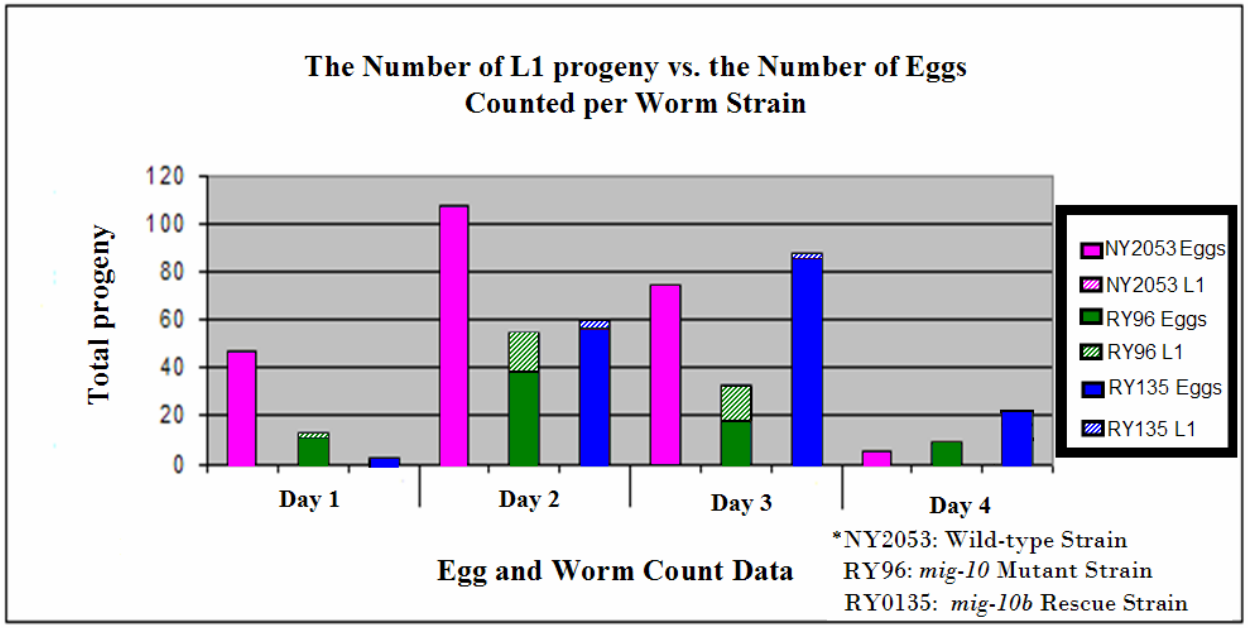

Figure 5: Progeny average for each worm type for Assay #1. The different strains correspond to different colors. The average total progeny for each strain is traced over a four day period. Solid bars represent the number of eggs laid, and cross hatch bars represent the number of L1s present.

These results suggest that RY0135 animals were partially rescued for the Egl and Wit phenotypes present in the mutant RY96 animals (Table 2). Both the eggs and L1 progeny were monitered during the assay. The relative proportion of hatched and unhatched worms was

significant because it reflects the severity of the Egl behavior. More severe mutants retain their eggs longer than the wild type worms; consequently, the progeny develop longer within the gravid worm and thus hatch sooner after they are laid.

		Total # of worms	Number of worms displaying phenotypic defects	
			Egl	Wit
NY2053	Day 1	5	1	1
	Day 2	4	0	0
	Day 3	4	0	0
	Day 4	4	0	0
RY96	Day 1	5	2	2
	Day 2	2	2	2
	Day 3	1	1	1
	Day 4	1	1	1
RY0135	Day 1	5	2	3
	Day 2	4	2	3
	Day 3	4	2	3
	Day 4	4	2	3

Table 2: Severity of Egl and Wit. Animals were observed daily to trace Egl and Wit behavior of the three strains. Four of five NY2053s displayed wild type egg laying behavior and had normal tails; the only abnormal worm died on the first day of observation. All five RY96s displayed one or both mutant phenotypes, and only one worm survived the duration of the assay. One RY0135 array worm died but the remaining four displayed one or both mutant phenotypes, but these phenotypes were more moderate than those displayed by the mutant RY96s.

There were fewer RY0135 rescue than RY96 mutant L1 progeny during the time course, indicating that a partial rescue had taken place. However, rescue is incomplete in comparison to the NY2053 strain because the RY0135 animals still appeared to have delayed egg laying (Figure 5). While it is possible that younger worms from this strain were accidentally selected, several worms from this rescue strain displayed the Egl and Wit phenotypes while the wild type worms did not, again indicating that the mutant rescue was not a total success. The experiment was repeated and a rating system was used to record the severity of the Egl and Wit phenotypes for each strain (Figure 6, Table 3, Table 4). Partial rescue of the RY0135 rescue strain was observed for each phenotype. The total animal population varied due animals that bagged mid-day, indicating that the animal was definitely Egl but may or may not have been Wit.

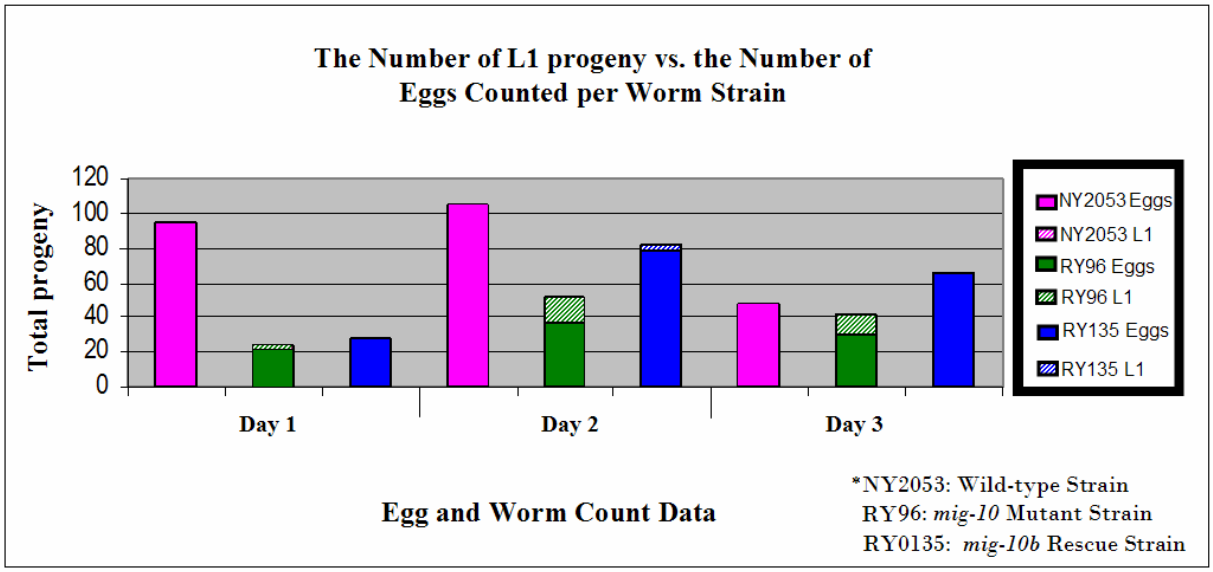

Figure 6: Progeny average for each worm type for Assay #2. The different strains correspond to different colors. The average total progeny for each strain is traced over a three day period. Solid bars represent eggs laid, and cross hatch bars represent L1s present.

Worm Line	Day	No. Egl animals / total animals (%)	Severity			
			0	+	+ +	+ + +
NY2053	1	0/3 (0)	3	0	0	0
	2	0/3 (0)	1	2	0	0
	3	0/3 (0)	2	1	0	0
RY96	1	0/5 (0)	0	5	0	0
	2	4/5 (80)	0	0	1	4
	3	5/5 (100)	0	0	0	5
RY0135	1	1/9 (11)	0	8	0	1
	2	1/8 (13)	1	2	4	1
	3	2/7 (29)	0	3	2	2

Table 3: Egl severity for Assay #2. Animals were observed at 8 hour intervals to determine Egl severity. The rating system indicates increasing severity; Egl animals that exhibited delayed egg laying and did not have an even row of eggs lining the body had the lowest (+) rating; Egl animals that had more severe delayed egg laying, appeared to be filled with eggs, and were not moving as quickly had the (+ +) rating; Egl animals that had bagged or appeared as if they about to bag had the highest (+ + +) rating.

Worm Line	Day	No. Wit animals / total animals (%)	Severity			
			0	+	+ +	+ + +
NY2053	1	0/3 (0)	3	0	0	0
	2	0/3 (0)	0	3	0	0
	3	0/3 (0)	0	3	0	0
RY96	1	0/5 (0)	0	3	2	0
	2	2/5 (40)	0	2	1	2
	3	0/5 (0)	0	1	4	0
RY0135	1	1/9 (11)	1	4	3	1
	2	0/7 (0)	1	3	3	0
	3	0/5 (0)	0	2	3	0

Table 4: Wit severity for Assay #2. Animals were observed at 8 hour intervals to determine Wit severity. The rating system indicates increasing severity; Wit animals had the highest (+ + +) rating.

The Egl/Wit assay was repeated once more. The rescue strain assessed in this final assay was RY0128, which contains a transgene with *mig-10a* driven by its native mig-10a promoter, and wild type and mutant controls were also examined (Figure 7). Egl and Wit severity was also recorded daily (Table 5, Table 6). These results suggest that RY0128 animals were not rescued for either Egl or Wit phenotypes present in the mutant RY96 animals. Both the eggs and L1 progeny were monitored during the assay to determine Egl rescue. Compared to the mutant strain, the rescue RY0128 worms exhibited no improvement in egg laying efficiency. Over the course of the assay, the RY0128 animals laid fewer eggs and exhibited more severe Egl and Wit behavior than their mutant counterparts.

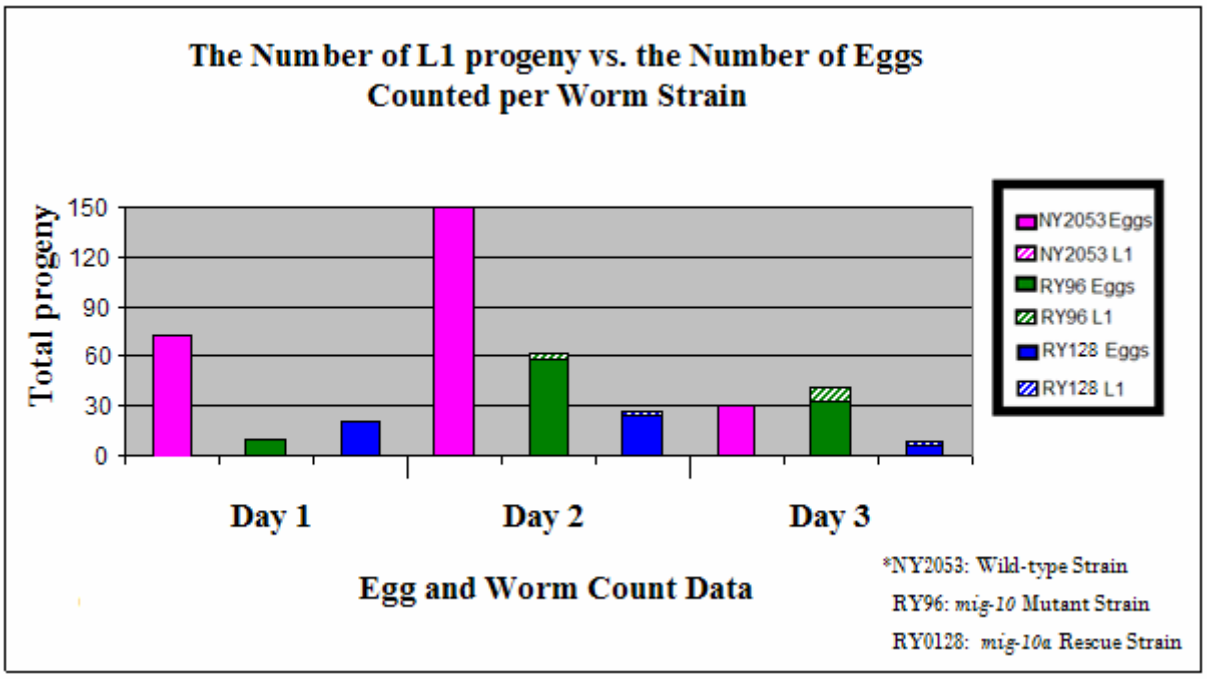

Figure 7: Progeny average for each worm type for Assay #3. The different strains correspond to different colors. The average total progeny for each strain is traced over a three day period. Solid bars represent eggs laid, and cross hatch bars represent L1s present.

Worm Line	Day	No. Egl animals / total animals (%)	Severity			
			0	+	++	+++
wild type	1	0/8 (0)	8	0	0	0
	2	0/6 (0)	4	2	0	0
	3	0/6 (0)	6	0	0	0
mutant	1	0/8 (0)	1	5	2	0
	2	1/7 (14%)	0	5	1	1
	3	0/7 (0)	0	2	4	0
RY0128	1	0/12 (0)	10	1	1	0
	2	0/9 (0)	5	4	0	0
	3	1/8 (13%)	3	3	1	1

Table 5: Egl severity for Assay #3. This assay was conducted once daily to compare the severity of the Egl defect in RY0128 worms to the wild type and mutant strains. Unlike the worms used in the previous assays, the wild type and mutant worms examined here did not contain the flp-20:GFP construct and so did not fluoresce. Refer to Table 3 for further details.

Worm Line	Day	No. Wit animals / total animals (%)	Severity			
			0	+	++	+++
wild type	1	0/8 (0)	3	4	1	0
	2	0/6 (0)	3	1	2	0
	3	0/6 (0)	3	1	2	0
mutant	1	0/8 (0)	5	3	0	0
	2	0/7 (0)	0	6	0	0
	3	0/7 (0)	2	4	1	0
RY0128	1	0/12 (0)	5	7	0	0
	2	0/9 (0)	2	4	5	0
	3	0/8 (0)	2	6	0	0

Table 6: Wit severity for Assay #3. This assay was conducted once daily to compare the severity of the Wit defect in RY0128 worms to the wild type and mutant strains described in Table 5. Refer to Table 4 for further details.

3.2 Construction of the ceh-23:*mig-10b[a]* Plasmids

In order to construct ceh-23:*mig-10a* and ceh-23:*mig-10b*, we isolated fragments from the starting plasmids mec-3:*mig-10a*, mec-3:*mig-10b* and ceh-23:GFP (Figure 8).

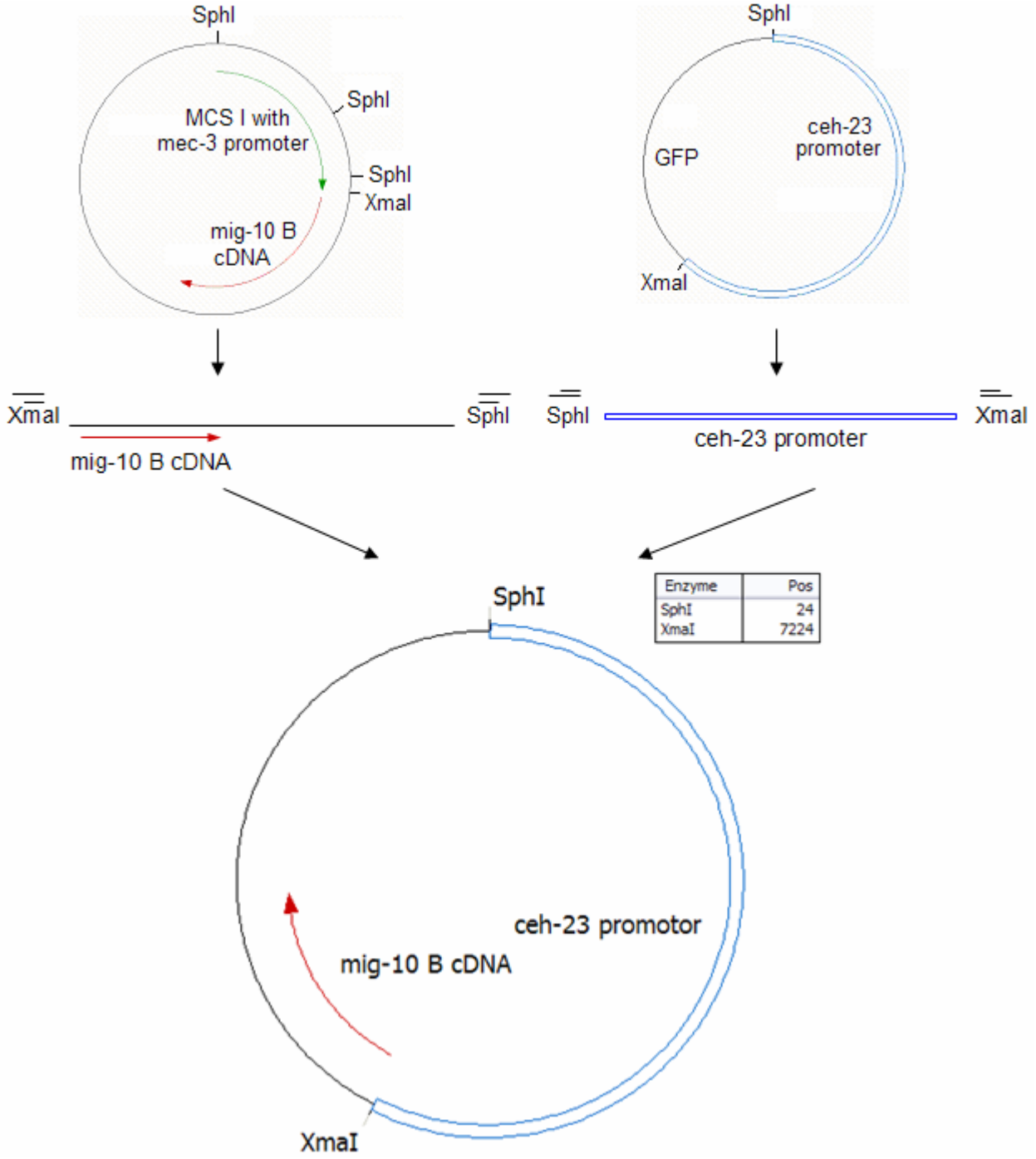

Figure 8: ceh-23:*mig-10* construct assembly.

3.2.1 Confirmation of Initial Constructs

PCR was used to confirm the presence of *mig-10a* or *mig-10b* in a mec-3:*mig-10* construct using primers specific to *mig-10a* and *mig-10b* (Figure 9). Ultimately the *mig-10* fragments were isolated from the electrophoresis gels and purified in order to carry out the final ligation with the ceh-23 promoter.

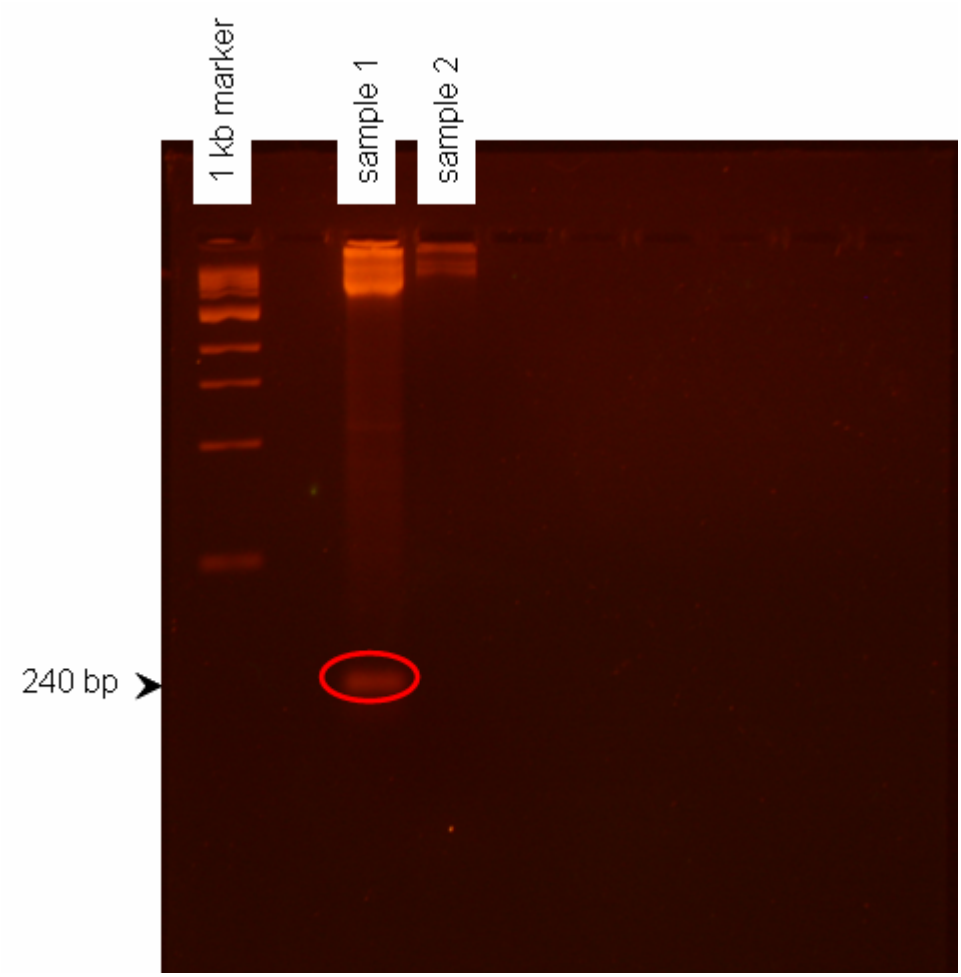

Figure 9: PCR confirmation of the *mig-10a* construct. Samples 1 and 2 are each a different PCR reaction. The tran-b and *mig-10a* primers were used on sample 1. The primers used on sample 2 were the tran-b and *mig-10b* primers. *mig-10a* DNA was correctly identified in the sample 1 lane. The highlighted band in the figure marks the position of the PCR fragment produced.

Two different digests were electrophoresed simultaneously in order to positively identify the ceh-23:GFP construct. The gel below indicates the band lengths of the fragments produced by each digestion (Figure 10). The ceh-23 promoter is 7196 bp in length (Figure C-3); the ceh-23:GFP construct was later redigested, isolated, and purified for the final ligation procedure.

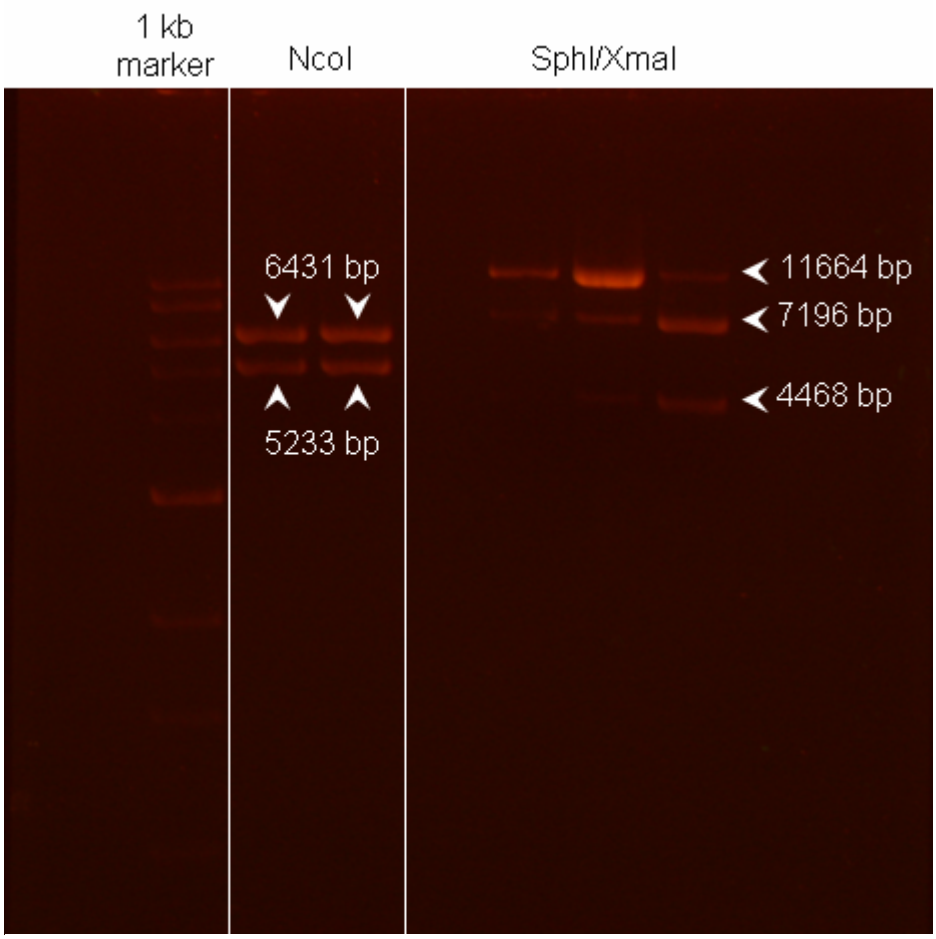

Figure 10: ceh-23:GFP restriction digests. Two digests of ceh-23:GFP. The first digest used NcoI, producing two bands (6431 bp and 5233 bp). In the second digest, SphI and XmaI were used to isolate the ceh-23 promoter (7196 bp) from the construct. A longer fragment of presumably 11664 bp appears in this digest as well and indicates a partial digest.

3.2.2 Confirmation of ceh-23:mig-10 Ligation

In order to evaluate the success of our ligation, we had to first confirm the presence of the two *mig-10* transcripts. PCR was performed as described in the Materials and Methods using transcript-specific primers (Figure 11).

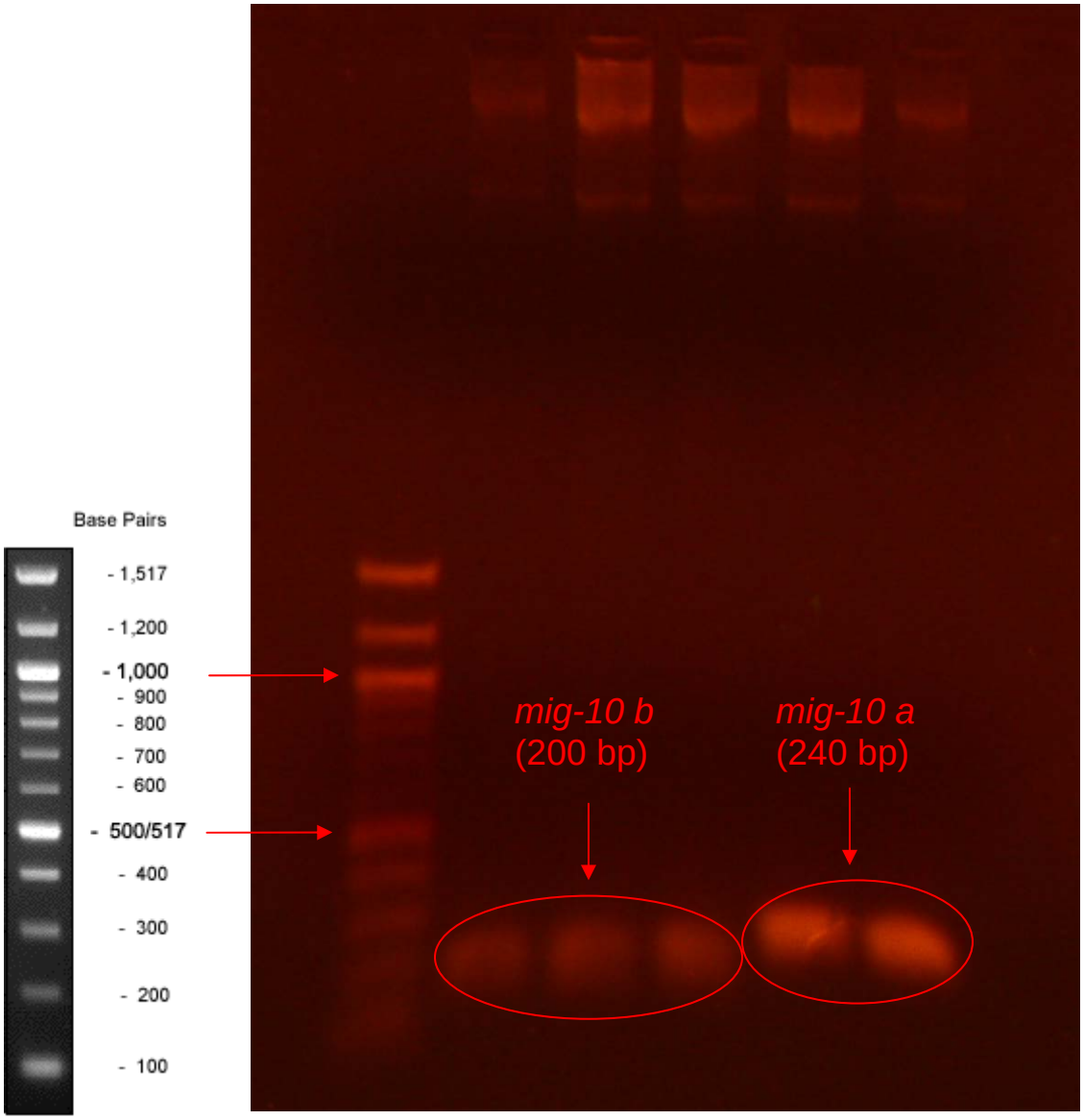

Figure 11: PCR confirmation of *mig-10* transcripts. Five different PCR reactions were electrophoresed. The tran-b and *mig-10b* primers were used on the first three samples. The last two samples were primed with tran-b and *mig-10a* primers. Each transcript was correctly identified in their respective sample lanes. The encircled regions above mark the positions of each PCR fragment produced.

The success of the ligation of ceh-23:*mig10* was also confirmed via restriction digest using the enzymes SphI and XmaI. Although each digest was partial, the expected ceh-23 and *mig-10* transcript bands were present (Figure 12).

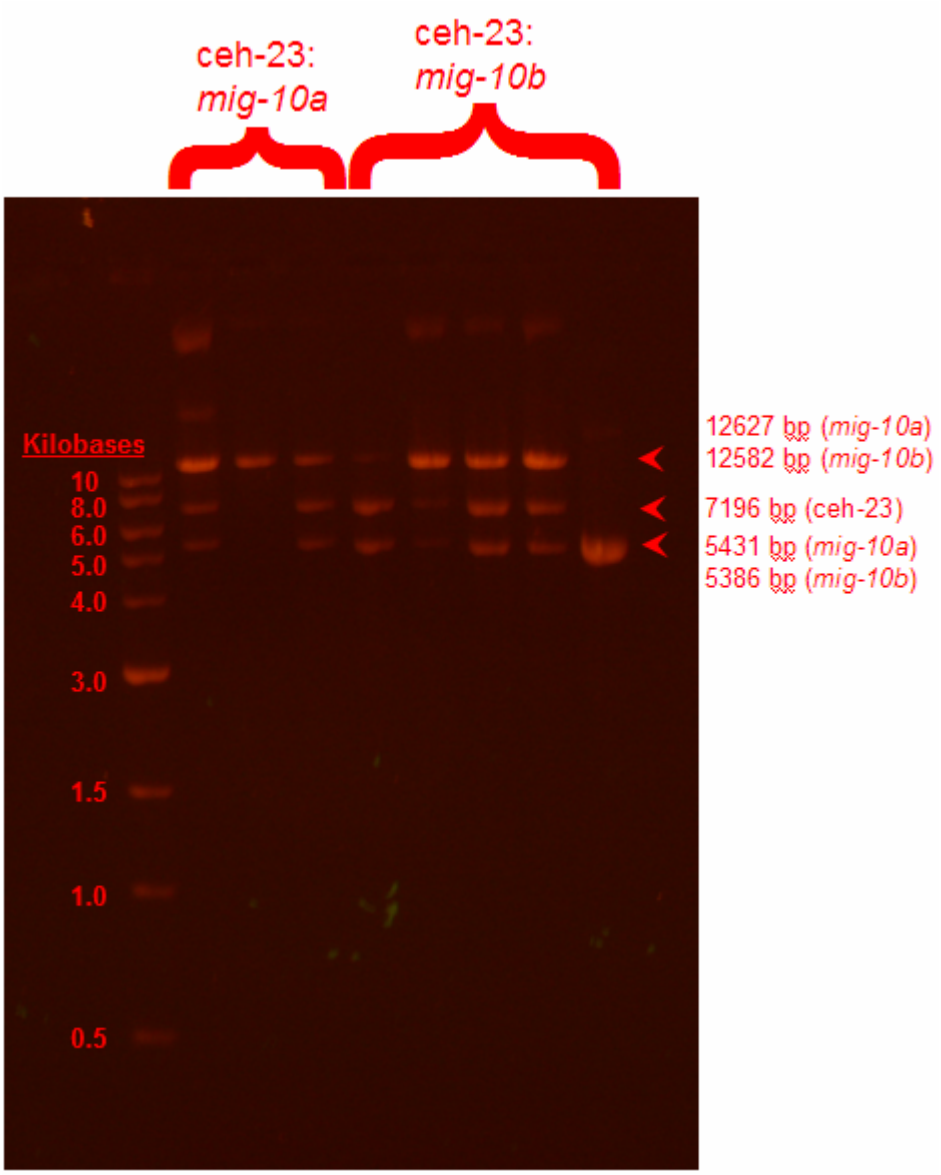

Figure 12: Restriction digest of ceh-23:*mig-10a* and *mig-10b* to confirm ligation success. The restriction enzymes SphI and XmaI were used to cut the purified DNA from the ligation product to confirm the presence of both ceh-23 and *mig-10*, but the digest was partial. The bands at the 12627 and 12582 bp positions are the ceh-23:*mig-10* constructs, which were not digested. Nevertheless, the presence of the ceh-23 promoter and *mig-10* transcripts was confirmed. ceh-23 is present at 7196 bp position in lanes (from left) 2, 4, 5, 7 and 8, *mig10a* is present at 5431 bp position in lanes 2 and 4, and *mig-10b* is present at the 5386 bp position in lanes 5, 7 and 8.

*3.2.3 Removal of 600 bp Piece from ceh-23:*mig-10b *Construct*

The restriction enzymes XmaI and PstI were used to digest ceh-23:*mig10a* and ceh-23:*mig-10b* to remove a 600 bp piece of ceh-23:*mig10*. The 600 bp piece contained a second ATG that needed to be mutagenized. The 600 bp band is not a very intense band, indicating that the digest was partial (Figure 13).

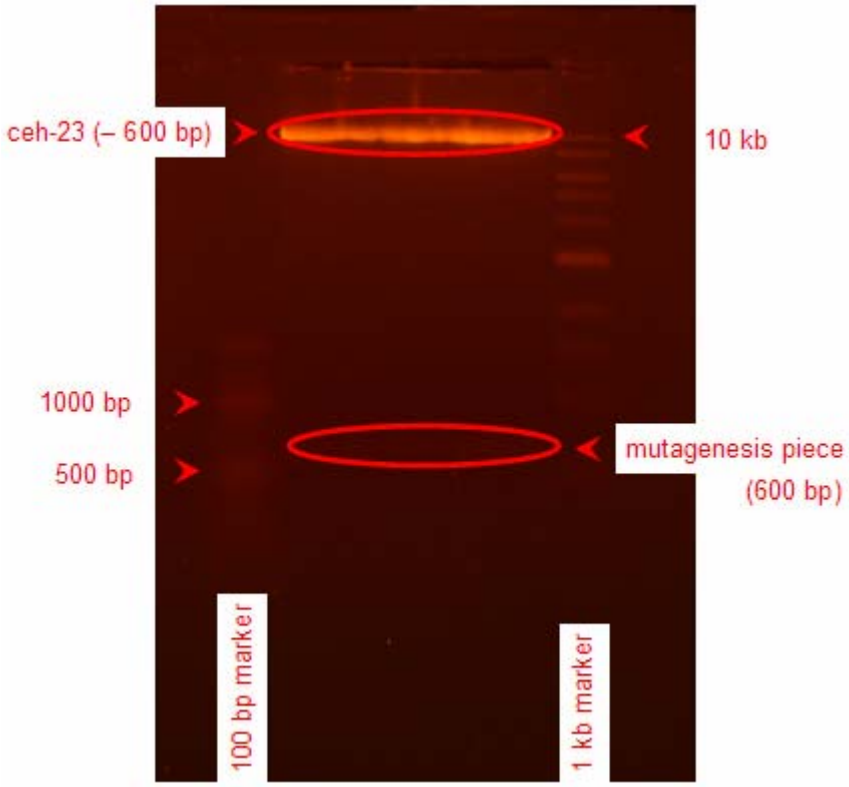

Figure 13: Removal of 600bp piece from ceh-23:*mig-10b* construct. A restriction digest was performed using the enzymes XmaI and PstI to remove a 600bp piece of the ceh-23:*mig-10b* construct. The 600bp piece is visible at the 600bp position. The partially digested ceh-23:*mig10b* construct is visible above the 10 kb marker.

3.3 Mutagenesis of ATG Site

The ceh-23 promoter contained the ATG for GFP; this start site needed to be deleted so that the ceh-23:*mig-10* constructs would begin translation at the correct AUG in the mRNA. The ATG site was mutated by two specially designed primers into a UAG site (Figure 14). Electrophoresis was performed to visualize the results of the PCR (Figure 15).

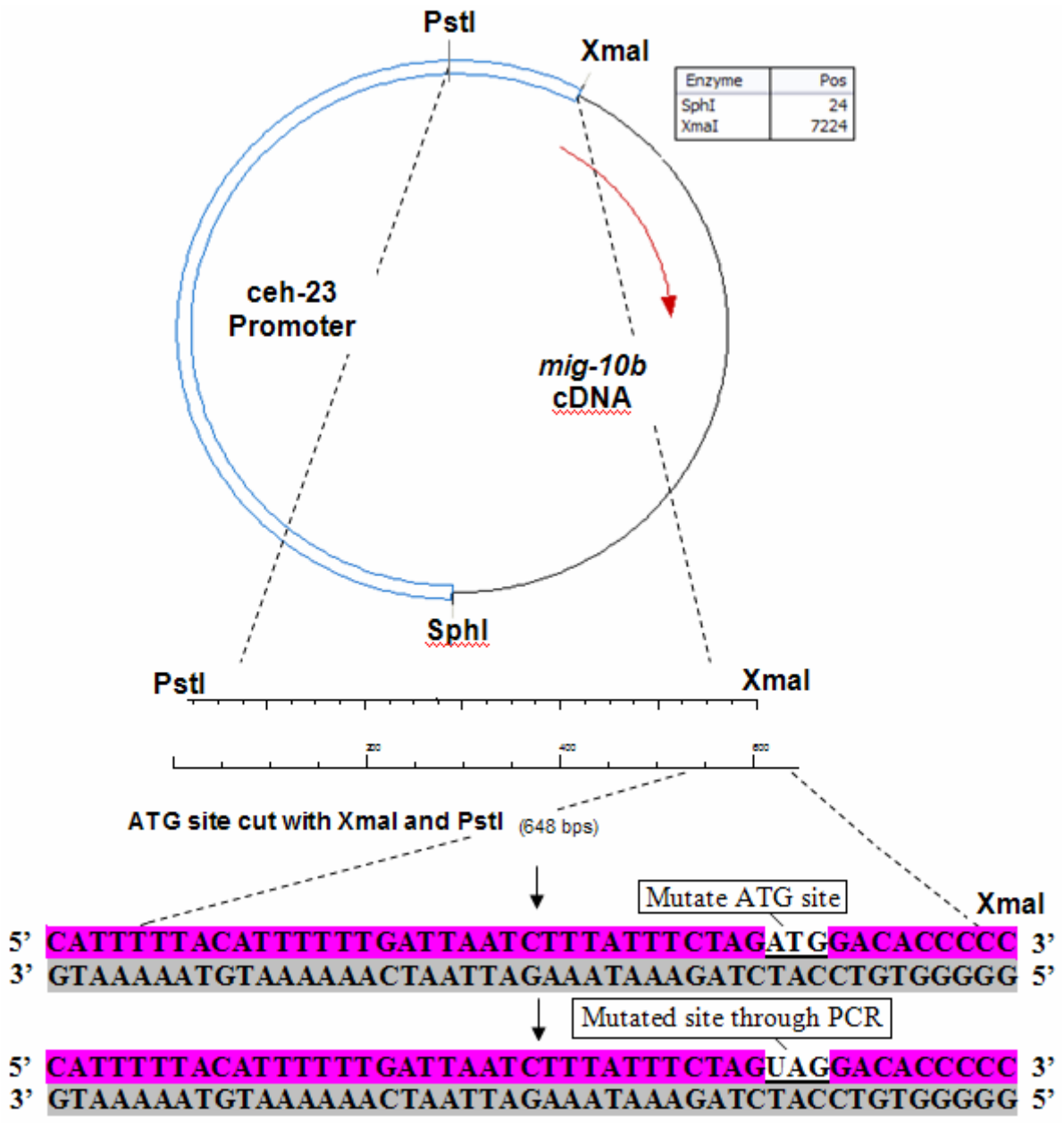

Figure 14: Mutagenesis of ceh-23 promoter start codon. The primers used were ceh-23PstI (5' GGAGCAGCTG CAGAAATCGAGAAGG 3') and ceh-23XmaI (5' GGCCAATCCCGGGGGGGTGTCCTACTAG 3'.)

After the completion of the mutagenesis step, the group was unable to ligate the mutagenized 600bp piece of the ceh-23 promoter with the ceh-23:*mig-10* construct.

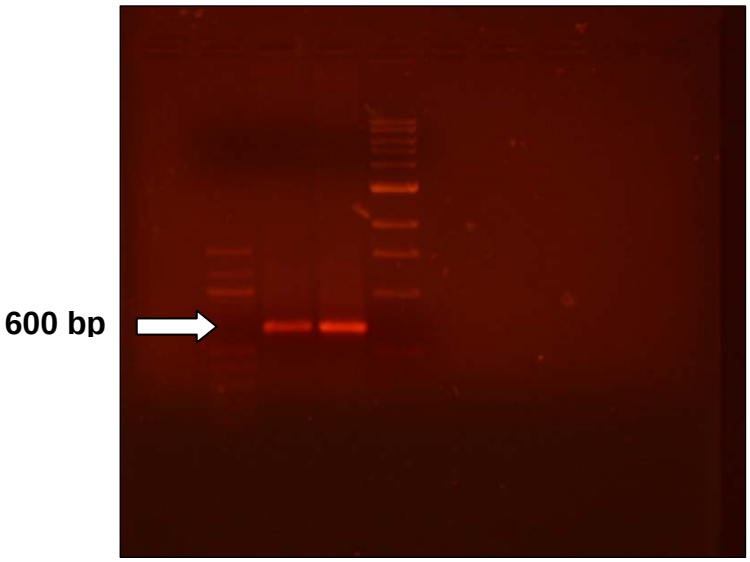

Figure 15: PCR of mutagenesis fragment. The primers were designed to amplify a 600 bp area of the ceh-23 promoter. The bands present on the gel are both at 600 bp position, signifying that the PCR worked correctly.

3.4 10 Worm PCR

Worm crosses were been carried out in an attempt to isolate the homozygote containing both the mpEx21 and mpEx18 arrays and HSN (*zdIs13*) markers in order to follow the progression of HSN migration within the mutant progeny. The objective was to visually determine whether incomplete HSN migration correlated with the Egl defect. A 10 worm PCR was performed on wild type, *mig-10* mutants and RY0128 array worms in order to determine success of the worm cross (Figure 16). The 10 worm PCR solutions were restriction enzyme digested with Hpy188I to verify whether the RY0128 array strain was homozygous for the mutant *mig-10* allele. The homozygous *mig-10* mutant produces three bands of lengths 558 bp, 190 bp and 79 bp, while the wild type *mig-10* gene is cut in four places, producing bands of lengths 366 bp, 192 bp, 190 bp and 79 bp (Appendix D). The result obtained suggests either that

the crossed strain was wild type for *mig-10*, with partial digestion accounting for the upper band, or that the strain was heterozygous for the wild type and mutant alleles.

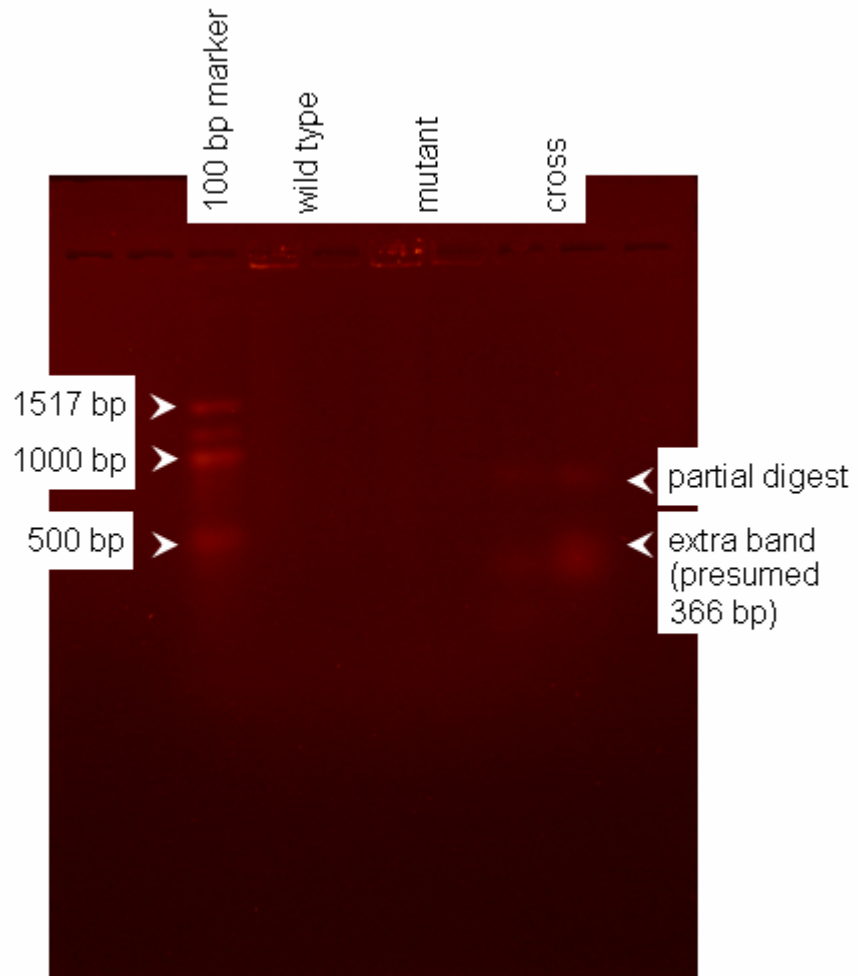

Figure 16: Verification of strain construction. Neither the wild type nor the mutant DNA was successfully digested. The worm cross DNA yielded inconclusive results due to a partial digest and the appearance of an extra band in the far right lane that was not present in the lane to the left.

4.0 Discussion

4.1 Native Promoter Constructs and Their Expression

These experiments tested the hypothesis that both *mig-10* transcripts may be necessary for rescue, and that rescue may only by achieved by expressing *mig-10* both cell autonomously and nonautonomously.

This project focused on the expression of two *mig-10* transcripts, *mig-10a* and *mig-10b*. Each transcript had been coupled with its respective native promoter and one of these constructs, each of which included a GFP marker and was known as an array, was later injected into mutant worms. The arrays were constructed using the native promoters in order to determine the normal expression patterns of each transcript. The worm strains RY0128 and RY0135 each contained one of the two rescue arrays and were assayed to determine the success of phenotypic rescue of the Egl and Wit defects.

Previous data indicated that the mig-10a promoter expresses GFP in the pharynx, vulva and in several migratory neurons, including the ALM, HSN and CAN. The mig-10b promoter, meanwhile, expresses GFP in six head neurons, the far posterior end of the intestine, and the vulva (Quinn et al.). These two controls indicate that the two transcripts of *mig-10* are expressed in different parts of the *C. elegans*' body. Consequently, these observations could justify the lack of complete rescue of the Egl phenotype resulting from the expression of only one *mig-10* transcript. Since the severity of the Egl phenotype is primarily associated with abnormal vulval function, and since both *mig-10a* and *b* are expressed in the vulva, perhaps both transcripts must be expressed simultaneously in order to achieve complete rescue of the Egl phenotype.

4.2 Partial Rescue Using Native Promoters of *mig-10a* and *mig-10b*

The data obtained from both the mig-10a:*mig-10a* and the mig-10b:*mig-10b* rescue assays contribute to our knowledge of where *mig-10* must be expressed in order to obtain Egl and Wit rescue. Each plasmid construct was assayed for *C. elegans* mutant rescue. The assay monitored the worm's egg laying and severity of Egl and Wit defects over a three to four day period. The results of the assay show partial phenotypic rescue of the RY0135 worm strain compared to the NY2053 and RY96 strains. The RY96 worms laid significantly fewer eggs than the wild type worms during most of the four day period. Moreover, an abnormally large number of RY96 mutant L1 progeny was recorded.

The wild type and mutant worms represented the positive and negative controls, respectively, for the RY0135 rescue strain assays examining egg laying patterns and Egl and Wit behavior. Partial rescue was observed because the RY0135 worms laid more eggs, fewer premature L1s, and were less severely Egl and Wit than the mutants. On the other hand, the rescue strain laid fewer eggs and exhibited more severe Egl and Wit behavior than the wild type worms, indicating that incomplete rescue had taken place.

A difference in the pattern of egg laying was also observed between the strains. The NY2053 worms lay eggs at a faster rate on Day 1, peak in egg laying on Day 2, and thereafter decrease their egg laying as they continue laying the remainder of their eggs. The RY96 and NY2053 worms display similar patterns of egg laying, but the mutants have a much lower scale of total daily and overall progeny.

The RY0135 worms had a slightly different egg laying pattern from the control strains; the animals laid only a few eggs on the first day and continued to increase their egg laying officiency until they peaked at about Day 3 and continued laying the remainder of their eggs on

Day 4. This mutant egg laying pattern appears to be very different from that of the controls. This discrepancy was thought to be caused by the selection of younger rescue worms that appeared to lay their eggs at a delayed rate. Due to this uncertainty, the second RY0135 assay was performed and caution exercised during the worm selection. This subsequent assay (Figure 6) produced similar results, confirming the partial rescue of the Egl and Wit defects.

Based on the results of the RY0135 assays, the mig-10a:*mig-10a* plasmid found in the RY0128 strain construct was hypothesized to also partially rescue the Egl and Wit defects. This hypothesis was ultimately rejected based on the results of the RY0128 strain assay. There was no Egl or Wit phenotypic rescue in the rescue strain of this assay, suggesting that the expression of *mig-10a* alone with its native promoter in the HSN and vulva is not sufficient to rescue the egg laying defect. This hypothesis implies that the expression of both *mig-10a* and *mig-10b* is necessary in the HSN and vulva to provide complete rescue of Egl. It is uncertain, however, if younger worm selection may have contributed toward inaccurate progeny counts.

4.3 Future Recommendations

We recommend that a future group repeats the RY0128 Egl/Wit assay to gather further data to compare to our results. The assay should include larger quantities of worms in order to eliminate statistical errors caused by the inexplicable disappearances and deaths of worms. Worms must also be carefully inspected prior to the start of the assay to ensure that they are of age (Appendix B).

Furthermore, it is possible that the mig-10a:*mig-10a* and mig-10b:*mig-10b* plasmids may have lacked some non-coding sequences required for accurate control of expression, which may also account for the poor mutant rescue obtained by each transcript. Therefore, an additional assay could be performed using plasmids containing part or all of the genomic DNA from each

mig-10 transcript. In this manner, splicing of the RNA transcripts will be permitted, which we predict will enhance protein translation and thus mutant rescue.

The observation of partial rescue in the RY0135 strain and no rescue in the RY0128 strain indicates that there may be a need for the simultaneous expression of both *mig-10a* as well as *mig-10b*. It is also hypothesized that expression of both protein isoforms must be both cell autonomous and cell nonautonomous. In addition it may be necessary for expression in both the HSN and vulva in order to obtain rescue of the Egl defect.

Manser et al. found that *mig-10* functions cell autonomously during development of the excretory canal cell (1997). Attempts were made to create a construct that would test whether expression in the CAN would achieve nonautonomous rescue of the foreshortened excretory canal defect. The ceh-23 promoter was coupled with *mig-10* to determine if expression is necessary both within the cell and in extrinsic cells. The ceh-23 promoter is thought to promote cell autonomous rescue of the CAN and cell nonautonomous rescue of the excretory cell. Future experimentation utilizing the ceh-23:*mig-10* construct will support this hypothesis if complete rescue of the migratory misplacement of the CAN is seen, as well as rescue of the foreshortened excretory canal defect.

The ceh-23:*mig-10* construct was digested to mutagenize a second start codon present in the ceh-23 sequence. However, after the mutagenesis step we were unable to ligate the 600bp ceh-23 insert with the construct due in part to the partial digest of ceh-23:*mig-10* using the enzymes XmaI and PstI. In order to continue this study these pieces must be ligated. If the ligation is successfully completed, purified ceh-23:*mig10* DNA must be prepared for worm injections. An assay should then be designed that follows the guidelines suggested in Appendix

A: Assay Suggestions, to observe whether rescue is achieved when the ceh-23 promoter expresses *mig-10* in the CAN and in the excretory cells of the *C. elegans*.

Appendix A:

Assay Suggestions

A.1 Importance of Thoughtful Planning

In order to carry out a successful assay of *C. elegans*, the experiment must be thoroughly planned to include appropriate controls and time constraints. For example, in order to carry out the Egl and Wit assay, it was important to include both a positive and a negative control with which to compare the strains containing the *mig-10a* or *b* arrays. In this manner, rescue success could be easily quantified based on the numbers of eggs vs. the numbers of L1 progeny recorded.

It was also critical to consider an effective time course for the Egl/Wit assay. In order to quantify the progeny, the adult worms had to be routinely relocated to fresh plates so that the counts would not be too overwhelming. On the other hand, counting progeny and transferring worms is a tedious process, and the appearance of the Egl and Wit phenotypes is progressive. The procedure, therefore, should not be carried out too often or too infrequently. In the context of the Egl/Wit assay, we have found that an eight-hour time course carried out over three days enabled our team to collect sufficient data regarding the success of Egl and Wit phenotypic rescue.

A.2 Data Collection

Once the experiment has been formulated, it is important to determine exactly how the data should be collected. Through trial and error, our team established an efficient data-collection system that enabled us to record our observations in an organized manner (Table A-1).

Assay Time: *4 pm*					
	Strain name: *NY2053 (wt)*				
worm	***A***	***B***	***C***	***D***	***E***
eggs	*34*	*30*	*35*	*- (gone)*	*29*
L1s	*0*	*0*	*0*	*"*	*0*
Egl severity	*0*	*0*	*0*	*N/A*	*0*
Wit severity	*+*	*0*	*0*	*N/A*	*0*
	Strain name: *RY96 (mig-10(ct41))*				
worm	***A***	***B***	***C***	***D***	***E***
eggs	*0*	*- (gone)*	*16*	*2*	*17*
L1s	*3*	*"*	*1*	*8*	*3*
Egl severity	*+++ (BAG)*	*N/A*	*++*	*++*	*+++*
Wit severity	*+*	*N/A*	*0*	*0*	*0*
	Strain name: *RY0135 (mig-10(ct41); mpEx21 array)*				
worm	***A***	***B***	***C***	***D***	***E***
eggs	*21*	*20*	*17*	*16*	*- (gone)*
L1s	*0*	*0*	*0*	*0*	*"*
Egl severity	*0*	*0*	*0*	*+*	*N/A*
Wit severity	*+*	*++*	*++*	*++*	*N/A*

Table A-1: Sample completed data collection table. This table promotes an organized assessment of the progeny and phenotypes of each individual worm. The 0/+ system is used to qualify the severity of Egl and Wit in each adult worm and is subject to change over time. Other notes may be included in order to explain the disappearance of an adult worm (ex. "gone", "BAGGED", etc).

Appendix B:

Mutant Worm Selection

There are many factors to consider when carrying out a worm cross. In the array/HSN cross, several crosses had to be carried out under specific conditions in order to obtain the final product. Since this series of crosses was primarily a troubleshooting endeavor, several problems have been resolved and are discussed in this Appendix.

B.1 Understanding the Procedure

It is important to keep track of each individual step in the procedure prior to beginning the crosses. Creating a schematic of each step in the cross, including the gender and genetic makeup of the worms, can enhance one's understanding of the logical sequence of events and overall goals of the cross (refer to Figure 6).

B.2 Know Marker Positions

Misconceptions regarding the positions and patterns of the markers were in part responsible for the failure of the array/HSN cross. While the RY0135 array fluoresces in neurons in the head, vulva and tail of the worm, the HSN strongly fluoresces in the anterior and posterior bulbs of the pharynx and less strongly in the HSN near the vulva (Figure B-1, A and B).

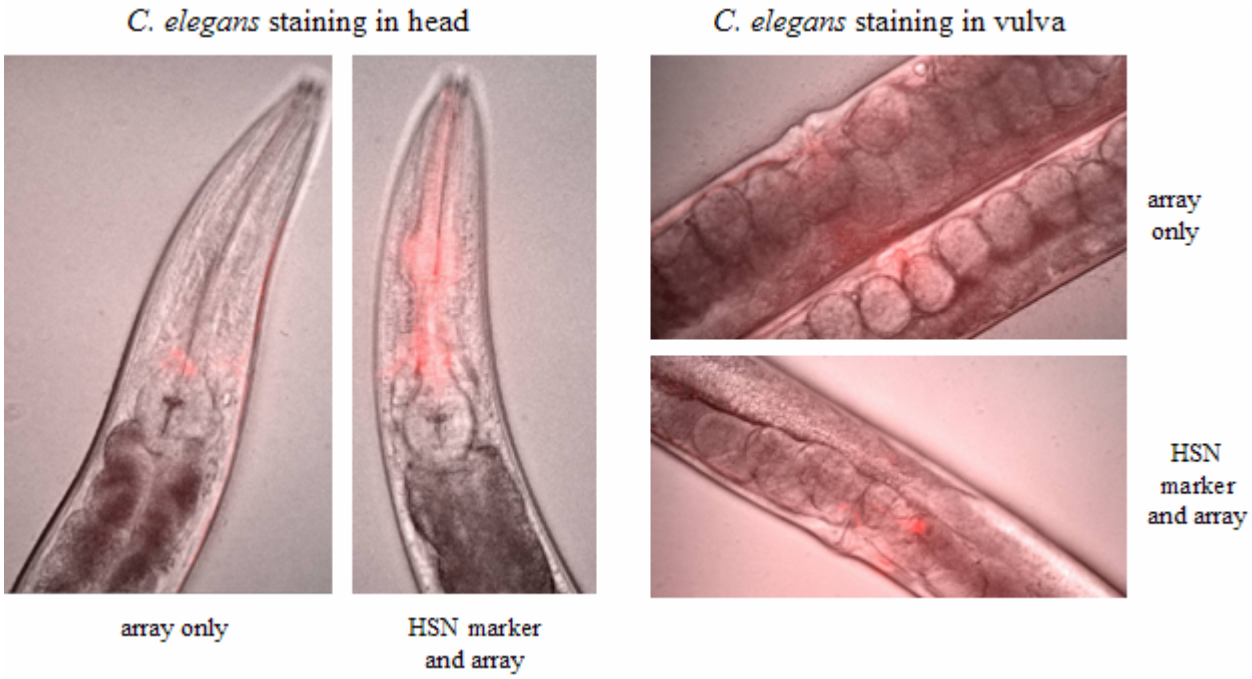

Figure B-1: GFP marker expression in *C. elegans*. The array and HSN markers have distinct expression patterns in the head and vulva of *C. elegans*. Understanding the exact locations of array and HSN marker expression can increase worm cross efficiency. These panels depicted above were created by blending Nomarski bright field and fluorescence images. Images created by Elizabeth Ryder.

B.3 Recognizing Age/Sex of Worm

The key to a successful cross is to plate worms at the correct ages. Male worms must be adults in order to impregnate the hermaphrodites, which must be at the L4 larval stage in order to produce cross progeny (Figure B-2).

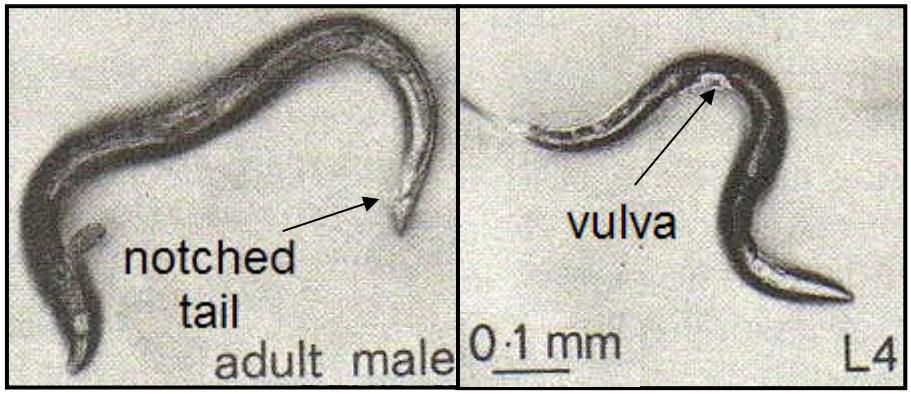

Figure B-2: Characterizing gender and age in *C. elegans*. The male *C. elegans* has a notched tail that easily distinguishes it from hermaphrodite worms. Hermaphrodite L4s are selected based on the appearance of a protruding vulva (denoted by the black dot encircled by a white patch). In addition, unlike adult worms, L4s are not gravid. Images adapted from Wood 1988.

Appendix C:

Cloning Vectors

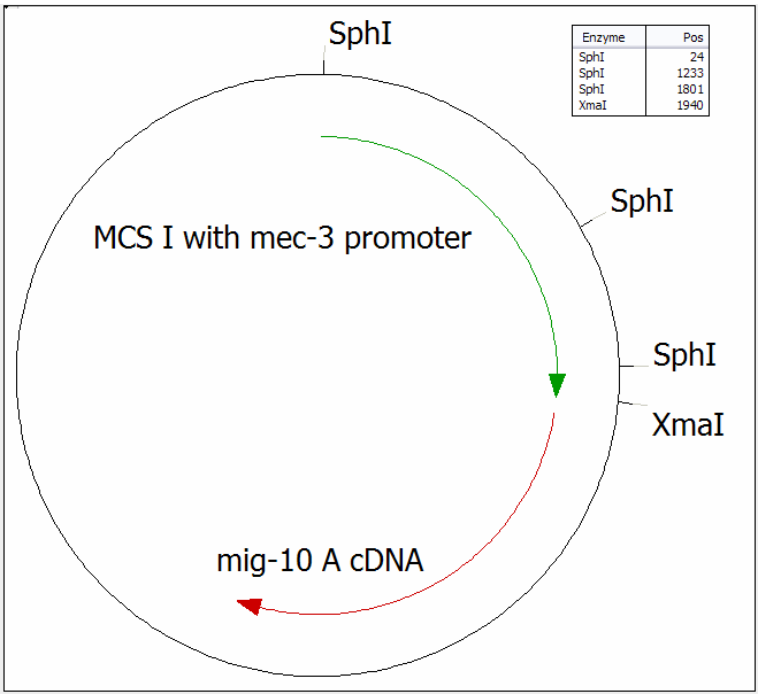

Figure C-1: pPD57.56 + *mig-10a* cDNA. *mig-10a* cDNA was cut out of this vector using SphI and XmaI. The mec-3 promoter was discarded and the *mig-10a* cDNA was used to create other constructs.

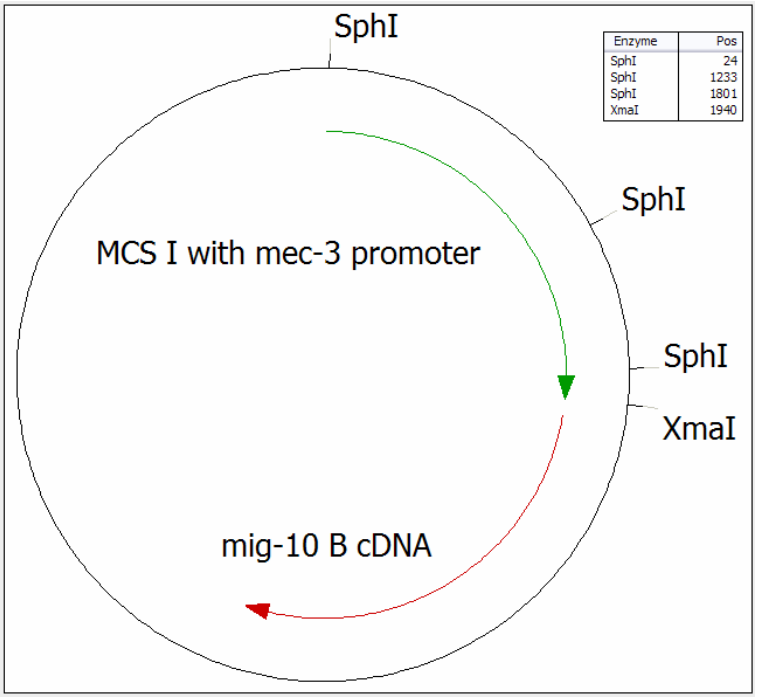

Figure C-2: pPD57.56 + *mig-10b* cDNA. *mig-10b* cDNA was cut out of this vector using SphI and XmaI. The mec-3 promoter was discarded and the *mig-10a* cDNA was used to create other constructs.

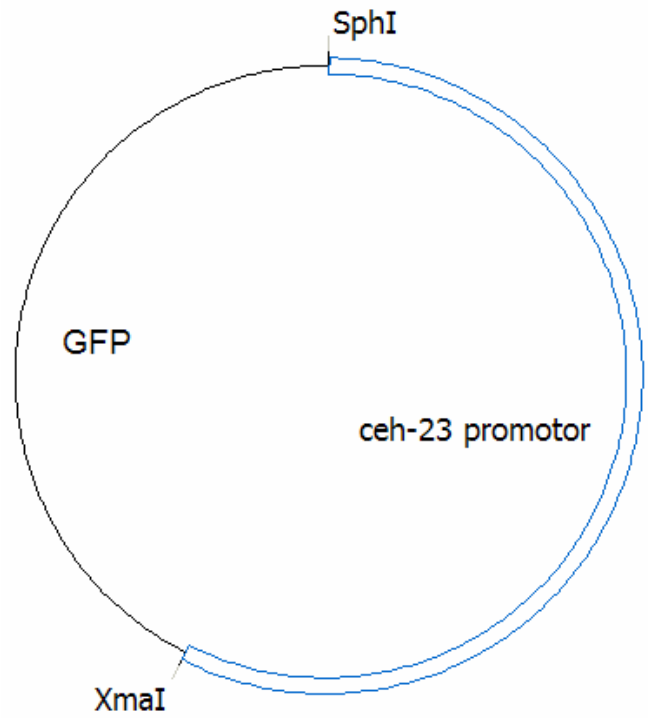

Figure C-3: ceh-23 + GFP. The ceh-23 promoter was isolated from the ceh-23:GFP construct via restriction digest with SphI and XmaI enzymes.

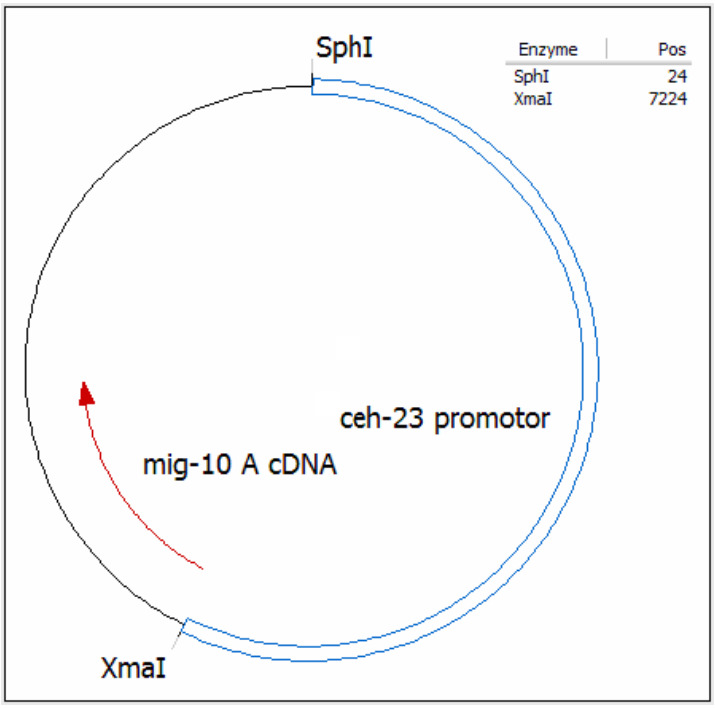

Figure C-4: ceh-23 + *mig-10a*. *mig-10a* cDNA was cut out of the "pPD57.56 + *mig-10a* cDNA" and ligated with the ceh-23 promoter + backbone.

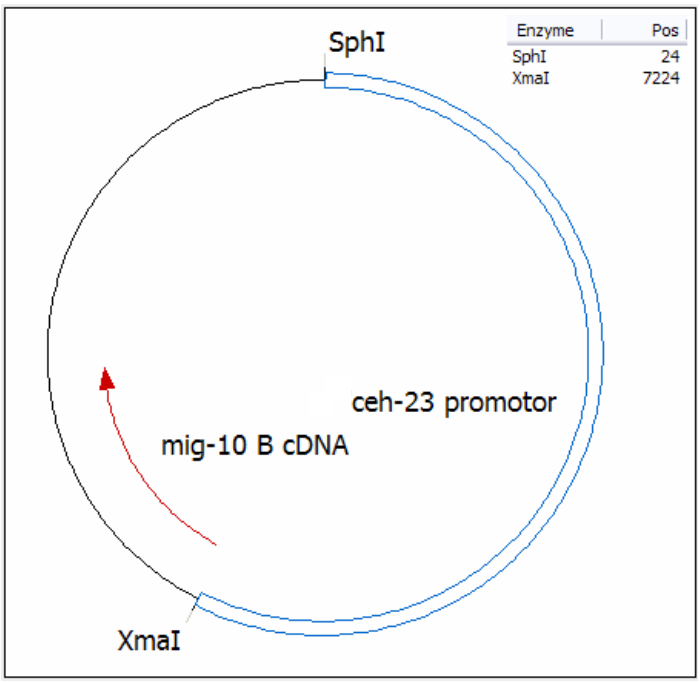

Figure C-5: ceh-23 + *mig-10b*. *mig-10b* cDNA was cut out of the "pPD57.56 + *mig-10b* cDNA" and ligated with the ceh-23 promoter + backbone.

Appendix D:

10 Worm PCR

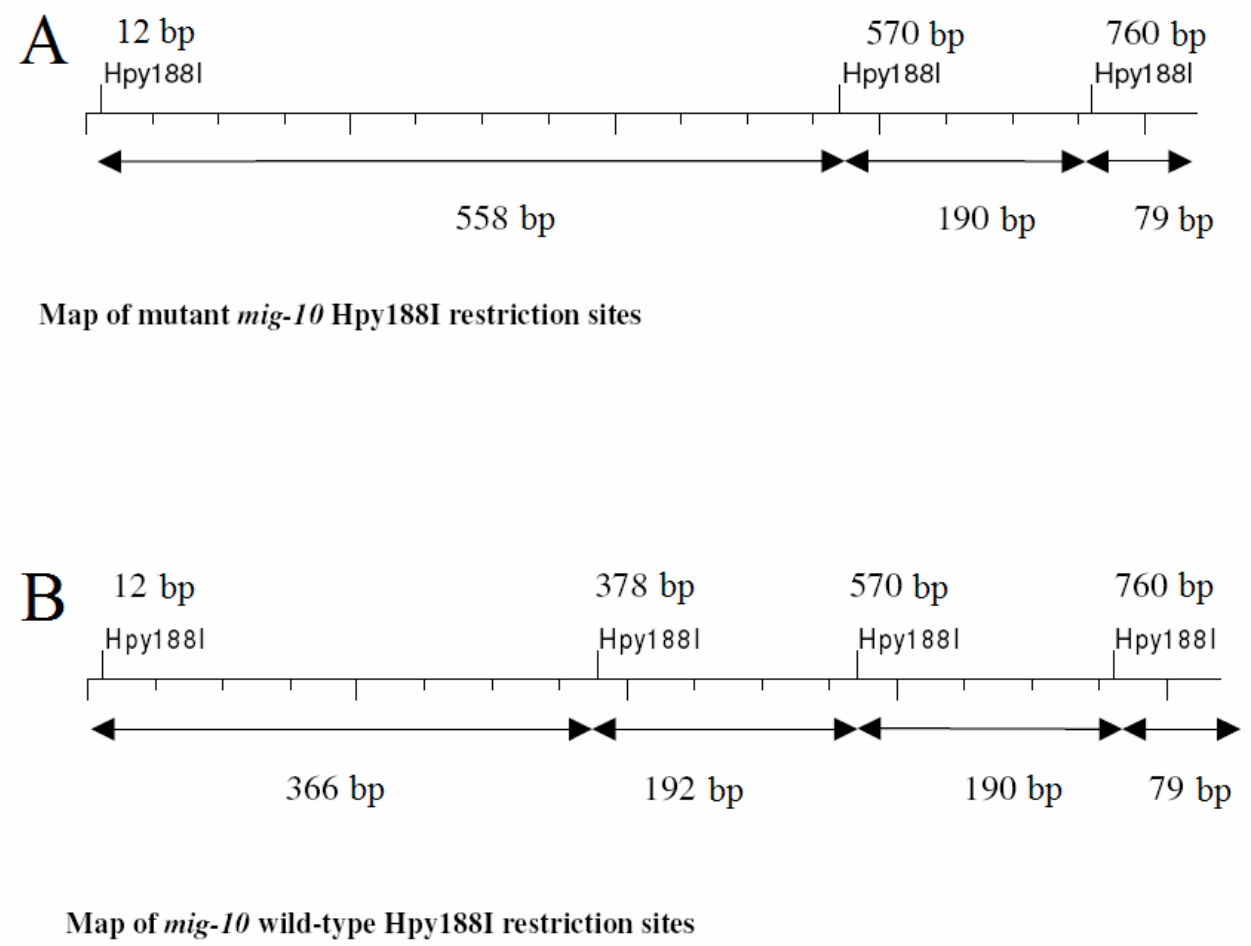

Figure D-1: Map of Hpy188I restriction sites on mutant and wild type *mig-10*. The MIG10-WT1 and MIG10-WT2 primers were used to amplify the *mig-10* alleles from the three worm strains examined in this experiment. The genetic sequence of MIG10-WT1 is 5' TGTTTGAATTTTCAGAATCCGC 3'; the MIG10-WT2 primer sequence 5' TGTTTCTTCTCACAATCCAACC 3'. The wild type *mig-10* gene contains one more Hpy188I restriction digest sequence than the mutant *mig-10* allele. The two alleles can be easily distinguished from one another when the digested products are electrophoresed. Diagram taken from the MQP of Romiya Glover and Stephanie Morin.

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