



# Isolation and Characterization of Novel Mutations in the *mig-10* gene of *Caenorhabditis elegans*

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# ABSTRACT

The purposes of this MQP were to identify and characterize novel missense mutations in the *Caenorhabditis elegans* gene *mig-10* which cause defects in neuronal migration, axonal growth, and excretory cell growth. Because of its fully penetrant mutant phenotype characteristic in the null mutant, the truncated excretory canal phenotype was used as a genetic model for developmental axonal growth. The mutants RY0920, RY0921 and RY0922 were isolated by using a simple screen. RY0920 strain was confirmed as new allele of *mig-10* by complementation test, PCR, and restriction digestion. Novel missense alleles of *mig-10* can help to define MIG-10 protein domains and residues important for neuronal migration function in *C. elegans*.

# Acknowledgements

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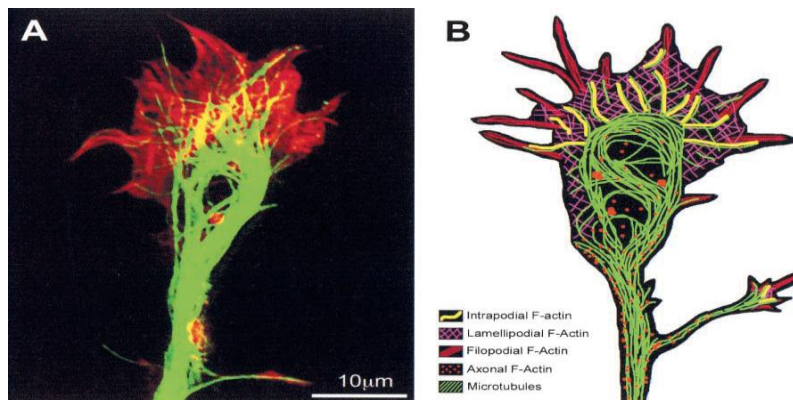
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# Introduction

## 1. Neuronal migration and axon outgrowth are important to the development of a functioning nervous system

In general, development of the nervous system requires a very dynamic array of signaling molecules and transduction pathways. It has been recognized that proper nervous system function relies on the formation of specific connections between varying neuronal cell types. In order to make these very specific connections, neurons in many organisms must first migrate and then send out axons. The process involves many guidance signals from the extracellular environment for these neurons to migrate to their final destination accurately. It is hypothesized that these neurons detect a series of attractive and repulsive guidance cues by four major conserved families of axon guidance molecule: netrins, slits, semaphorins and ephrins. Certain guidance molecules are acting to attract neurons and others are repelling neurons, depending on the context of the neural migration. For example, guidance molecules netrin and slit can function either as attractants or repellents to direct axons to their final targets (Bear et al., 2007).

Axon outgrowth is also guided by guidance molecules of the extracellular matrix. The extended end of a developing axon is a special structure called a growth cone (Fig.1) which contains finger-like filopodia, extended from sheet-like lamellipodia, exploring and detecting the changes in its extracellular environment. Specifically, the receptors on growth cones help recognize specific guidance cues. These cues enable the axons either to move toward or away from their certain targets accordingly. Once a growth cone senses a guidance cue, the receptors in the growth cone send a signal to tell the axon the correct orientation for migration (Bear et al., 2007).

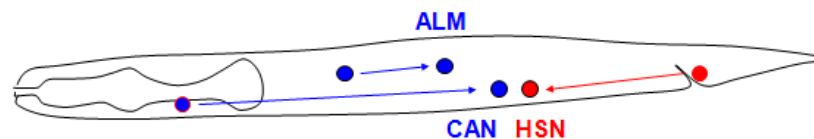


**Figure 1: Structures of a neural growth cone involved in axon guidance:** Comparison of filopodial and lamellipodial growth cones. A fixed growth cone has been stained with phalloidin and antibodies to tubulin in order to fluorescently label F-actin (red) and microtubules (green) (Picture taken from Dent et. al., 2003)

In our study in *C. elegans*, the long process of the excretory cell, called the excretory canal, was used as a model of axon guidance. The excretory cell in *C. elegans* is located on the ventral side near the developing pharynx. A complicated network of tubular epithelia is formed from a single excretory cell. This tubular formation is a fascinating example of cell morphogenesis which is mediated through interactions of specific receptors on the membrane with the extracellular environment. Canal tips might be stimulated and guided during the development of excretory canal by utilizing the same cues and mechanism as do the neural migration and outgrowth (Yu and Bargmann, 2001).

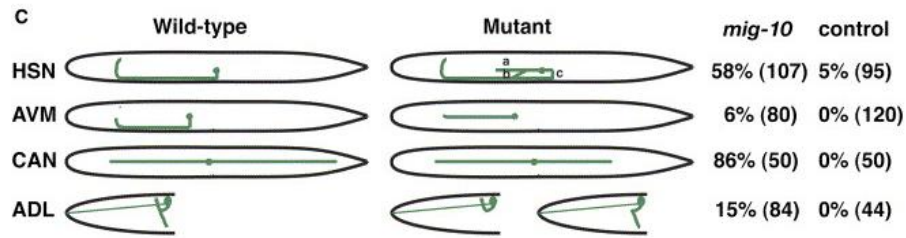
## 2. The *mig-10* Gene Functions in Neuronal Migration and Axon Outgrowth

The *mig-10* gene of *C. elegans* plays an important role in the regulation of the embryonic migration of several neurons: the Canal Associated neurons (CAN), Anterior Lateral Microtubule cells (ALM), and Hermaphrodite Specific neurons (HSN). CAN and ALM are known to migrate in the direction of anterior to posterior to the position approximately half the length of the worm. HSN migrates in the opposite direction to about the same location as CAN and ALM (Fig. 2). Mutations within the *mig-10* gene can shorten these neuron migrations in both directions, supporting the hypothesis that *mig-10* is likely involved in the basic mechanism of neuronal migration, rather than specific directional signaling.



**Figure 2: Migration directions of CAN, ALM and HSN cells of *C. elegans*** (Figure taken from Ficociello and Ryder, 2008)

The *mig-10* gene is also essential in the process of axon guidance and proper development of the excretory canal (Manser and Wood, 1990). If there is a mutation in this gene, defects are created not only where the ability of certain neurons to migrate and axon outgrowth are compromised but also the excretory canal cell is shortened in length. In Figure 3 below, the percentages of defective neurons in *mig-10(ct41)* are compared to the percentage of defective neurons in wild-type animals. On the left hand side are diagrams of wild-type and mutant *C. elegans*, and on the right are the percentages and counts for the numbers of animals in this experiment. This data supports the theory that the mutation does in fact hinder axon outgrowth and neuronal migration as compared to wild-type genes, especially in HSN and CAN (Chang et al, 2006).

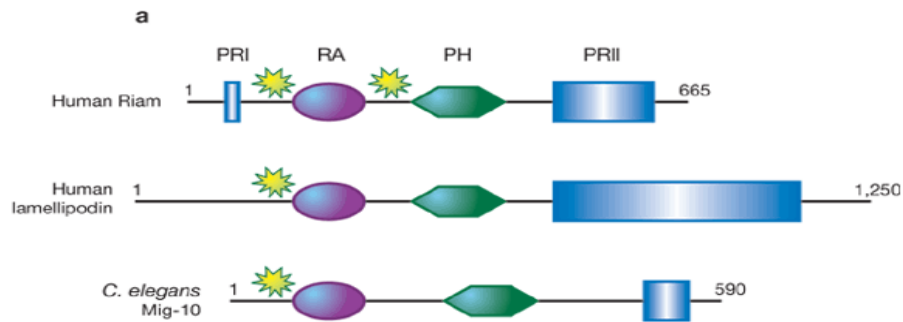


**Figure 3: Axon guidance defects in *mig-10* mutants** (Figure taken from Chang et al, 2006)

As seen under a fluorescence dissecting microscope, in wild-type animals, when their excretory canal cell is labeled by a transgene *bgIs312* expressing green fluorescent protein (GFP), the excretory canal extends nearly the full length of the animal and glows fluorescent green, whereas the *mig-10* mutant phenotype is visible as a much shortened fluorescent excretory canal. *mig-10* (*ct41*) is a null allele containing a stop codon within the third exon caused by a single base pair change (C→T). In *mig-10* (*ct41*) mutant animals, the excretory canal is severely truncated, which is the only fully penetrant aspect of the mutant phenotype. Other migratory defects include incomplete migration of CANs, ALMs, HSNs, and ccL mother cells. In addition, some of the adult animals exhibit a withered tail, a protruding vulva, and egg laying defects. On the other hand, *mig-10* (*e2527*) is a splicing mutation (TTTCAG→TTTCAC) which likely results in reduced MIG-10 protein production. In this mutation, only ALM migration is affected with high penetrance, whereas all three sets of neurons are affected with high penetrance in *ct41* (Manser et al, 1997).

### 3. Structure and Interactions of MRL Proteins

“MRL proteins” are the Mig-10/RIAM/Lamellipodin protein family. These proteins offer a connection between signaling, adhesion, and cell motility by providing a link between the plasma membrane and the actin cytoskeleton, which is crucial to cellular shape and movement (Legg and Machesky, 2004). MRL proteins contain pleckstrin-homology domains (PH) (protein domains consisting of approximately 120 amino acids and occurs in a wide range of proteins involved in intracellular signaling or as part of the cytoskeleton) and Ras-association (RA) domains as well as a proline-rich C terminus. In Figure 4, the structures of the three proteins which make up the MRL protein family are shown.



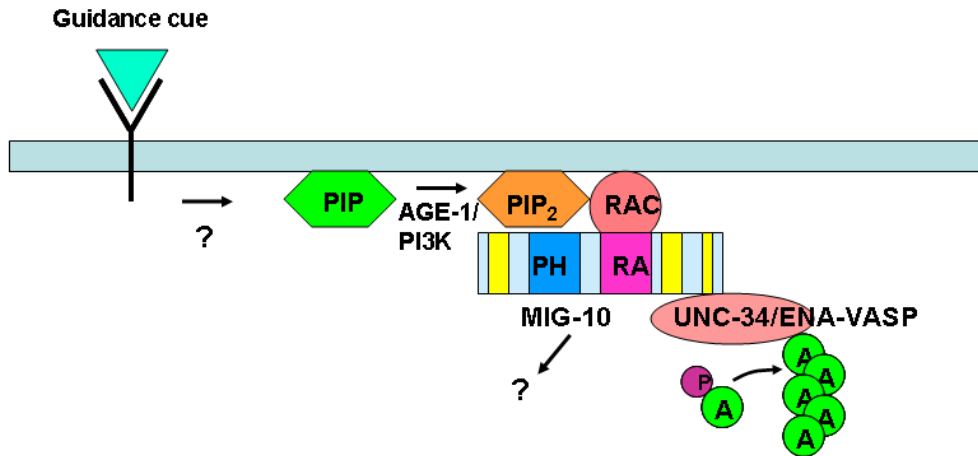
**Figure 4: RIAM and Lamellipodin are part of the MRL protein family (Mig10/RIAM/lamellipodin).** PRI and PRII represent the proline-rich domains, RA represents the Ras-association domain, PH represents the pleckstrin-homology domain and the yellow star represents the putative coiled-coil domain. In observing Figure 4, it can be derived that these proteins share very similar structures, which is why they are grouped together into one family of proteins. (Figure 4 taken from Legg and Machesky 2004)

#### 4. Model for the mechanism of MIG-10 protein

MIG-10 has been identified as playing a role in both the pathways of slit-dependent and netrin-dependent axon guidance. The MIG-10 protein is a cytoplasmic adaptor protein that functions downstream of these cues, specifically UNC-6/Netrin and SLT-1/Slit (Manser et al, 1997; Quinn et al, 2006). A mutated *mig-10* gene results in the phenotype of incomplete migration of CAN, ALM, and HSN, as well as the phenotype of a truncated excretory canal (Manser and Wood, 1990). Furthermore, over-expression of MIG-10 in the absence of both guidance cues UNC-6 and SLT-1 cause axons of neurons such as AVM and PVM to migrate to different orientations or directions, displaying a multipolar phenotype. When MIG-10 is over-expressed in the presence of either UNC-6 or SLT-1, the multipolar phenotype is suppressed and a monopolar phenotype is expressed. Additionally, it has been found that axonal guidance can be enhanced by the over-expression of *mig-10* (Quinn, et al., 2006). Together, these studies suggest that MIG-10 alone promotes the activity of axon outgrowth without a directional response, however, in the presence of guidance cues such as UNC-6 or SLT-1 the outgrowth activity is guided in a directional path and this directional guidance response is enhanced by MIG-10 over-expression (Quinn et al, 2006).

When MIG-10 is expressed in HEK293 cells, it co-localizes with filamentous actin and creates filopodial protrusions (Quinn et al., 2006). Additionally, when *mig-10* is over-expressed in *C. elegans* in the absence of guidance cues netrin or slit, it causes multi-polar axonal outgrowth. This suggests that at the cellular level, MIG-10 is creating actin filamentation mediated outgrowth, wherever it is expressed. In vivo, MIG-10 mediated outgrowth is controlled by cellular polarization of slit and netrin guidance cues (Quinn et al., 2006). However, the link between guidance cues and MIG-10, as well as MIG-10 and the actin polymerization machinery, is somewhat elusive. What is known is that MIG-10 most likely interacts with UNC-34, an Ena/Vasp protein, which is directly

involved with actin polymerization machinery (Chang et al., 2006). Still, other potential interactors for *mig-10* remain speculative.

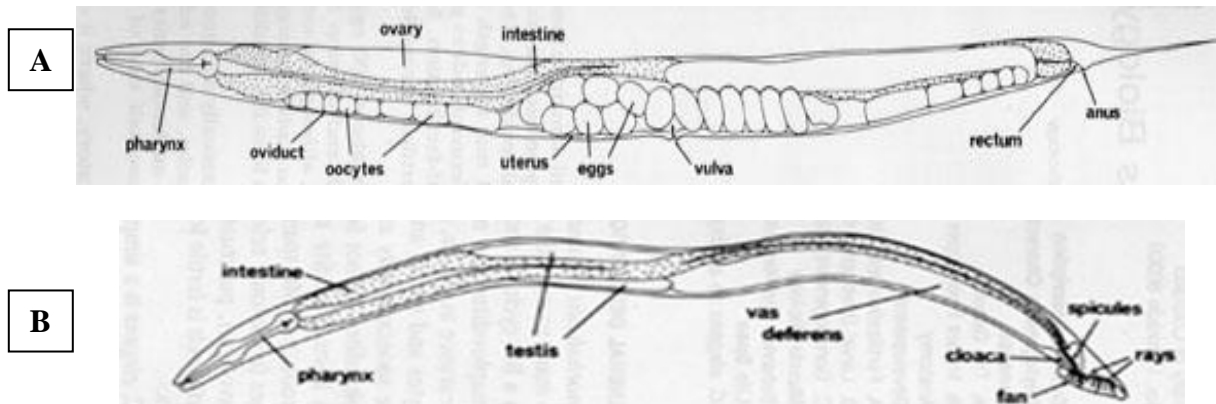


**Figure 5: MIG-10 orients the location of actin polymerization: a speculative model for MIG-10 signaling cascade.** Extracellular guidance cues recruit a PI3 kinase along with a Ras family GTPase which localize MIG-10, and hence the actin polymerization machinery. RA, ras association domain; PH, pleckstrin homology domain. Ras is a monomeric GTPase; PI3K, Phosphatidylinositol-3 kinase; PIP (phosphatidyl inositol); PIP2 (a phosphorylated version of PIP); PIP2 is recognized specifically by MIG-10’s PH domain. UNC-34 is an ENA/VASP protein. (Figure taken from Ficociello and Ryder 2008).

## 5. *C. elegans* – A Model System

### 5.1. Background and anatomy

*Caenorhabditis elegans* or *C. elegans* (Caeno meaning recent, rhabditis, meaning rod, and elegans meaning nice) is a free-living, non-parasitic nematode often used in laboratories for experimentations around the world. *C. elegans* does not have the typical male/female sexual classes; the animals instead consist of a sperm producing male, and a self-fertilizing egg and sperm-producing hermaphrodite. Shown below in Figure 6, panels A and B illustrate the anatomical structures for both the hermaphrodite and the male of *C. elegans* (Brenner, 2008).



**Figure 6: Anatomy of a hermaphrodite *C. elegans* (A) and a male *C. elegans* (B)** (Figure taken from Brenner, 2008)

*C. elegans* are very small, on average around one millimeter in length, and easily seen under a microscope. They lack pigment, causing them to be transparent, which allows for easy observation of internal structure under the microscope at high magnification. Due to their small size, they are able to be cultivated in large numbers within a single habitat (prepared plate with spotted “food”); this makes cultivation easy as well as inexpensive (Brenner, 2008). The plates which *C. elegans* are normally grown on are called “Nematode Growth Medium” plates, which contain a specific strain of *E. coli* bacteria called “OP50”, which the *C. elegans* feed on for their duration of time on the plates (Fay, 2008).

*C. elegans* have five pairs of autosomes, and a sixth pair of chromosomes, which is a pair of sex chromosomes that determines whether the organism presents as a hermaphrodite or as a male. Sex in *C. elegans* is determined by the ratio of sex chromosomes to autosomes in the genome. If the sixth chromosome pair is XX, then *C. elegans* will be a hermaphrodite, and if the sixth chromosome pair is XO, a male is produced instead (Brenner, 2008).

Embryogenesis takes around fourteen to sixteen hours, and is followed by four larval stages; the animal becomes an adult after approximately 35 hours as seen in Figure 7 (Fay, 2008). Not all worms however follow this specific pattern of a life cycle. The “dauer” larval stage is an alternative life form that is sometimes adopted when food is too scarce or a plate is overcrowded with too many worms. This larval stage is characterized by very thin worms that are not able to eat, however may stay alive for three months. This type of larval stage is often considered “non-aging” as they can live for months without eating, then re-enter the L4 stage when more food is available and continue to live on for up to fifteen more days (Wormatlas, 2006).

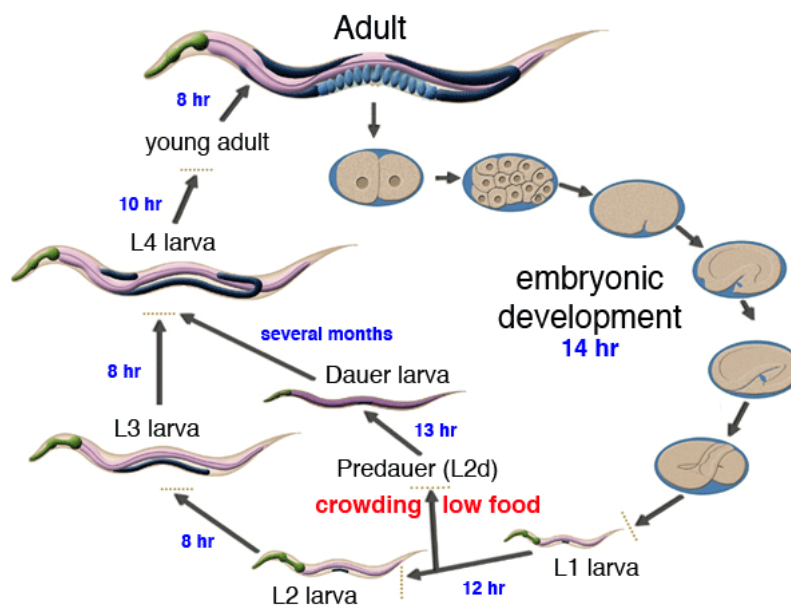


Figure 7: Life Cycle of *C. elegans* (Figure taken from Wormatlas, 2006)

## 5.2. Why use *C. elegans* for a model system

A model system is an extremely simple organism able to be handled in large numbers. Sydney Brenner introduced *C. elegans* in 1963 as a model organism to perform research for developmental biology and neurobiology. The *C. elegans* genome was completely sequenced in 1998, making it the first multi-cellular organism with a completely sequenced genome.

There are many aspects of *C. elegans* which makes them a very useful model system, one of them being the short life cycle of *C. elegans*. From egg to egg-laying parent takes around three days, with a lifespan of about two to three weeks in a stable, healthy environment. This short life cycle facilitates biological study because it allows for a greater amount of observation over a shorter amount of time than other model systems, such as mice (Brenner, 2008). Also, each healthy adult is able to produce about three hundred of their own progeny (Wormatlas, 2006), allowing for a large scale screen to be performed using a small number of parent organisms in a very short amount of time.

*C. elegans* is a multi-cellular eukaryote, meaning it shares cellular and molecular structures and control pathways with higher organisms. This makes information learned from *C. elegans* directly applicable to higher organisms, including humans, with which *C. elegans* are forty percent homologous. In saying that these two organisms are forty percent homologous implies that forty percent of the *C. elegans*' one hundred million base pair genome is similar in structure and anatomical position (Biology Online, 2008) to that of the thirty times larger human genome. In many organisms, this may mean common ancestry (Brenner, 2008).

## 6. Genetic Screens

Genetic screens are an essential tool to help discover and select individual worms that exhibit a phenotype of interest. In our study, the desired phenotype to identify novel mutations in *mig-10* was a truncated excretory canal in *C. elegans*. The excretory cell in *C. elegans* is located on the ventral side near the developing pharynx. The shortening of the excretory cells is the only fully penetrant mutant phenotype in the null mutant (Manser et al., 1997). Furthermore, it is a very easy phenotype to see as it is important for ease and speed of the screening.

There are several screening methods that can be used, such as non-complementation genetic screens and simple screens. The non-complementation screen begins with a heterozygous strain for a recessive mutation in the gene of interest. This screen looks at the progeny of the F1 generation for the desired phenotype and then at the F2 progeny of these worms to get the homozygous alleles of new mutation. The advantage of the non-complementation screen is that the phenotype will only result from new mutations in the desired gene. Non-complementation occurs when two mutations,

in the same gene, but on opposite chromosomes, result in a mutant phenotype. In our case, if a new mutation *mig-10* is produced it would fail to complement the old mutation present on the other chromosome, resulting in the Mig phenotype (Jorgensen and Mango, 2002). Past MQP research showed that complications involved throughout the whole process in non-complementation genetic screens became a drawback, despite the advantages it offers (Leblanc, de Rham and Zhang, 2008). The disadvantages in this type of screening include difficulty in recognizing phenotypes because of more than one genetic marker being required, making it easier to overlook a potential putative; genetic markers may also cause low strain viability and the possibility of unwanted recombination (Leblanc, de Rham and Zhang, 2008).

For our project, the approach of simple screen may offer more advantages to increase the chance of finding putative mutation in *mig-10* gene. The desired phenotype to be screened for F<sub>2</sub> generation is the truncation of the canal cell (the homozygous form of mutant gene), which is easy to distinguish in the simple screen because of it is not complicated by the presence of other mutations. In this screen, a mutagen called ethyl methane sulphonate (EMS) is used to induce point mutations in the sperm or oocytes of wild-type hermaphrodites. Although it is likely for nonsense mutations to occur from EMS, missense mutations, which will yield more information about the protein, are likely as well. A missense mutation is a point mutation that will cause a change in a single base pair that can cause a substitution of a different type of amino acid in MIG-10 protein. The disadvantages of this screen include the identification of mutations that are not in the gene of interest and the only method to figure out the desired ones is doing time-consuming complementation tests.

## 7. *Project Objectives*

The goals of this project were to identify and characterize novel missense mutations in the *mig-10* gene of *C.elegans* to help define protein domains and residues important for neuronal migration function. A simple genetic screen was used for this project to identify novel mutations in the F<sub>2</sub> progeny generation, which resulted in the phenotype of a visibly truncated excretory cell, visualized by a transgene (*bgIs312*) expressing GFP in that cell. We hoped to isolated missense alleles rather than null alleles because a missense mutation would cause a change of a single amino acid with different properties in the MIG-10 protein. New alleles may cluster in particular domains of MIG-10 that are important for function. New alleles may affect particular amino acid residues of importance. Another advantage of missense alleles of the *mig-10* gene is that they might help in determining whether MIG-10 interacts with the same partner proteins for all of its functions. A missense allele might affect some aspects of the phenotype but not others.

# Methods

## 1. Maintained strains

Worms were grown on NGM plates spotted with OP50 *E.coli*. Strains maintained throughout the project are shown in Table 1. Strains were maintained by transferring three to four worms at the L4 stage to a new plate once or twice a week.

Strain name	Genotype	Purpose
	<i>bgIs312</i>	Starting strain for simple screen GFP transgene marks excretory canal Displays wild type excretory canal phenotype
RY108	<i>mig-10(ct41); bgIs312</i>	Displays truncated excretory canal phenotype
BW315	<i>mig-10(ct41)</i>	Complementation test Control for PCR and restriction digestion
CB1490	<i>him-5(e1490)</i>	Strain produces ~30% male progeny Backcross and complementation test

**Table 1: Maintained strains**

## 2. EMS mutagenesis and simple screen

Three to five gravid *bgIs312* adults were picked to four to six NGM agar plates that were previously spotted with OP50 *E.coli* bacteria. The progeny from these gravid adults gave rise to the “P0” generation. Three days later the P0 progeny were washed off of the plates using M9 buffer at least two times and were pipetted into a 15mL centrifuge tube to be spun down. The worms were centrifuged for one minute in the Allegra™ 6KR centrifuge at 330 rpm or 244xg. After the first spin cycle, the tube was examined under a microscope to ensure the worms were in a pellet in the bottom of the tube. The supernatant was removed, taking extra care not to disturb the pellet of worms. 10mL of M9 buffer was then added to the pellet, and the tube was inverted several times and spun down and as before, followed by the removal of the supernatant also as before. The worms were then resuspended in 2mL of M9 buffer, to which was added 2 mL of ethylmethanesulfone (EMS) solution (20uL of EMS in 2mL of M9 buffer). Care was taken to wear gloves and dispose of anything that touched the EMS into a 50mL tube with NaOH pellets. The worms were then gently rotated for four hours on a nutator. After the four hours elapsed, the worms were spun down again just as before. The worms were washed three to four times with M9 buffer, using care since the supernatants contained EMS. The supernatants were pipetted into the tube of NaOH pellets as well as any tips that came in contact with the EMS. After the final centrifugation, the worms were resuspended in two to three drops of M9 buffer and then the worms were pipetted to two to three

new dishes away from the food. After an hour had passed, all the L4 hermaphrodites were picked from the plates and were re-plated at five for each plate and were incubated overnight at 20 degrees Celsius.

The following day in the morning, from these plates a number of gravid P0s (five to six) were picked to as many plates as necessary. The worms were allowed to lay approximately fifty eggs each; these eggs were the F1 progeny. The remaining parent worms were picked off so that only the F1 eggs were left. The plates were left for two to three days at 20 or 25 degrees Celsius to allow the F1 worms to hatch and lay at least 300-500 F2 eggs, and the F1 parents were washed off of the plates. These F2 eggs were then left for two to three days at 20 or 25 degrees Celsius, until the F2 eggs had hatched and were ready to be screened.

Once there were L4 F2 worms on each plate, screening could begin. Using a fluorescence dissecting microscope, we tried to examine all worms on each quadrant of a plate before moving on to the next plate in order to identify any visible putative *mig-10* mutants, with a truncated excretory canal. These mutants had a very truncated excretory canal (the long glowing green process that extends the length of the worm's body in wild type animals) (Ryder and Ficociello 2008).

### **3. Worm PCR**

#### **3.1 Lysis Step**

2.5uL lysis buffer and enzyme (50mM KCl, 10mM Tris, pH 8.2, 2.5 mM MgCl<sub>2</sub>, 0.45% NP-40, 0.45% Tween 20, 0.01% DNA free gelatin and 60 µg/mL Proteinase K) were placed into the cap of each PCR tube. Ten worms for each strain were picked into each tube cap. Then the tubes were microcentrifuged for 5 seconds. The tubes were kept in a -80°C freezer for at least 10 minutes. Before adding 22.5 uL of PCR cocktail to each tube, all the worm tubes were heated for 1 hour at 60°C to activate the enzyme and following with the inactivation of Proteinase K for 15 min at 95°C.

#### **3.2 PCR Amplification**

The primers were *mig-10* WT1 (5' TGTTTGAAT TTTCAGAAT CCGC 3') and *mig-10* WT2 (5'TGTTTCTTCTCACAATCCAACC3'). The PCR reaction mixture/cocktail (25uL) contained 1X Long Template PCR System Buffer 3 (Roche Brand, Catalog #1742663), 0.25mM dNTP, 0.3uM Primer *mig-10* WT1, 0.3uM Primer *mig-10* WT2 and 3.75U of Taq Polymerase (New England BioLabs, Catalog #M267L), and 2.5uL template DNA preparation from lysis step. The short PCR amplification began with initial denaturing at 94°C for 10 min, then the amplification

was performed for 34 cycles, consisting of denaturing at 94°C for 30 s, annealing at 60°C for 1 min, and primer extension at 72°C for 2 min, with a final extension at 72°C for 10 min.

### 3.3 Restriction Enzyme Digest

10uL DNA amplified products from each tube were digested with 5U Hpy188I endonuclease (New England BioLabs, Catalog #R0617L) in a reaction mixture (15ul) containing dH2O and 1X NEBuffer 4 (New England BioLabs, Catalog #B7004). The 1X NEBuffer 4 consists of 20mM Tris-acetate, 10mM potassium acetate, 10mM Magnesium Acetate and 1mM dithiothreitol. Each tube with digestion reaction was incubated at 37°C overnight.

### 3.4 Gel Electrophoresis

The samples were electrophoresed in 2% agarose gel (1g) with 50 mL of 1X TBE (Tris-borate-EDTA) buffer. The 100bp DNA Ladder (New England BioLabs, Catalog #N3231L) was used as a marker in this gel electrophoresis. The gel was run at 110-130 Volts for 1 hour.

## 4. Primer design and DNA sequencing

We used Primer Quest from IDT DNA to design five sets of primers. The schematic illustration for locations of these primers can be seen in Figure 8 below.

The sequence for set 1 forward primer is AAATGTATCACGATCGACGGCGGA and the reverse primer is TGGTGATAAGAAAGAGCCGGTGGT.

Set 2 forward primer is AGGCAGTCCTAATTTGCCAGCTTC and the reverse primer is TGAAGCAGCTGAGTTGATGGAGGT.

Set 3 forward primer is TGGACAGGTAACTCCACCCACAA and the reverse primer is AGCAACTGAAGTGCTTCTCCGTCT.

Set 4 forward primer is AAGCTTTATTTTCATGCGTCGCCCCG and the reverse primer is AGTGCAAGTCTAATAGGGAGGCCA.

Set 5 forward primer is TACGATGAGCAACCGACTGGAACA and the reverse primer is TGGAGTACAAGGCTTTGGTGGAGT.

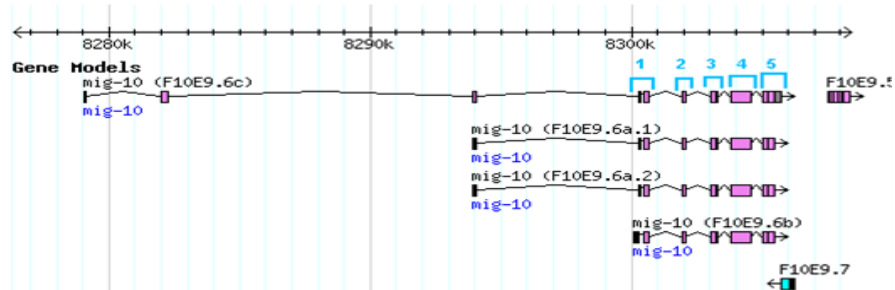


Figure 8: Initial sequencing primer pairs shown in blue. (Figure taken from Wormbase)

# Results

In order to identify new missense mutations in the *mig-10* gene, we performed a simple genetic screen, looking for the desired phenotype of truncated excretory canal cell. To determine whether isolated mutants carried alleles of the *mig-10* gene, the complementation test was performed with the tester strain of known mutation *mig-10 (ct41)*. Putative mutants were backcrossed to remove any deleterious mutations, and then characterized using standard PCR, restriction digestion, and DNA sequencing.

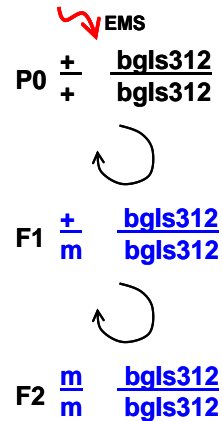
## 1. Simple Screen

It can take screening about 5000 haploid genomes to get one mutation in each gene that produces the truncated mutant phenotype. In order to calculate the number of haploid genomes screened, we estimated that each mutagenized P0 would lay approximately 50 eggs (F1 progeny). Each F1 would lay about 10 eggs (approximately 500 F2 progeny/plate). F2's picked from same plate were likely not independent mutations (same F1 parent with two independent sets of mutagenized chromosomes). Therefore, each F2 plate screened is estimated as 100 haploid genomes. We screened an average of 12 to 15 plates per simple screen. Therefore, the total haploid genomes screened for these plates should be about 1200-1500 for each screen, or about 5000-6000 haploid genomes for the project (Table 2).

Screen #	# of Haploid	# of Putative	# of Mutants that Propagated
1	750	1	0
2	600	3	0
3	900	3	1 (RY0921)
4	950	8	2 (RY0920 and RY0922)

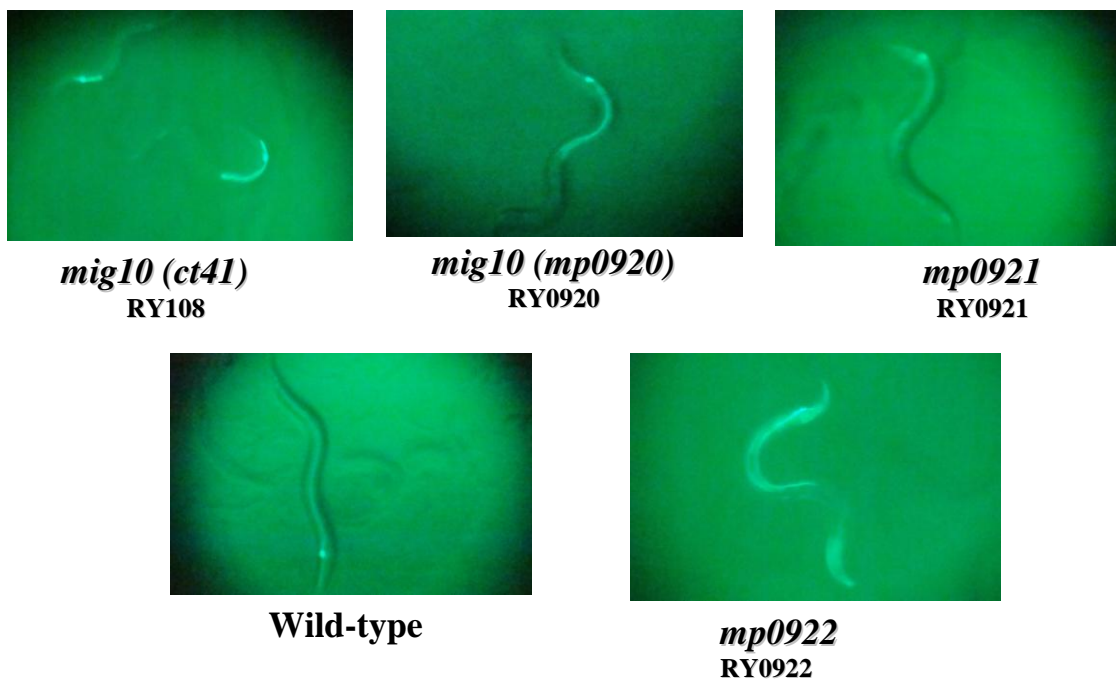
**Table 2: Mutagenesis Results**

We conducted a total of four simple screens (Fig. 9).



**Figure 9: Simple screen to examine F2 generations for desired phenotype of truncation of excretory canal:** *bgls312* is an integrated transgene marker used to visualize the excretory canal. A strain of wild type worms with this marker was mutagenized by EMS to screen for mutant worms with a truncated excretory canal. (Figure taken from Ficociello and Ryder 2008)

We isolated a total of fifteen putative mutants with truncated excretory canals (Table 2 above). Of these, there were three mutants had no progeny, while nine mutants gave rise to wild type progeny, suggesting they were false positives. Three independent mutant strains with truncated excretory canals (alleles *mp0920*, *mp0921*, and *mp0922*; Fig.10) were analyzed further using complementation tests.



**Figure 10: Micrographs of *C. elegans* showing different phenotypes.** From left to right: Mig phenotype of *mig-10 (ct41)*; *mig-10 (mp0920)*; *mp0921*; Wild Type phenotype; and *mp0922*. All strains shown in Figure 10 contained the *bgls312* transgene, which was used as an indicator for *mig-10* phenotype of truncated excretory canal. There is some variability in the phenotype of the mutant RY0921. *mp0921* phenotype looks very similar to wildtype phenotype at L3-L4 stage and the truncation phenotype appears at a later stage of young adult.

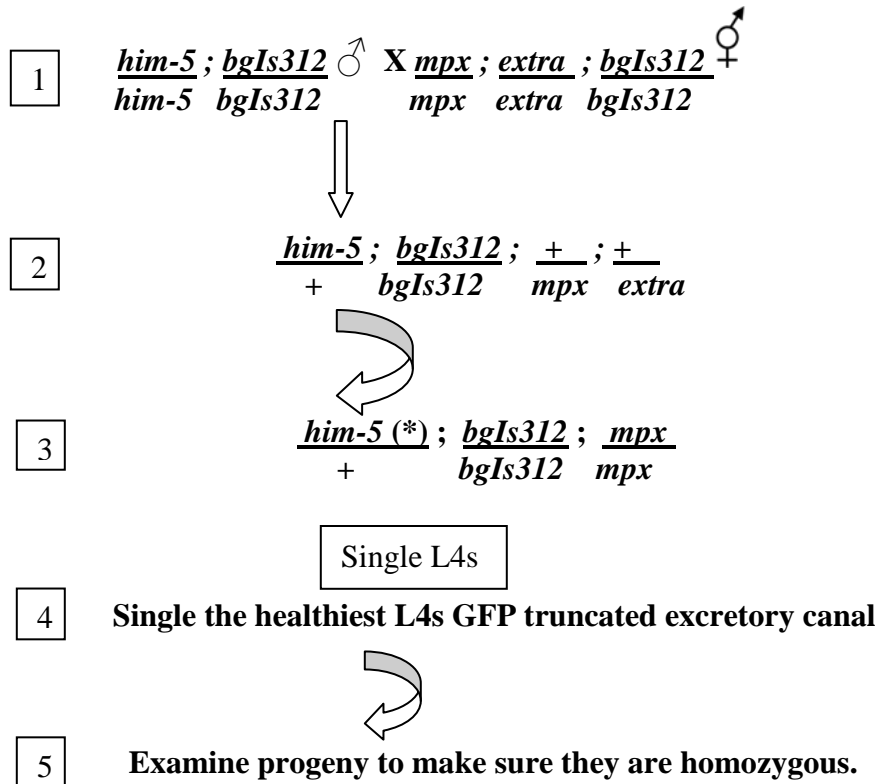
The severity of the three new found putatives was estimated based on the full length of labeled canal cell of wild-type phenotype (Table 3). They all appear to be less severe than *mig-10* (*ct41*) phenotype.

<b>Strain</b>	<b>Approximate average truncation of excretory canal</b>
RY0920	~40%
RY0921	~66%
RY0922	~50%
RY108	~10-30%

**Table 3: Strain Severity**-These results were acquired after all of the strains were backcrossed at least one time.

## 2. Backcrossing

Putative strains were backcrossed to isolate the mutant gene from any deleterious mutations arising during mutagenesis (Figure 11). The backcrossing process was done several times for each strain to achieve a better result for eliminating these deleterious mutations. *mp0920* was backcrossed three times while *mp0921* and *mp0922* were backcrossed one time each.



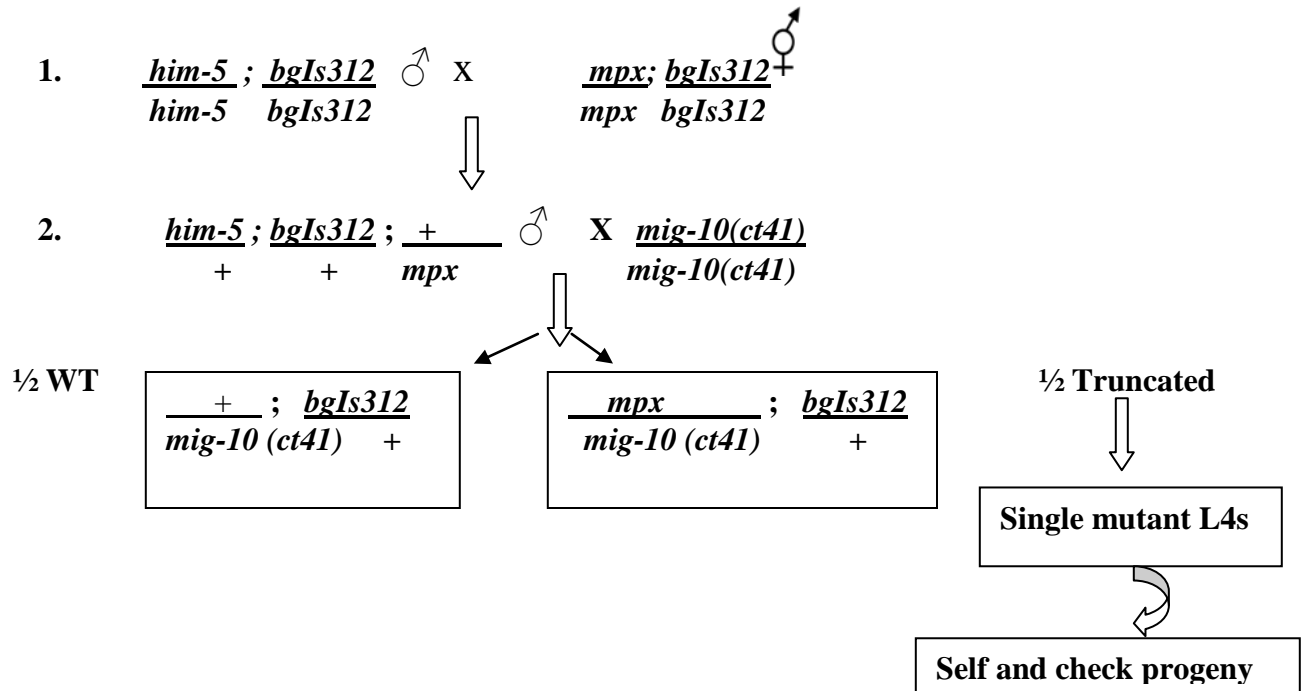
**Figure 11: Summary of Backcrossing.** *bgIs312* is an integrated transgene marker that visualizes the excretory cells. “Extra” designates extraneous mutations (e.g. dumpy or other undesired mutants). L4s to single out from Steps 2 should be all GFP full-length excretory canals (wild-type). Worms that have truncated excretory canals or dumpy phenotypes should also be followed up to check if they are self-progeny. (\*) here can be +/+ or +/*him-5* or *him-5/him-5*. In step 1, 3-4 L4 putative mutants from the simple screen were crossed with 8-10 *him-5; bgIs312* males at 20C. Two days later, the P0 parental adults were removed from the mating plate. In step 2, on the fourth and fifth days, several wild type L4s were picked to a new plate, allowed to self-fertilize, and the next generation was examined for mutant worms. In step 3 and 4, animals with extraneous/deleterious gene will not all homozygose at this step, it is ideal to single out the healthiest looking mutant worms because they do not homozygose deleterious mutations. In step 5, in order to ascertain whether each putative strain was homozygous for new mutant gene after the backcross, several worms with mutant and wild type phenotype were singled to examine the mutant ratio in the next generation. It was observed that the phenotype was not fully penetrant in some alleles or homozygous mutants can have a wild type appearance in the progeny generation. Therefore, in the next generation, the number of worms with mutant and wild type phenotype on each plate was observed. The separation and choosing healthy putative is important for follow-up experiment.

### 3. Complementation tests

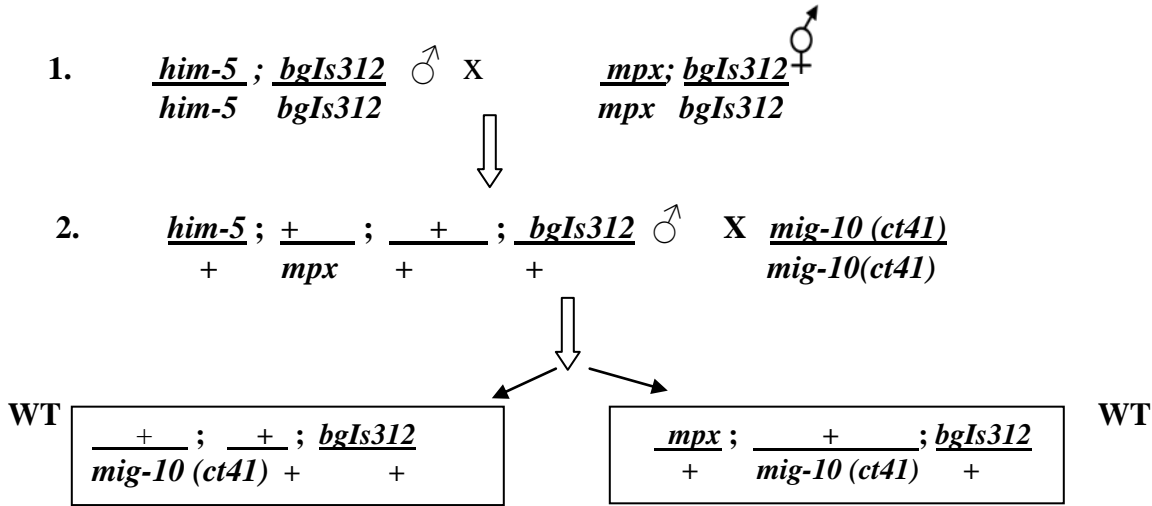
Complementation tests were performed to determine whether the mutant gene responsible for the shortened excretory cell mapped to *mig-10* or a different gene. Non-complementation occurs when two mutations, in the same gene, but on opposite chromosome, result in a mutant phenotype. If a new mutation *mpx* is produced, it would fail to complement the old mutation *ct41* present on the other chromosome, resulting in the Mig phenotype.

The summary for each case of the results is described as in Figure 12 below.

#### Case 1: Assuming mutation is an allele of *mig-10*



**Case 2: Assuming mutation is not an allele of *mig-10***



**Figure 12: Complementation Test Process-** *mpx* designates isolated alleles that show a similar phenotype to *mig-10*, a shortened excretory canal. The curved arrow represents animals that were self-fertilized. BW315 strain are known *mig-10 (ct41)* mutant worms that do not have integrated transgene *bgIs312* marker that visualizes the excretory cells. The first step to the complementation tests was to cross 8-10 *him-5* males with 3-4 *mpx;bgIs312* L4s. Then, after two days, the parental adults were removed from the mating plate. The male progeny *him-5; mpx;bgIs312* (8-10 worms) were then crossed to *mig-10 (ct41)* L4s (3-4 worms). The adults from that mating were also removed from the plate two days later. On the third through fifth days, the mutant crosses were examined under the UV dissecting microscope to determine the percentage of cross progeny with shortened excretory canals on the plates. Cross progeny were identified by the *bgIs312* marker. If *mpx* were an allele of *mig-10*, about 50 percent of the cross progeny would be mutant (Case 1). If *mpx* were not an allele of *mig-10*, however, then the percentage of mutants would be zero, unless there was an interaction between the genes or the mutation was dominant (Case 2). If present, mutant L4s from the F2 generation were singled and allowed to lay the F3 generation. The F3 generation was examined three days after it was laid. If all progeny were mutant, then *mpx* should be an allele of *mig-10*. If there was variability among the F3 mutants such as no fluorescence or variation in the length of the truncation, several of the severe mutants were singled to determine whether the severity was inheritable.

#### 4. Isolate and identify new mutations: PCR and enzyme digestion figures.

Each mutant allele isolated in the screen was tested using PCR and restriction enzyme digestion to ensure that it was a new mutation, different from the *mig-10(ct41)* allele.

PCR reactions were run using primers specific to the *mig-10* gene. This was followed by a digestion reaction with the Hpy188I restriction enzyme and gel electrophoresis. The digested samples did not have similar sized DNA fragments to the DNA fragments of the known *mig-10* (*ct41*) mutation. As shown in Figure 13, all three putative mutants were confirmed as new mutations with DNA fragments of different sizes than the *mig-10* (*ct41*) mutant.

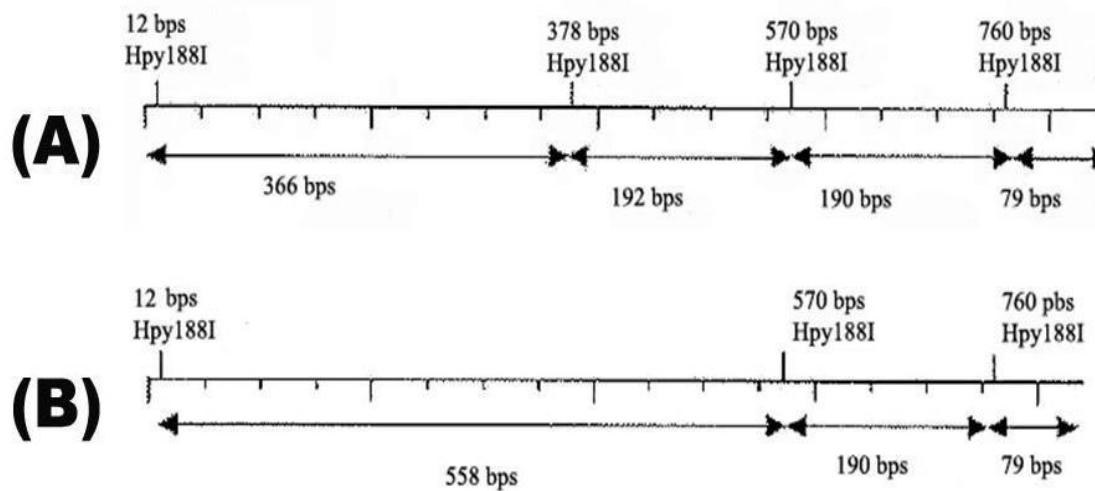
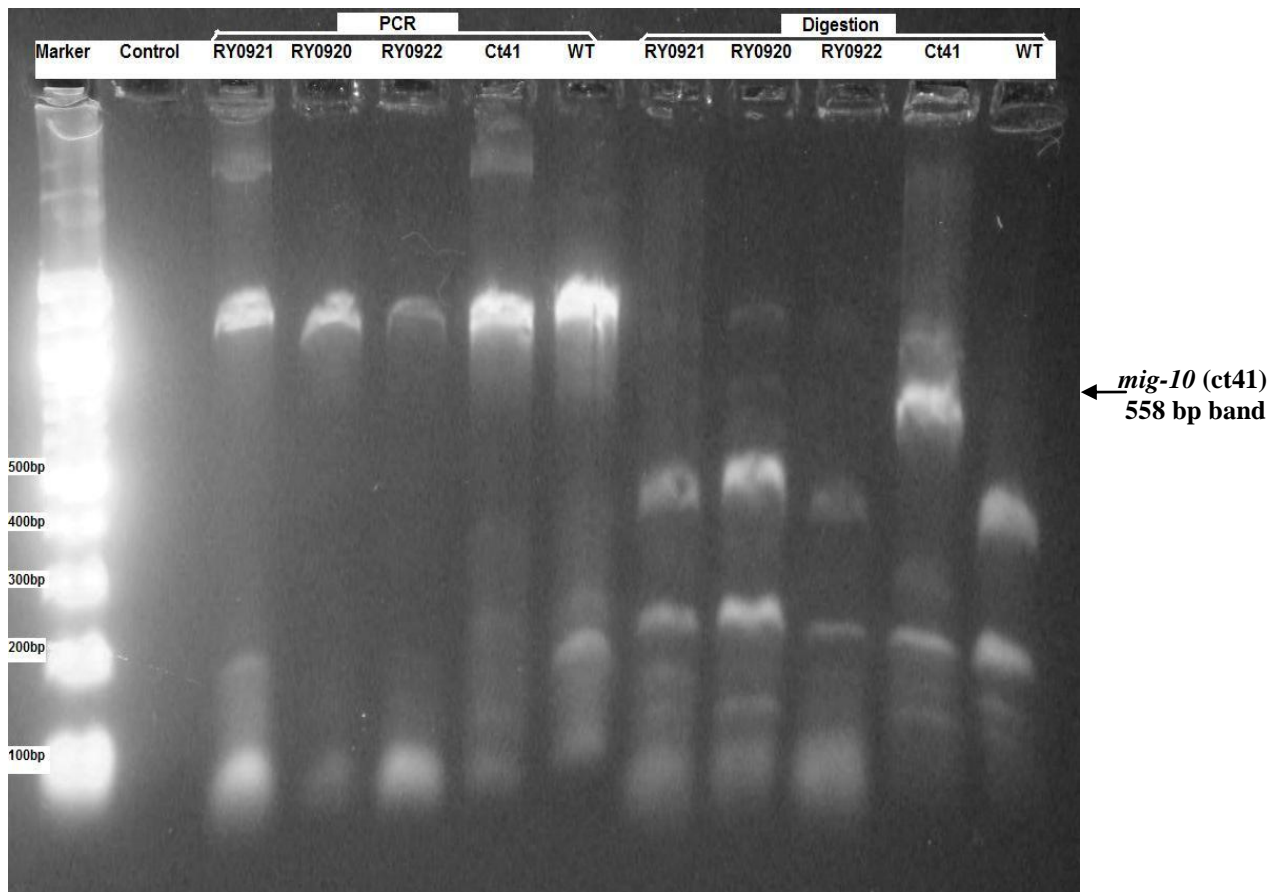


Figure 13: Restriction map of (A) wild type *mig-10* gene and (B) known *mig-10* (*ct41*) mutation (Figure taken from WPI thesis by Stoval, 2004)



**Figure 14: PCR and enzyme digestion of putative mutants, known mutation *mig-10 (ct41)*, and wild-type.** From Left to Right: Lane 1: marker; Lane 2: PCR negative control (containing no DNA); Lane 3, 4, and 5: undigested sample of three putatives (RY0921, RY0920, RY0922) having truncation phenotype; Lane 8, 9 and 10: digested sample of three putatives having truncation phenotype; Lane 6 and 11: undigested and digested *mig-10 (ct41)* mutation; Lane 7 and 12: undigested and digested wild-type *mig-10*.

## 5. Characterization of putative strains: DNA sequencing.

In order to identify the sequence change in the *mig-10* allele isolated in the screen, sets of primers were designed for DNA sequencing for most of the coding regions of the *mig-10 (mp0920)* allele (see Part 4, Methods). Initial attempts to amplify PCR products with these primers were unsuccessful, and were not continued due to time constraints.

# Discussion

The goals of this project were to identify and characterize novel missense mutations in the *mig-10* gene of *C.elegans* to help define protein domains and residues important for neuronal migration function. For this study, the desired phenotype was a visibly truncated excretory cell, visualized by a transgene *bgIs312* expressing GFP in that cell. We isolated a new allele *mp0920* of *mig-10* mutation by using mutagenesis in a simple screen and began attempting to sequence the isolated mutant strain.

All of the putative mutants were backcrossed several times to isolate the one gene responsible for the truncated excretory canal and to make the strains healthier. A complementation test showed that *mig-10 (ct41)* failed to complement *mp0920*, thus signifying that this strain was probably an allele of *mig-10*. The complementation tests for *mp0921* and *mp0922* confirmed that these are not mutations in the *mig-10* gene.

We found that the simple screen was a sufficient method of finding putative mutants. However, there were a number of problems. Although the truncated excretory canal cell phenotype was easy to recognize, the likelihood of false positives was significant, especially when the worms starved and grew older. The mutagenesis procedure was quite lengthy. The F2 generation should be screened as soon as the worms that hatched first are L4's, where the fluorescent canal cell most visible, and before the worms grew older and the fluorescent canal cell becomes less visible. The plates could be screened for a maximum of three days. With 300-500 worms on the plate, after three days the plate was quite starved and the worms on the third day could be at the young adult stage with less GFP shown. In addition, many worms crawled deeper underneath the gel and hence were not very visible. Both of these factors impeded the process of finding mutants.

To remedy these problems in future screens, we suggest washing off and moving F2 worms to 2-3 new fresh plates from each original plate before screening. We also suggest lengthening the time available for screening by growing the animals at different temperatures. The optimal temperature for *C.elegans* to grow normally is at 20<sup>0</sup>C. Because of the lengthy process of the simple screen, we stored some plates at 15<sup>0</sup>C to slow down their growth to be screened at a later time. We also stored some plates at 25<sup>0</sup>C to speed up the worms' life cycle to screen at an earlier date.

In addition, we had issues with the viability of mutagenized *C.elegans*. We made some adjustments with the protocol such as increasing the number of worms to be mutagenized (from 3-4 to 5-6 worms per plate to generate P0 generation).

We were in the process of designing and determining the proper conditions for primers to work in PCR before sending the PCR products for DNA sequencing. However, we were not able to finish this work because of the time restriction posed by the school year. This part of the project should be continued because the analysis should help to characterize this new mutation. If this new mutation was characterized, it would be possible to use this mutation to study the signal transduction pathway of the *mig-10* gene, and possibly the active domains of the protein.

For future work, we suggest that continuing to characterize the novel *mig-10* allele *mp0920* by DNA sequencing to determine where the mutation occurred in the gene. Furthermore, it is important to accurately quantify the phenotype of the new allele such as estimating percentage of penetrance, severity of canal length in comparison with known *mig-10* (*ct41*) phenotype, as well as evaluating the effect of this new mutation on neuron and axon migration (i.e. position/distance from target locations). Specifically, in order to characterize the effect of the new mutation on neuronal migration and axon guidance, other transgenic markers for specific neurons or axons should be used.

In conclusion, we were able to isolate and confirmed a new allele of *mig-10* gene by a simple screen, complementation test and PCR. In spite of its problem, the simple screen is a better choice than a non-complementation screen, which was used in the past, because we were able to identify a new mutant. We found that the phenotype or truncation severity of this new mutant is weaker than the known *mig-10* (*ct41*) mutation. This may suggest that we have identified a missense mutation rather than a null mutation, which is a positive outcome. The improvements we have recommended could make the next steps of this project, specifically the simple screen, more efficient, and hopefully resulting in the identification of several alleles, which might have different effects on MIG-10 function, thus elucidating the mechanism of its function.

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