



**Phenotypic and Molecular Characterization of the
mig-10(mp0920) mutant allele in
Caenorhabditis elegans.**

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Abstract

The MIG-10 protein plays a role in axon guidance in *C. elegans*. The *mig-10(mp0920)* mutant was characterized phenotypically by measuring the migration of the ALM and AVM neurons as well as the anterior and posterior processes of the excretory cell. The excretory processes of the *mig-10(mp0920)* mutants showed an intermediate truncation between wild-type and the null mutant *mig-10(ct41)*, while neuronal migration was unaffected by *mig-10(mp0920)*. Three exons of *mig-10(mp0920)* were sequenced and found to be wild-type.

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Introduction

Nervous system

The study of the formation of the nervous system during embryonic development is an important topic in the field of neurobiology. The nervous system is composed of a network of neurons. This network is formed when neurons and axons travel along a path set out by extracellular guidance cues. These guidance cues, such as Netrins, Slits, Semaphorins and Ephrins, activate growth cone receptors. In the growth cone, F-actin and microtubules localize asymmetrically through a mechanism which is initiated by the activation of the guidance receptor. The growth cone migrates along the path of the asymmetric localization (Quinn & Wadsworth, 2008). Depending on the guidance cue, the axon migration may be attracted or repelled, initially to the correct target or away from the wrong path respectively (Yaron & Zheng, 2007).

A pathway that leads to a mis-wiring of the neurons, and thus a dysfunctional nervous system, can be associated with developmental disorders such as Down's syndrome and autism. Understanding the mechanisms behind the development of the nervous system can better our efforts in treating developmental disorders and neurodegenerative diseases (Quinn & Wadsworth, 2008).

Research on the nervous system has mostly involved guidance cues and their surface receptors. The mechanisms involved downstream of these are not yet fully understood (Yu & Bargmann, 2001). MIG-10 is a component of this downstream mechanism. In this project, the nervous system of the *C. elegans* was used in order to study the protein MIG-10, an ortholog of Lamellipodin (Lpd), a vertebrate protein which is involved in axon outgrowth.

***C. elegans* as a model organism**

Caenorhabditis elegans (*C. elegans*) is a model organism due to its small size and short life cycle. The genome of *C. elegans* has been completely sequenced and its small nervous system has been well characterized; this allows for it to be used for genetic analysis (Riddle, 1997). This model organism has proteins, such as MIG-10, that are orthologs to vertebrate proteins, like Lpd, that are involved in axon outgrowth. All of these advantages can be used toward providing information about the mechanism downstream of the guidance receptors (Quinn & Wadsworth, 2008).

MIG-10 Protein

The *mig-10* gene was discovered when screening for mutations that affect neuronal migration. Phenotypes such as “withered” tails from *mig-10* mutants indicated an abnormal canal-associated neuron (CAN). Mutations of the *mig-10* gene show phenotypes of truncated migration of these types of neurons: CANs, anterior lateral microtubule cells (ALMs) and hermaphrodite-specific neurons (HSNs). The migration of CANs and ALMs are anterior to posterior and HSN migration is posterior to anterior. Also, truncation of the posterior excretory canal was noted in the *mig-10(ct41)* mutant. This result shows that the *mig-10* gene is also involved in excretory canal growth as well as neuronal migration (Manser and Wood, 1990). These phenotypes are consistent with our current model of MIG-10 function in mediating actin polymerization during migration (Quinn and Wadsworth, 2008).

Different mutations of the *mig-10* gene have been studied, such as the null allele, *mig-10(ct41)* which has been studied extensively. The mutant *mig-10(mp0920)* was isolated through EMS mutagenesis, which typically causes point mutations. In comparing the *mp0920* allele to

the *ct41* allele, the truncation was less severe phenotypically and therefore suggests that this allele is possibly a missense mutation (Kukla, 2009). This study on the effect of mutations of the *mig-10* gene, has the potential of improving our understanding of the model for the pathway of MIG-10 and how it functions; with the possibility of finding a significant alteration of the wild-type gene, the role MIG-10 plays in axon guidance can be furthered.

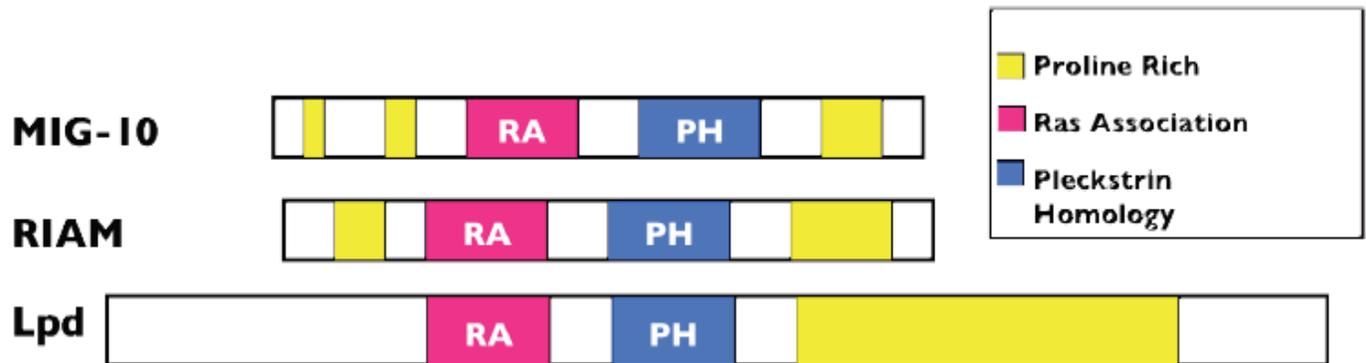


Figure 1: Domain regions of MRL Protein Family (Fiociello and Ryder, unpublished)

MIG-10 Function

In *C. elegans* MIG-10 is a cytoplasmic adaptor protein that belongs to the MRL family. The MRL family includes MIG-10, RIAM and Lpd. Within the MRL family, the proteins share RA and PH domains, and a proline-rich region (Lafuente et al., 2004). Figure 1 shows the RA, PH and proline rich domains that the MRL family shares. MIG-10 functions downstream of the guidance cue UNC-6 (netrin) and SLT-1 after they bind to their guidance receptors, UNC-40 (DCC) and Robo, respectively (Chang et al, 2006; Quinn et al, 2008). MRL proteins are involved in regulating actin polymerization (Krause et al., 2004).

The role of MIG-10 in outgrowth has been shown by experiments involving overexpression. When MIG-10 is overexpressed and the guidance cues are not present, the outgrowth is misguided into multiple processes. With the addition of the guidance cues, the

outgrowth is redirected into a single process. These results indicate that both MIG-10 and its guidance cues are required for promoting directed outgrowth (Quinn et al., 2008).

MIG-10 has also been shown to be involved in actin polymerization. In the Chang et al., 2006 study, *mig-10;unc-34* double mutant showed more severe deficits in axon guidance than the single mutants. These results show that MIG-10 works with UNC-34 (Ena/VASP ortholog), even though they both have distinct functions. Also in this study, a null mutation of AGE-1, a PI3K ortholog, suppressed excessive axon growth when MIG-10 was overexpressed. With this information, it is asserted that MIG-10 acts downstream of AGE-1 (Chang et al., 2006).

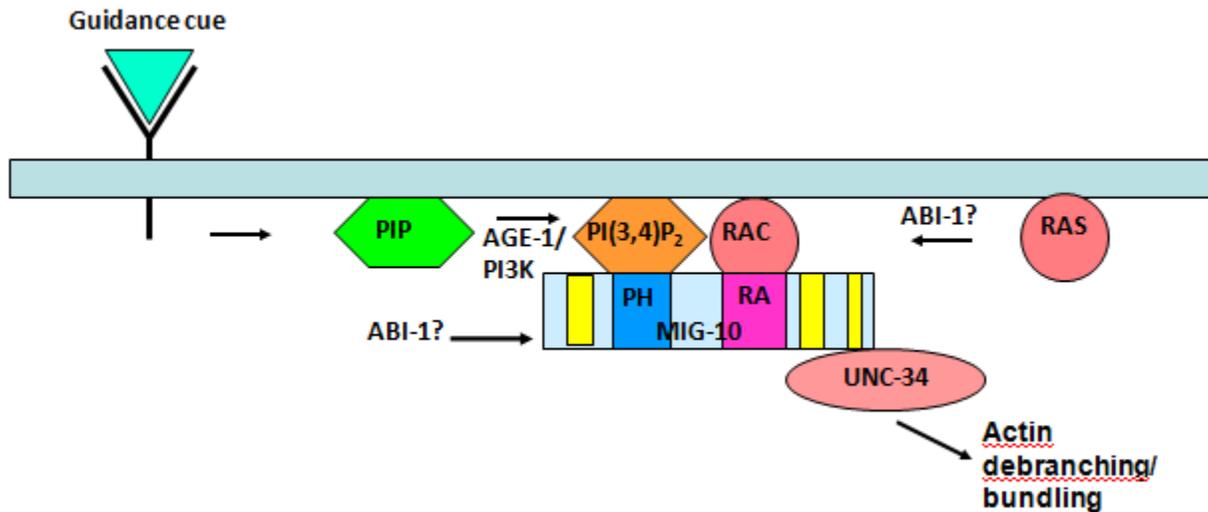


Figure 2: Proposed Model for MIG-10 function. (Fiociello and Ryder, 2007).

Guidance cue activates guidance receptor which activates CED-10 (Rac1 GTPase) and AGE-1 (PI3 kinase). The resulting activation localizes MIG-10 to the cell membrane.

Figure 2 shows a proposed model of the signaling pathway for MIG-10. This pathway shows that a guidance receptor, UNC-40(DCC), is activated by a guidance cue, UNC-6 (netrin). An activated UNC-40 (DCC) then activates AGE-1 (PI3K) and CED-10, an ortholog of Rac1 GTPase. PI3 kinase phosphorylates PIP to PI(3,4)P2. After this, MIG-10 is localized asymmetrically to the cell membrane through its Ras association (RA) and pleckstrin homology (PH) domains. The RA domain of MIG-10 associates with the Ras-related protein, and the

pleckstrin homology domain of MIG-10, associates with PI(3,4)P2. UNC-34 then binds to MIG-10 and mediates actin polymerization (Chang et al, 2006; Quinn and Wadsworth, 2008).

Actin Polymerization

Actin polymerization is crucial for cell migration; it is the process through which actin filaments are assembled. The machinery behind this process begins with growth factors on the surface of the lamellipodia, binding receptor tyrosine kinases (RTKs). This binding activates the RTKs' cytoplasmic kinase domain, and the auto-phosphorylation of tyrosine residues. The PI3-K region of the SH2 domain contains a regulatory subunit (p85), as well as a catalytic subunit (p110). The phosphorylated tyrosine residues bind the SH2 domain via p85. PI3-K then binds GTP-loaded Ras via p110 (Disanza et al., 2005).

When activated, PI3-K converts PIP2 into PIP3. PIP3 initiates the binding of guanine-nucleotide exchange factors (GEFs) such as Sos-1, which, when presented along with Eps8 and Abi1, acts as a Rac-GEF. These factors go on to activate Rac (Disanza et al., 2005). Rac regulates capping proteins, which are responsible for capping actin side branches and for direct the generation of uncapped filaments (Disanza et al., 2005).

Abi1 regulates WAVE, a molecule with a WH2 domain. WAVE goes on to activate the Arp2/3 complex, which is responsible for the nucleation of actin filaments (Disanza et al., 2005, & Shakir et al., 2008). UNC-34 also plays a role in actin polymerization. This Ena/VASP protein has been shown to prevent capping of actin filaments. UNC-34 associates with the MIG-10 protein that has localized to the cell membrane. This interaction supports MIG-10's function in actin polymerization (Krause et al., 2003).

Project Goals

A. Backcrossing

In order to use the *mig-10(mp0920)* strain, the neuron marker, *flp-20::GFP* was backcrossed in. This backcrossing allows for phenotypic quantification of the migration of neurons. The excretory canal marker, *pgp-12::GFP* had been previously backcrossed into *mig-10(mp0920)*, and was also be used to phenotypically quantify the migration of the excretory canal. Backcrossing helps to eliminate any undesired mutations.

B. Phenotypic characterization of *mig-10(mp0920)*

Through the use of marker transgenes, the phenotype of the mutation was determined. In order to quantify truncation of the excretory canal and the migration of the cell bodies, the *mig-10(mp0920)* mutation was compared to a wild-type strain. The markers *pgp-12::GFP*, *flp-20::GFP* and *mec-4::GFP* were used to label the excretory canal and the neuronal cells respectively. Lengths of the processes were quantified by measuring from the back of the pharynx to the middle of the vulva, in L4 stage *C. elegans* for ALM and AVM migration, and from the cell body to the end of the process for the anterior and posterior migration of the excretory canal.

C. Molecular characterization of *mig-10(mp0920)*

The main goal of this MQP project is to determine if there is any alteration in the sequence for the *mig-10(mp0920)* mutant allele. Through the process of PCR and sequencing, any base changes can be addressed. Several pairs of primers were used to allow sequencing of all the exons of the gene. If the *mig-10* mutated allele (*mp0920*) were to be found to be a

missense allele, this would help to identify the domain of the MIG-10 protein that is involved in the neuron and axon migration as well as where in the mechanism it plays a part.

Methods

Maintaining Strains

E. coli. spotted agar plates were used to maintain the worms. Three L4 hermaphrodites of each strain were plated onto a new plate to maintain the strain, and kept at either 20°C or 15°C. To maintain the wild type strain (N2M), 5 male worms were plated with 3 hermaphrodites.

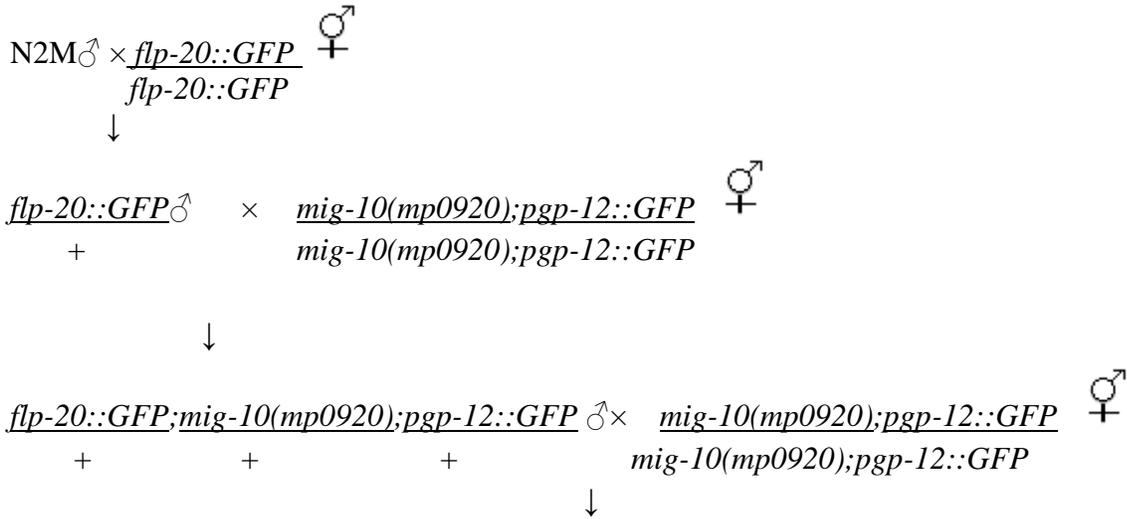
Mounting Worm Slides

Worms were washed off plates using M9, pipetted to centrifuge tubes, and allowed to settle. Agarose pads were made by melting aliquots of 2ml, 2% agarose and adding 20ul 1M sodium azide. Drops of this mixture were placed onto slides and were covered with another slide to form the pads. The settled worms (3ul-5ul) were pipetted onto the pads and a cover slip was placed on the slide.

Crossing in Excretory Canal Marker (*flp-20::GFP* and *mec-4::GFP*)

The initial *mig-10(mp0920)* strain that was used had been backcrossed 3 times before. Wild type male worms were crossed with *flp-20::GFP* hermaphrodites. The male progeny of this cross was crossed with hermaphrodites from *mig-10(m0920);pgp-12::GFP* which was already a three times backcrossed homozygous strain. The male progeny from this cross with the *flp-20::GFP* marker present was crossed into the *mig-10(mp0920);pgp-12::GFP* homozygous hermaphrodites. Three hermaphrodites from this progeny were plated to self-cross and several worms were singled in order to homozygous for the *pgp-12::GFP* and *flp-20::GFP* markers. Their progeny were singled until the strains were homozygous for the *pgp-12::GFP* and *flp-20::GFP* markers. Throughout the cross, the worms that had both the *pgp-12::GFP* marker and

the *flp-20::GFP* marker, were selected for crossing. The other neuronal marker, *mec-4::GFP*, was also crossed into the *mig-10(mp0920);pgp-12::GFP* strain the same way. Figure 3 is showing the crossing in of the *flp-20::GFP* marker.



Picked progeny with truncated excretory canals (as marked by *pgp-12::GFP*)

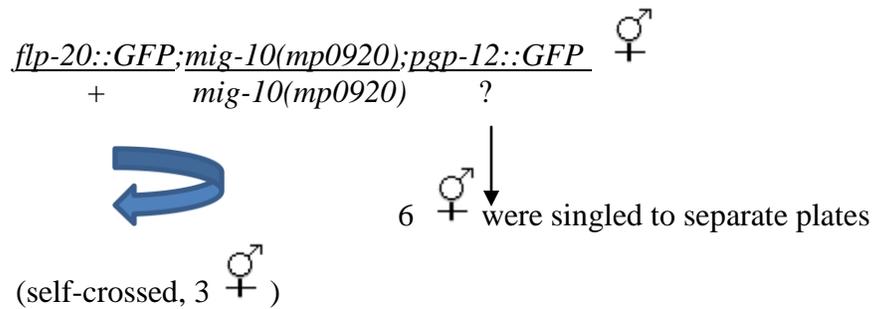


Figure 3: Backcrossing in *flp-20::GFP* marker for neurons

Initial strain, *mig-10(mp0920);pgp-12::GFP*, was 3 times backcrossed.

Final strain: *mig-10(mp0920);flp-20::GFP;pgp-12::GFP*(homozygous for the markers)

Legend: downfacing arrow= ongoing process, curved arrow= self-cross

Gene Sequencing

A digest buffer plus enzyme was made by pipetting 7.5ul of 10 mg/ml proteinase K into 0.5ml of a lysis buffer (50mM KCl, 10mM Tris, pH 8.2, 2.5mM MgCl₂, 0.45% NP-40, 0.45% Tween 20, 0.01% gelatin, 0.15mg/ml Proteinase K). A quantity of 2.5ul of this mixture was added to the cap of a PCR tube. Approximately 10 worms were picked to the cap of the tube as well. The PCR tube was centrifuged for 10 seconds and 50ul of mineral oil was pipette on top of the formed pellet. This tube was stored at -80°C for at least 30 minutes before running the lysis protocol (1 hour at 65°C and 15 minutes at 95°C to inactivate the Proteinase K).

The samples in the PCR tubes were thawed and a master PCR mix was made. The master mix consisted of 2.5ul 10x Thermopol buffer, 0.5ul dNTP (stock's 10mM in each dNTP), 1.0ul Vent, 1.0ul forward primer (5um stock) and 1.0ul reverse primer (5um stock), 2.5ul of the worm lysate, and 16.5ul dH₂O.

Primer pairs 1,2,3,4,5 and 9 were used for the PCR protocol. The PCR products program consisted of a 95°C incubation stage to denature the DNA, the program also had a 60°C stage in which DNA was annealed. The primers were extended during the 72°C stage. The cycle repeated 35 times and then was kept at 4°C when completed. These samples were then kept at -20°C.

The PCR samples were run on a gel. 5ul of 10mg/ml ethidium bromide was pipetted and mixed into 100ml of 0.8% agarose in TAE. The gel was run for 1 hour at 90 volts. The brightest bp strands on the gel were cut out using a UV light and a razor blade. The samples were placed into tubes and kept at -20°C. Primer pair 1 failed to produce a PCR product. The successful PCR products were either gel purified or through a nucleotide purification method.

The PCR products that we run on a gel were purified by following the QIAquick Gel Extraction Protocol. The QIAquick PCR Purification Protocol was followed for the other products. The concentrations of the purified DNA samples were calculated. The sample was sent out to Genewiz for sequencing.

Quantification of Phenotypes

A fluorescent microscope was used to take photos of the worms. A camera was attached to the microscope, and the program ImageJ was used. Photos of the L4 worms of a strain were taken under incandescent and fluorescent light. These images were merged and the color channels were modified to show the fluorescent markers along the worm. To quantify the phenotypes, the processes were measured, in pixels, using ImageJ.

The anterior projection of the excretory canal was measured from the center of the cell to the anterior end, and then was compared to the distance between the nose of the worm to the posterior end of the pharynx. The posterior projection of the excretory canal was measured from the cell body to the posterior end of the excretory canal. This measurement was compared to the length of the whole worm, from nose to tail, for normalization of the data.

The migration of the ALM1, ALM2, and AVM processes were determined by measuring from the middle of the vulva to the middle of the cell body. These measurements were compared to the length between the middle of the vulva to the posterior end of the pharynx for normalization of the data.

Statistical Analysis

The data collected from the measurements of the processes, were analyzed using the program, SPSS. Analysis of variance (ANOVA) and post hoc tests were used to determine any

differences in ALM, AVM migration, and excretory canal truncation between the *mig-10(mp0920)*, *mig-10(ct41)*, and the wild type worms.

Results

The new *mig-10* allele, *mp0920*, had been previously isolated from a genetic screen. In order to characterize this allele, phenotypic and molecular information was gathered. For their phenotypic characterization, *C. elegans* were characterized at their L4 stage. The wild-type allele and two mutant alleles, *ct41* and *mp0920*, were used for this report.

To characterize for the phenotype of the allele, strains with markers for the excretory canal and the neurons were used. Each strain contained the excretory canal marker *pdp-12::GFP*, and either *flp-20::GFP* or *mec-4::GFP* to mark for the neurons.

For the molecular characterization, the *mig-10(mp0920)* gene was sequenced at portions of the gene.

mig-10 molecular characterization

Regions of the *mig-10(mp0920)* gene were sequenced and compared to the wild-type *mig-10c* transcript, which is 26,725 base pairs long and has 2,340 bp coding region. The transcript for the *mig-10c* isoform is shown in Figure 4. Several regions were sequenced; only two sequencing results were successful. The PCR products generated by primer pair 5 sequences for exons 10 and 11; exon 1 corresponds to primer pair 9. These exons were wild-type. The last seven exons and introns of the *mig-10c* sequencing data is located in Appendix B, along with the N-terminus region and the results of the PCR products that corresponded to primer pairs 5 and 9.

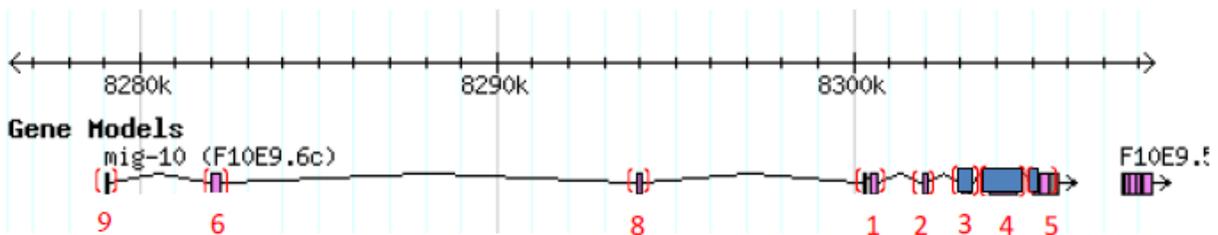


Figure 4: Transcript for *mig-10c* Primer locations

Primer locations are indicated by their numbers. Each coding region is encased in brackets (wormbase.org 2010, as modified by Kukla, 2009).

mig-10 phenotype quantification

Excretory canal migrations are truncated in *mig-10* mutants

The posterior migration of the excretory canal was measured from the cell body to the end of the process and normalized for the size of the worm by dividing by the total length of the worm (Figure 5A). Analysis of the length of the posterior canal migration showed that differences in migration between all three worm strains were significant. (ANOVA followed by post-hoc tests; $p < .001$). As shown in Figure 5B the posterior migration of the *mig-10(ct41);pgp-12::GFP* mutant was severely truncated. The *mig-10(mp0920);mec-4::GFP;pgp-12::GFP* mutant showed intermediate truncation between the null mutant and the wild-type.

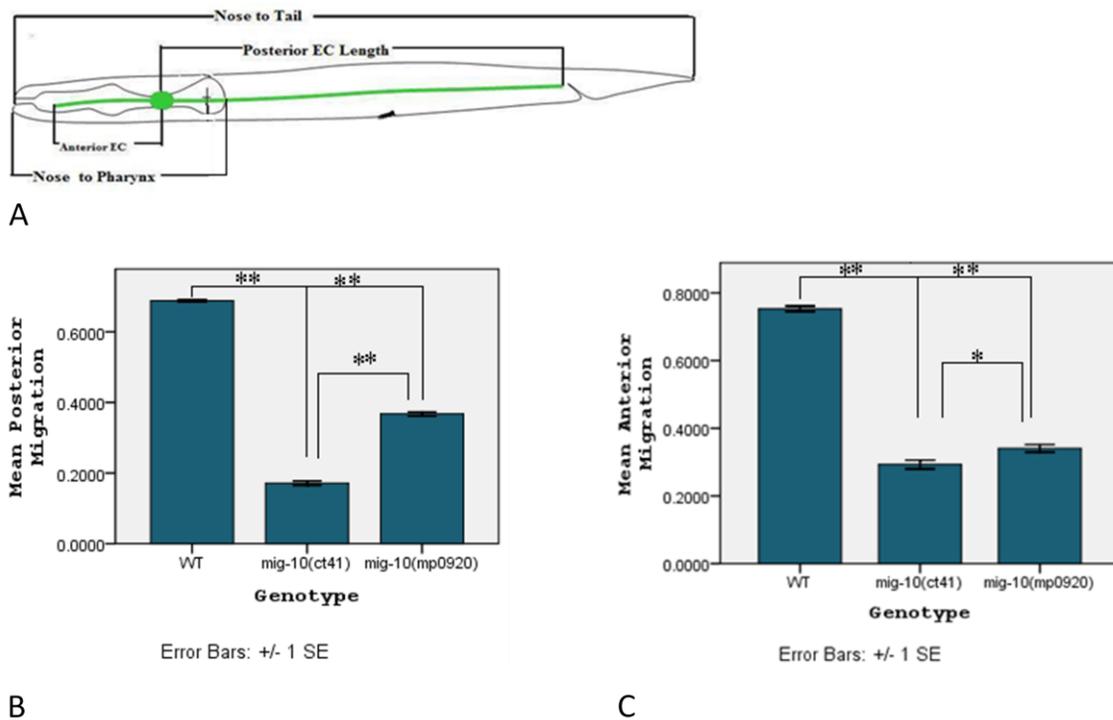


Figure 5: Posterior and anterior processes of the excretory canal are truncated in *mig-10* mutants
A) Schematic of worm measurements. All worm strains have the *pgp-12::GFP* marker for the excretory canal. B) Posterior migration = posterior EC length / Nose to Tail C) Anterior migration = anterior EC / Nose to Pharynx. *, $p < 0.01$, **, $p < 0.001$ (Bonferroni Post Hoc test). WT, $n=40$; *mig-10(ct41)*, $n=35$; *mig-10(mp0920)*, $n=52$.

The anterior migration was measured from the middle of the cell body to the end of the process and normalized by dividing by the distance from the back of the pharynx to the tip of the nose of the worm (Figure 5A). The anterior canal migration was also shown to be truncated in both mutant strains (Fig. 5C). ANOVA and Post Hoc tests were performed and showed that each mutant and the wild-type were significantly different with a p-value less than 0.001 (Fig. 5C). Although both mutants were also significantly different from each other ($p < 0.01$), the absolute difference in truncation between the two mutant strains was small (Fig. 5C).

Neuron migrations are truncated in *mig-10(ct41)* and are WT in *mig-10(mp0920)* mutants

ALMs

The ALM neurons' migrations were measured from the middle of the vulva to the cell body. This measurement was then subtracted from the distance from the back of the pharynx to the middle of the vulva in order to show more easily, the truncation severity between strains. Due to the ALMs' posterior migration, subtracting this distance will account for the actual distance migrated, which is shorter when migration is truncated. To normalize the data, the measurement was then divided by the distance from the back of the pharynx to the middle of the vulva. Figure 6A is a depiction of the areas of measurement for ALM1 and ALM2.

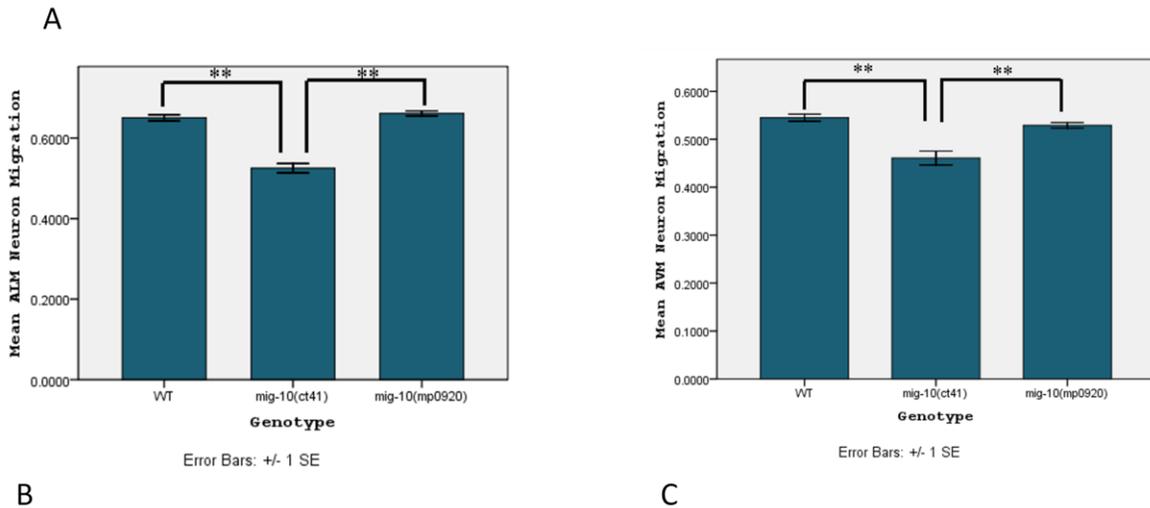
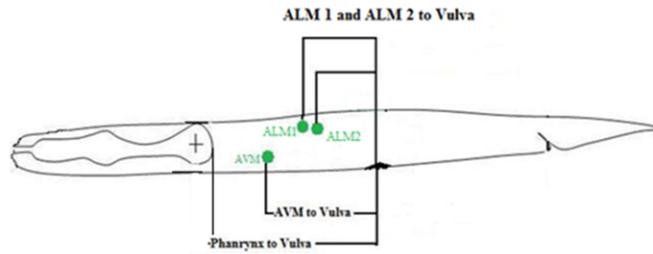


Figure 6: ALM and AVM Neuron Migration is truncated only in the *mig-10(ct41)* mutant

A) Schematic of worm measurements. All worm strains have a GFP marker for the ALM and AVM neurons (either *flp-20::GFP* or *mec-4::GFP*)

B) $\text{ALM neuron migration} = \{(\text{pharynx to vulva}) - (\text{ALM to vulva})\} / (\text{pharynx to vulva})$

C) $\text{AVM neuron migration} = (\text{AVM to vulva}) / (\text{pharynx to vulva})$. **, $p < 0.001$ (Bonferroni Post Hoc test). WT, $n=57$; *mig-10(ct41)*, $n=47$; *mig-10(mp0920)*, $n=46$.

ALM1 was designated as the ALM that was furthest away from the vulva and ALM2 was designated as the ALM closest to the vulva. The measurements for ALM1 and ALM2 were then averaged for each animal. Worm strains carrying either the *mec-4::GFP* or the *flp-20::GFP* marker were used interchangeably as wild-type controls. Figure 6B is a graph of the mean ALM migration data for the wild-type and both mutants.

ANOVA and Post Hoc Tests were also conducted in order to determine the significance of the measurements (Appendix A). The results show that the wild-type and *mig-10(mp0920);mec-4::GFP,pgp-12::GFP* strains had no significant difference in ALM migration ($p=1.00$ from Bonferroni) and thus *mig-*

10(mp0920) had no effect on ALM migration. The *mig-10(ct41);flp-20::GFP* mutant ALM migration was significantly shorter than both the wild-type and *mig-10(mp0920);mec-4::GFP;pgp-12::GFP* strains ($p < 0.001$). These results show that the *mig-10(ct41);flp-20::GFP* mutant's migration of the ALM neurons is truncated.

AVM

For the AVM neuron migration, measurements were taken from the middle of the vulva to the cell body and were normalized by dividing by the distance between the back of the pharynx to the middle of the vulva (Figure 6A). Figure 6C is a graph of the mean AVM neuron migration.

ANOVA and Post Hoc Tests were also run to determine any significant differences in migration (Appendix A). With a p-value less than 0.001, these results show that the migration distances of the wild-type and the *mig-10(ct41);flp-20::GFP* mutant were different. The AVM migration of the *mig-10(ct41);flp-20::GFP* and the *mig-10(mp0920);mec-4::GFP;pgp-12::GFP* mutants were also significantly different from one another. The *mig-10(ct41);flp-20::GFP* AVM neuron migration was truncated, while the *mig-10(mp0920);mec-4::GFP;pgp-12::GFP* mutant showed results similar to the wild-type strain ($p = 0.751$ from Bonferroni).

Discussion

The goal of this project was to characterize the phenotype and genotype of the *mig-10(mp0920)* mutant allele. The *mig-10(mp0920)* mutant was compared to the wild-type strain and the *mig-10(ct41)* mutant. The characterization was done in order to improve the model of the pathway of the MIG-10 protein (of *C. elegans*) and how it functions.

For the phenotype characterization, the posterior migration of the excretory canal of the *mig-10(mp0920)* mutant showed an intermediate truncation severity when compared to the null mutant, *mig-10(ct41)*, and the wild-type strain. The anterior migration of the excretory canal for all three strains was significantly different from one another. The ALM and AVM migrations of the *mig-10(mp0920)* mutant were similar to the wild-type strain and were different from the *mig-10(ct41)* mutant in which its ALM and AVM neuron migrations were truncated.

The intermediate phenotype of the posterior process of the excretory canal suggests that the *mig-10(mp0920)* allele is possibly not a null mutation, as its truncation is not as severe as the null mutant, *mig-10(ct41)*. This finding suggests that the *mig-10(mp0920)* allele has a mutation in the coding region of the gene. In order to find a possible mutation, portions of the *mig-10(mp0920)* allele were also sequenced.

Several primer pairs were used for PCR products. The PCR products that used primer pairs 2, 3, 4, 5 and 9 were successful. Even with several attempts and gel purification, only 2 primer pairs ended up having good results. The PCR products that were created using primer pairs 5 and 9 had good sequencing that was used for comparing the *mig-10(mp0920)* allele to the wild-type allele. Primer pair 5 sequenced for the 10th, and 11th exons and primer pair 9 corresponded with the 1st exon. Upon comparison to the wild-type, the sections sequenced showed no differences, and therefore were classified as wild-type.

A possible mutation in the *mig-10(mp0920)* allele cannot be ruled out at this point. The *mig-10(ct41)* mutant was used as a control for the PCR process. The control worked for the majority of trials, but the *mig-10(mp0920)* mutant did not. Primer pair 1 failed to produce any PCR products. This may have been caused by using a static temperature setting for the PCR protocol. All products were obtained through the same PCR as detailed in the Methods section of this paper. This might not have been an ideal strategy to use for all of the primer pairs. Experimenting with temperature settings to design individual PCR programs for the different primers may be necessary.

The overall problem for the PCR products was caused by their failure to sequence correctly. The successful PCR products, corresponding to primer pairs 2, 3, and 4 sequenced poorly. This may have been due to human error, such as errors in calculations as well as mistakes while following protocol. While not intentional or apparent, there was still a chance for error to occur. Further sequencing of this allele should be conducted.

Another possible experiment to conduct would be to increase the sample size of the worm strains for the phenotype characterization experiment. The phenotype results gathered in this report differed from those found in Jared Kukla's MQP report. In Kukla's report, the ALM2 migration and the anterior migration of excretory canal in the *mig-10(mp0920)* mutant was similar to the other mutant and different from the wild-type. These differences may be due to different factors between the reports; this report dealt with a strain which had been backcrossed multiple times in order to remove any extraneous mutations, both the ALM migrations were averaged into one AVM result and a larger sample size was used. An even larger sample size of the different worm strains would improve the data results.

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Appendix A: Post Hoc Tests

Table 1: Post Hoc Tests for Posterior EC Migration

Multiple Comparisons								
Dependent Variable: PostProp								
	(I) genotype	(J) genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Tukey HSD	pgp-12::GFP WT	mig-10(ct410);pgp-12::GFP	.516470	.0082909	.000	.496802	.536137	
		mig-10(mp0920);mec4::GFP;pgp-12::GFP	.320572	.0075335	.000	.302701	.338442	
	mig-10(ct410);pgp-12::GFP	pgp-12::GFP WT	-.516470	.0082909	.000	-.536137	-.496802	
		mig-10(mp0920);mec4::GFP;pgp-12::GFP	-.195898	.0078317	.000	-.214477	-.177320	
	mig-10(mp0920);mec4::GFP;pgp-12::GFP	pgp-12::GFP WT	-.320572	.0075335	.000	-.338442	-.302701	
		mig-10(ct410);pgp-12::GFP	.195898	.0078317	.000	.177320	.214477	
	Bonferroni	pgp-12::GFP WT	mig-10(ct410);pgp-12::GFP	.516470	.0082909	.000	.496349	.536591
			mig-10(mp0920);mec4::GFP;pgp-12::GFP	.320572	.0075335	.000	.302289	.338855
mig-10(ct410);pgp-12::GFP		pgp-12::GFP WT	-.516470	.0082909	.000	-.536591	-.496349	
		mig-10(mp0920);mec4::GFP;pgp-12::GFP	-.195898	.0078317	.000	-.214905	-.176891	
mig-10(mp0920);mec4::GFP;pgp-12::GFP		pgp-12::GFP WT	-.320572	.0075335	.000	-.338855	-.302289	
		mig-10(ct410);pgp-12::GFP	.195898	.0078317	.000	.176891	.214905	

Based on observed means.
 The error term is Mean Square(Error) = .001.
 *. The mean difference is significant at the .05 level.

Since $p < 0.001$ for all pairwise comparisons, this allows for the null hypothesis that each strain is similar to one another, to be rejected. Thus, this table shows that each strain of worm is significantly different from one another. Both the mutants are different from one another as well as from the wild-type for the posterior migration of the excretory canal.

Table 2: Post Hoc Tests for Anterior EC Migration

Multiple Comparisons							
Dependent Variable:AntProp							
	(I) genotype	(J) genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	pgp-12::GFP WT	mig-10(ct410);pgp-12::GFP	.459894*	.0166079	.000	.420497	.499291
		mig-10(mp0920);mec4::GFP;pg	.412127*	.0150907	.000	.376329	.447925
	mig-10(ct410);pgp-12::GFP	pgp-12::GFP WT	-.459894*	.0166079	.000	-.499291	-.420497
		mig-10(mp0920);mec4::GFP;pg	-.047767*	.0156882	.008	-.084982	-.010552
	mig-10(mp0920);mec4::GFP;pgp-12::GFP	pgp-12::GFP WT	-.412127*	.0150907	.000	-.447925	-.376329
		mig-10(ct410);pgp-12::GFP	.047767*	.0156882	.008	.010552	.084982
Bonferroni	pgp-12::GFP WT	mig-10(ct410);pgp-12::GFP	.459894*	.0166079	.000	.419588	.500200
		mig-10(mp0920);mec4::GFP;pg	.412127*	.0150907	.000	.375503	.448750
	mig-10(ct410);pgp-12::GFP	pgp-12::GFP WT	-.459894*	.0166079	.000	-.500200	-.419588
		mig-10(mp0920);mec4::GFP;pg	-.047767*	.0156882	.009	-.085841	-.009694
	mig-10(mp0920);mec4::GFP;pgp-12::GFP	pgp-12::GFP WT	-.412127*	.0150907	.000	-.448750	-.375503
		mig-10(ct410);pgp-12::GFP	.047767*	.0156882	.009	.009694	.085841

Based on observed means.

The error term is Mean Square(Error) = .005.

*. The mean difference is significant at the .05 level.

Since $p < 0.001$ for all pairwise comparisons, this allows for the null hypothesis that each strain is similar to one another, to be rejected. Thus, this table shows that each strain of worm is significantly different from one another. Both the mutants are different from one another as well as from the wild-type for the anterior migration of the excretory canal.

Table 3: ALM Average Post Hoc Tests

Multiple Comparisons							
Dependent Variable:ALMAVPhorProp							
	(I) genotype	(J) genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
TukeyHSD	flp::GFP/mec-4::GFP WT	mig-10(ct41);flp-20::GFP	.124968	.0120545	.000	.096426	.153509
		mig-10(mp0920);mec-4::GFP;pgp-12::GFP	-.010805	.0121261	.647	-.039516	.017906
	mig-10(ct41);flp-20::GFP	flp::GFP/mec-4::GFP WT	-.124968	.0120545	.000	-.153509	-.096426
		mig-10(mp0920);mec-4::GFP;pgp-12::GFP	-.135773	.0126892	.000	-.165817	-.105728
	mig-10(mp0920);mec-4::GFP;pgp-12::GFP	flp::GFP/mec-4::GFP WT	.010805	.0121261	.647	-.017906	.039516
		mig-10(ct41);flp-20::GFP	.135773	.0126892	.000	.105728	.165817
Bonferroni	flp::GFP/mec-4::GFP WT	mig-10(ct41);flp-20::GFP	.124968	.0120545	.000	.095776	.154160
		mig-10(mp0920);mec-4::GFP;pgp-12::GFP	-.010805	.0121261	1.000	-.040170	.018561
	mig-10(ct41);flp-20::GFP	flp::GFP/mec-4::GFP WT	-.124968	.0120545	.000	-.154160	-.095776
		mig-10(mp0920);mec-4::GFP;pgp-12::GFP	-.135773	.0126892	.000	-.166502	-.105043
	mig-10(mp0920);mec-4::GFP;pgp-12::GFP	flp::GFP/mec-4::GFP WT	.010805	.0121261	1.000	-.018561	.040170
		mig-10(ct41);flp-20::GFP	.135773	.0126892	.000	.105043	.166502

Based on observed means.
 The error term is Mean Square(Error) = .004.
 *. The mean difference is significant at the .05 level.

Since $p > 0.05$ for the wild-type and the *mig-10(mp0920);mec-4::GFP;pgp-12::GFP* strain, the null hypothesis cannot be rejected. Therefore, this table shows that the wild-type and the *mig-10(mp0920);mec-4::GFP;pgp-12::GFP* are similar for the migration of the ALM neurons. Also, this table shows that the *mig-10(ct41);pgp-12::GFP* and the wild-type strains are significantly different ($p < 0.001$ for all pairwise comparisons) from one another for the migration of the ALM neurons.

Table 4: AVM Post Hoc Tests

Multiple Comparisons							
Dependent Variable:AVMProp							
	(I) genotype	(J) genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	flp::GFP/mec-4::GFP WT	mig-10(ct41);flp-20::GFP	.084115*	.0136658	.000	.051758	.116471
		mig-10(mp0920);mec-4::GFP;pgp-12::GFP	.015870	.0137470	.482	-.016678	.048419
	mig-10(ct41);flp-20::GFP	flp::GFP/mec-4::GFP WT	-.084115*	.0136658	.000	-.116471	-.051758
		mig-10(mp0920);mec-4::GFP;pgp-12::GFP	-.068244*	.0143853	.000	-.102304	-.034184
	mig-10(mp0920);mec-4::GFP;pgp-12::GFP	flp::GFP/mec-4::GFP WT	-.015870	.0137470	.482	-.048419	.016678
		mig-10(ct41);flp-20::GFP	.068244*	.0143853	.000	.034184	.102304
Bonferroni	flp::GFP/mec-4::GFP WT	mig-10(ct41);flp-20::GFP	.084115*	.0136658	.000	.051020	.117209
		mig-10(mp0920);mec-4::GFP;pgp-12::GFP	.015870	.0137470	.751	-.017420	.049161
	mig-10(ct41);flp-20::GFP	flp::GFP/mec-4::GFP WT	-.084115*	.0136658	.000	-.117209	-.051020
		mig-10(mp0920);mec-4::GFP;pgp-12::GFP	-.068244*	.0143853	.000	-.103081	-.033407
	mig-10(mp0920);mec-4::GFP;pgp-12::GFP	flp::GFP/mec-4::GFP WT	-.015870	.0137470	.751	-.049161	.017420
		mig-10(ct41);flp-20::GFP	.068244*	.0143853	.000	.033407	.103081

Based on observed means.
 The error term is Mean Square(Error) = .005.
 *. The mean difference is significant at the .05 level.

Since $p > 0.05$ for the wild-type and the *mig-10(mp0920);mec-4::GFP;pgp-12::GFP* strain, the null hypothesis cannot be rejected. Therefore, this table shows that the wild-type and the *mig-10(mp0920);mec-4::GFP;pgp-12::GFP* are similar for the migration of the AVM neuron. Also, this table shows that the *mig-10(ct41);pgp-12::GFP* and the wild-type strains are significantly different ($p < 0.001$ for all pairwise comparisons) from one another for the migration of the AVM neuron.

Appendix B

Last 7 Exons and Introns of the Unspliced *mig-10c* sequence

6,005bp long, from 20,720bp to 26,725bp

aaatagaaatcccctcctgtccaatatacataatattatcacctgataacgtaaatgatattagtgccttgcgtatgtattatttgcgtcctattgat
aatatgataagaattgacgcaaaaacacataaaataaacgaaaaattgaaaaatcacggctaattttatgggttccttataattctccagtttc
tctccctattttctccgatgaaaaatttgaattgaaacaaatttcgtatcaatttctctccatcaatataatgctttctctgtttattgtataca
cttctctttcacacctcttttaagacttgataagaattttaaactttaaacttgaagcgcacactcacatctaaaatgtatcacgatcg
acggcggaccgggttcaggcaatatgagacgtatatgaaacagaaacgtatgaacaatttaataataaattattatattcaagagtattatcc
tatgataaattgtttgaatttcagaatccgcagattggcagttggatgaacttctgaagaattggaagctctgaaactcaactcaactctag
taatgggtggagatcaactttacttggtaattttatgttttgaatttttgaactccaacaattttatataaattgaaacaccagaaa
acctacgagatggcaattttacaaaattcaactctaaattttcaggagtgctcgggattccagcttcttctagccgggaaaatgtgaagtca
atatccacactgccacctccaccaccggctcttcttaccacaaactccacaacaacctcaactttacatcatcaataatcatttgggttacc
agaatggtattcatcagtttggatttttggtaaaatttgaaaaaatagataagtatataacgtttcctagtttgaatttggcatctattt
ttcaaatttaaagaatgctcataaccatagaaaaatgaattctccaatttgttatactgaaacacacctgttcttctgtcctctgaacagcag
acctagccccatcttgtataaagtgaatacgaacgcctttgtcacaatccccctctcctttgcacaagaaattgatttcttctgtggcg
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cacatttaccacataccttatactttatccgaaaatttcatctgatccaaatgagcagacacctatcggaacatgtacataagctatttgg
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catgtgcttgagatatcaatctagatggttactgataactaattgatctactttgtcaacaacaaaatgcaaccttccaaaatctaattttca
cggctagagaggcataaaaatataaaaactttagtggagatctaattgaaaatttaatttgaacagagtgacgaagatcacgagaaggt

tgttgagaatatccaaatgtgggttcaagattcaccgaacaagctttatttcatgcgtgcccggacaagtacgccttcatctctcgtcctgaac
tctacctcactccaaaaacttctgatcacatggaaatccgtctggagatcaatggacaatc gatgtgaaacaaaagtttgcagtgaaat
ttccaccgtgaaccagtagtaccaccagagatggaaggatTTTTATAtttGAAATCAGATGGAAGAAAATCATGGAAAAACATTATTCTGTGTT
AAGACCAAGTGGTTGTATTATGCACCAAGAGTAAGAAACCAACAACAAAAGATTTGACATGCTGATGAATTFACACTCGAATCAAGTTATA
CTGGAATCGGATGGGAGAAGAATAACAATCACC GACTCCTGGTGTATTCAATAAAATGACTGCATTGCAAAATGAAGAGAAGTCAATTTAT
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AAAGACTTGCCAAATAAGAAGAGAAACTCTGGACCAGCATCAAGTATGCTGCTGCTTCATCTCAACTGCTATTTCTGAAGTCCACATAGTT
TGAGTCATCATCAGCGTACACCTCTGTGCTTCATCAATTCAATTGAGTTCTCATATGATGAATAATCCAACGCATCCTTTATCAGTGAATGTCAGG
AATCAGTCACCAGCTCTTTTCAGGTAAGAATATTA ACTACC GATTTCTTATTAATATGGCCTCCTATTAGACTGCACTCCCTATTAGTGTG
CACTCCCTAATAGTCTGCGTCTACTTTTTGTACAACTACCTACTAATTTGAGATTTTACAACATTTTTGCCTACTTGAACAAATTTTTGG
TAAATTTCTATGGAATTGAAGTTATTGTACCTTTTTGTTGCAATGCAATCAAAATTTCTGAATTTTACATAAGATACGAGTTTTTCAGAAAAAATA
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ATCTCATCATCTCGAACGTCAGCGAAATTAGAGATTCAATACGATGAGCAACCGACTGGAACAATCAAAGAGCACCTCTAGATGTGCTCC
GTCGAGTATCAAGAGCTCCACCTGTCCCAACGATTCGTAAGTGTTTTTATTAGAATTTCCCCATAAAATATTATTTCAGCCCAAGAAGAAAT
CGGATTCGGACGAAGAGTTCCAGCGCCACCTCCAGTTGCCTCTGTGATGAGAATGCCGCCACCAGTGACTCCACCAAAGCCTGTACTCCA
TTAACTTCAAGAAAGCACCTCCACC GECTCCGAAGAGATCTGACACAACAAAATGCAATCAGCTTCACCTATGGCCCCGGCGAAAAATGA
TTTGAAGCTGCGTTGGCGAGAAGCGGTGAGAAAATGGCAACCATGGAGTGTAGACAACATTAGAATACTGGATATTTACTCTTTCAA
AATTCTGTATTACC GGTTGTGCTGGAACCTGAACCTGAAAATCTTTACATGAACAATTTTTGTA ACTCTTTACTACAACTCCACCACCAAC
TCACATCGCTTGACACACTTTACAGAGAAATGCCTTTATTACTGATAATGTCATGTATTCTGAAAATTTTTGTTGAAAATTAATAAA
AAAAATTTGAGCATTGCATTTT

Sequence for *mig-10(mp0920)*, mig-10F5 primer, 948bp long, no alterations

nnnnnnnnntgnnnnntatnnnagaagantacggatTTTTGGAAATCCTCAAATTCATTTGATATAATTCAGTAACTCTTGCCAAC
aatctcatccatctcgaacgtcagcgaattagagattcaatacgatgagcaaccgactggaacaatcaaaagacaccttagatgtgctc
cgtcgagtatcaagagcttccacctgccccaacgattcgtaagtgtttttatttagaattccccataaaatattttcagcccaagaaga
atcggattcggacgaagagttccagcggccacctccagttgcctctgtgatgagaatgccgccaccagtgactccaccaagccttgactc
cattaacttctaagaaagcacctccaccgctccgaagagatctgacacaacaaaattgcaatcagcttcacctatggccccggcgaaaaat
gatttggaagctgcgttggcgagaaggcgtgagaaaatggcaaccatggagtgtagacaacatttagaatactggatattcatactcttca
aaaattctgttattaccgggtgtgctggaacctgaaaattctttacatgaacaatttttgaactctcttactacaaactccaccacca
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nnattcatcactaaggngatctcttcgccgtaactatnnaactgaaatcngtctannacgnnacaacacnctngccctactgngtgat
nacangnatancagnggtgagtnnngtannnnnngnagcacnntnnangannntntngnatannannantacctaanaagaannat
anntggancacgcntnn

Sequence for *mig-10(mp0920)*, newR5 primer, 687bp long, no alterations

NnnnnnnntnnnnnnnagagttacaaaaattgttcatgtaaagaatttcaggttcaggtccagcacaccggtaataacaagaatTTT
gaaagagtatgaaatatccagtattctaaatgttgtctaacactccatggtgccattttctcacgcttctcgccaacgcagcttccaaatcattt
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aggctttgggtgagtcactgggtggcgccattctcatcacagaggcaactggaggtggcgtggaaactctcgtccgaatccgattctctg
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acatctagaggtgctcttttgattgtccagtcgggtgctcctgattgaatctctaatcctgctgacgttcgagatggatgagattgtggcaag
agttactgaaattatatcaaattgaaatttgaggattccaaaaaataaccgtatttctctattaatatggctgcaataactattttcgatggtctn
nnnnnnnnnaannnnnaaaan

N-terminus of *mig-10c* (source: wormbase.org)

TTTTTATTCTTTCTGTGTGTGCGGGGGAGGGGGTATCCCTGACTTGATCATCGCTTTGCATTTGTTAGCAACGATCCCACAGAGGGAA
GGTCAATCACTTTTATGCAGATGTTGTTGTTTTCGAGGAGACGGAAAGAAAATTTGGTGTAGATTATTAGTGGTCAATTGGGTTTCCTTCTGTCT
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TTAACTGTAAGTAGGTGCAATAATTTGCTATATTTGATAGTTGAACAGTTTTCTGTTGAGCTTAACTGAAATATAAGCGTCAATGTA
GAGTAGTGTGGTCAACATAGGAAAATACGGTATTAGAGAGCGGAAATCTCAATTTGTTTTGTATTGTTTTATTGGATTAGCTTGACAAGTCG
TGTGTTGAATTTTCTAGATTGCAAGGTTTATAATTTTAACTGAGAATAAAAAGATTGTTCTACATAACAATACATTTTTAAGACTGAAAAAGT
GGATTATTGTGACAATAAAGTAGAAATCTATGAAAAAATCAATAAAGTCTGTTCTGCTGATTGAAAAGTATTCAGAATTTAGTAT
ACAAATATTATTCGCAAGTTTGGCAATACTTTGGATTTCTAAAAGTCGAAAACGAGCAATTTTGTGCTAGATTTTGTATTGACGAATT
TTTTAGTTTTTTCTTAAAAAGTTTTGACCCTTAAAGTTTTCTTCCCTTCAATTTTTCTTCTTATACGACTTCTCAAGTTCAACTTAA
AACAAAGTACATGTACATTTCCGGTAAACTTTGTGCTCAGAAGATCCATTTCTTTTGTACATTTATCAAGATTGAATCCAAAATTCAGCCAA
TATGGACAGTTCGAAGAGGAATCGCATCTGGAAGTTGACAGTGACGAAGAAGATCAACTTTTTGGTGAAAAGTGTGAGTCTTATTGTGGTA
ACCAAAGAAATGCAGTGGTCCGTAACACTGACTECCAAATGTTCTCGTAATFACCTATGCACACTTTCAAGTGTGGCGTTGATCTTAG
CCAATTTGAAACGTTTAGATGTTAAATGGGAAAGTTTTTATTATAGAAAAAGTTTGGAAAAAATCGAGTCACTGAATAGTTG
AAGAACGAAAAATAAACTTCCAAAAATCAAAAACATTTAGTGTTCGAAAATATAGTGTTTTTTGTGGTATGTTTGACAAAAGCTAAAC

