# GENETIC ANALYSIS OF CLASSICALY AND EMBRYOLOGICALLY MUTAGENIZED *C. ELEGANS*

By
Kevin Scott Chatley
&
Christian Hansen Berg

Submitted in partial fulfillment of the
requirements for Departmental Honors in
the Department of Biology
Texas Christian University
Fort Worth, Texas

May 3, 2013

GENETIC ANALYSIS OF CLASSICALY AND

EMBRYOLOGICALLY MUTAGENIZED

*C. ELEGANS*

Project Approved:

Phil Hartman, Ph.D.
Department of Biology
(Supervising Professor)

Dean Williams, Ph.D.
Department of Biology

Ron Pitcock, Ph.D.
John V. Roach Honors College

TABLE OF CONTENTS

ACKNOWLEDGMENTS

This project would not have been possible without the help of many individuals. We would both like to thank Dr. Phil Hartman, who mentored us through this project and helped us to obtain a finished product. Without his continuous help and advice, this project would not have been possible. We would also like to thank those who worked previously for the goal of this project, David Neal, Joe Rodriguez, James Barry, Whitney Finstand, and Numan Khan, who carried out the beginnings of this project by isolating the mutants, performing genetic crosses, and beginning to extract and analyze the results. We are likewise appreciative for Karim Zeglam, a fellow TCU student, who grew and maintained our various worm stocks over the past year. Lastly, we are grateful for Dr. Dean Williams and Dr. Ron Pitcock for taking the time and effort to read through and edit this project, as well as to provide us guidance throughout our research. Thank you again for all the help to make this project successful.

# INTRODUCTION

In biology, model organisms are used to study specific biological workings that can provide additional understanding of other organisms (Fields et al, 2005). For example, many experiments are performed on mice, a model organism, in order to better understand the workings of the different aspects of the human body without any potential harm coming to humans. Because all living things are descended from a single common ancestor, described by the theory of evolution, we are able to study model organisms and apply the findings to other organisms (Fox, 1986).

A very common and useful model organism is *Caenorhabditis elegans*. It was first used as a model organism by Sydney Brenner prior to 1974. He chose to use *C. elegans* as a model organism because of its simplicity. They have short generations and lifespans, about three and a half days and two weeks respectively. *C. elegans* populations are easy to grow up as well as useful for genetic analysis. They can also be frozen for long periods of time and are still viable after they thaw, which allows for them to be stored for long periods of time (Brenner, 1974). Compared with *Drosophila melanogaster*, a common model organism being used before *C. elegans* as well as today, *C. elegans* has very few cells. *Drosophila melanogaster* has $10^5$ neurons, while *C. elegans* only has 800 total cells, 400 of them being neurons, making the worm one of the simplest organisms known to have a nervous system (Brenner, 1974). This simplicity allows for *C. elegans* to be studied efficiently.

*C. elegans* became the first multicellular organism to have its entire genome sequenced. The sequences have shown to be highly conserved, allowing studies on its genome to be greatly effective (Consortium, 1998). The entire sequenced genome is

about 100 million base pairs long in six chromosomes, and about 20,000 protein-coding genes (Reboul, 2001). The genome of *C. elegans* is always being updated, as DNA sequencing is not a perfect process. However, most changes are minor.

Not only as the *C. elegans* genome been extensively studied in the lab, other areas of the *C. elegans* biology have been studied as well. The development and other processes of *C. elegans* have been learned. Every single cell's developmental fate has been determined (Sulston, 1977). Many important proteins and genes that are crucial in the early development of the worm have been identified along with their knock-out phenotypes (Stoekius et al, 2009). This allows for effective study of developmental biology using *C. elegans.*

Because *C. elegans* is a diploid organism, deleterious mutations may be induced in the worm without killing it. Chemical mutagens are very effective on the worm, in particular ethyl methane sulfonate, or EMS. Because of the efficiency of inducing mutations as well as preserving the strains of *C. elegans* allows for multiple mutations in multiple genes. Multiple mutations allow to provide knock-out phenotypes. While most of these induced mutations are recessive and loss-of-function, there are a few gain-of-function mutations that have been documented (Hodgkin, 2005).

*C. elegans* have two sexes, hermaphrodites and males. The vast majority of all *C. elegans* populations are hermaphroditic. Hermaphrodites are self-reproducing, and since they make up such a large portion of the population in the lab, the lab populations tend to be driven towards homozygosity (Neal, 2012). Therefore, genetic analysis on the hermaphrodites alone would not yield useful results. However, because there is a minimal number of males in the lab population that mate with the hermaphrodites,

genetic markers are transferred throughout the generations, making genetic analysis possible (Brenner 1974).

Because of the specific reproductive traits of the worm, researchers are able to easily study *C. elegans* mutants. In classical mutagenesis, these mutations are induced to the germline of an adult worm. The worm will then pass on the trait to its offspring, known as the F1 generation. This F1 generation will possess the trait as heterozygotes. After a round of self-fertilization the first homozygous mutants begin to appear in the F2 generation (Neal, 2012). This process and its expected results are well understood by researchers.

There is another mutagenesis process that has been explored at TCU over the last 3 years. Instead of exposing *C. elegans* to a mutagen as an adult, the researchers have been exposing mutagens to early *C. elegans* embryos in the one to four cell stage. These worms were specifically exposed to EMS. One of the differences with using this method over the classical method is that the mutant phenotypes can first appear in the F1 generation, signifying the mutation was induced early on in embryogenesis (Finstad, 2011).

EMS is used in C. elegans genetic experiments because it is efficient in creating different alternations in gene sequences (Anderson, 1995). EMS has shown to be particularly effective in inducing G/C to A/T transitions (Riddle and Blumenthal, 1997). If other mutations are desired, other mutagens should be used. For example, UV light seems to be the most efficient in generating deletion mutations (Anderson, 1995). Classical EMS mutagenesis protocols have shown the mutation rate to range from $10^{-4}$ to $5 \times 10^{-4}$. Researchers at TCU have calculated a mutation frequency of $3.3 \times 10^{-3}$ for

worms exposed to EMS as embryos (Neal, 2012). The spontaneous *e665* mutation rate, a specific point mutation in the unc-58 gene that is studied in this project, is approximately $10^{-6}$ (Barry, 2010).

In *C. elegans*, a large number of genes encoding for potassium channels belong to the TWK family (Wei et al, 1996). Specifically, these channels have four transmembrane domains as well as two highly conserved P regions. This family of potassium channels has been identified in mammals, *Drosophila*, *Arabidobsis,* and *C. elegans* (Salkoff et al, 1999). A large number of TWK channels were identified using sequence analysis of the *C. elegans* genome (Salkoff et al, 1999). TWK channels are shown to be activated by general anesthetics (Kindler et al, 1999). When the temperature of the external environment changes, the TWK channels have also shown to change their activity (Kunkel et al, 2000).

The researchers at TCU have been studying a point mutation in a gene that codes for a subunit of a TWK potassium channel. The specific *C. elegans* strain used was CB665, which particularly has a point mutation *e665* in the *unc-58* gene (Finstad, 2011). This TWK potassium channel protein is found in motor neurons and interneurons. The e665 mutation causes the worm to have a very distinct phenotype, which has been described as having a "shaker" morphology and uncoordinated side to side movement (Park and Horvitz, 1986). However, secondary mutations in the *unc-58* gene can offset the orginal e665 mutation and are clearly identifiable.

Embryogenesis in *C. elegans* is invariant among individuals. The cells divide with conventional timing making it easier to study. Embryogenesis consists of very rapid cell divisions which are not delayed due to things such as DNA damage. DNA damage

normally inhibit cell division in eukaryotes because of the presence of cell cycle checkpoints (O'Farrell et al, 2004).

Cell cycle checkpoints work to halt the continuation of cell division when harmful factors, such as DNA damage, appear during the process. In *C. elegans* these checkpoints are activated in germ-line cells but are not functional in the embryo (Holway et al, 2006). In the adult worm mismatch repair genes code for a group of sensor proteins. These proteins are important in DNA repair in the cell cycle, as they prevent mutations from building up in many organisms (Lans, 2011). The proteins routinely examine DNA in the cell and if they sense the DNA to be damaged, the proteins can suspend the cell cycle until the damage is fixed or in extreme circumstances, trigger apoptosis, or programmed cell death (Harris et al., 2006).

*C. elegans* embryos have a cell cycle that is rapid and goes between S and M phases, with the G1 and G2 phases lacking (Edgar and McGhee, 1988). The G1 and G2 stages are reintroduced to the developing cells later in development, depending on the cell lineage (Sulston et al., 1983). Because the cell cycle is so rapid early in development, certain cell cycle control mechanisms are lacking. These mechanisms include proteins Cyd-1, Cyclin D, Cdk-4, and Cdk 4/6, all of which have demonstrated to be related to other regulators in mammals including humans during the G1 stage of the cell cycle (Koreth and van den Huevel, 2005).

Information obtained by studying *C. elegans* has proven useful to developing the theory of evolution as much of the information can be applied to other organisms (Cutter 2010). Evolution occurs slowly over millennia and mutation provides the genetic

material for natural selection to act upon. Without mutation evolution would not be possible and there would not be the diversity of life that the Earth sees today.

This project is a continuation of previous research done at TCU. 21 strains of *C. elegans* were mutagenized embryonically and then had their DNA extracted. They were found to have a mutation frequency of $3.3 \times 10^{-3}$. All but two of the strains were extracted successfully. Once sequenced, these strains were found to have many more mutations than expected. Along this small section of 1000 base pairs along the X chromosome, one to four mutations were found in each strain (Neal, 2012). That is higher than the 11 mutations expected in classical mutagenesis experiments (Sarin et al., 2010). In order to fully understand if these mutations found are indeed more than classical mutagenesis, this project was set up. We hypothesize that there was some error in those results, and our goal is to figure out how much if any.

Using EMS mutagenesis, this project will attempt to figure out if there is any difference between classical mutagenesis and embryonically mutagenized revertants and resolve the importance of any variance in the different *C. elegans* strains. Because this is a continuation of a previous project at TCU, we will attempt to mimic the previous project as much as possible to see if our results differ. While we sequenced the same strains as the previous project, we also went ahead and sequenced strains that were classically mutagenized in order to have another source of data to compare to. The gene that was sequenced in each strain was the unc-58 gene on chromosome X. If there is a difference in the genomes of the adult and embryonic strains, the hope is that the project will provide insight to mutagenesis in other organisms and provide valuable medical information.

# MATERIALS AND PROCEDURES

As from David Neal's thesis (2012):

"**Strain Maintenance and Freezing**

Each of the 21 embryonically mutagenized mutant strains was maintained on an agar plate measuring 60 mm by 15 mm. The plates contained a thin bacterial lawn of *Escherichia coli OP50* containing the nutrients required for worm survival. The plates were monitored twice per week, and stocks were transferred approximately twice per month. Careful monitoring of the plates was needed to limit the effects of fungal contamination.

**DNA Extraction**

In order to obtain pure DNA for sequencing, DNA was extracted from each of the individual mutant strains. To collect a sufficiently large number of worms for extraction the stock plates were chunked with a portion being added to two separate 100 mm by 15 mm agarose plates seeded with bacteria. Agarose was used to negate the enzyme-inhibiting effects of contaminants in agar. The worms were allowed to grow for approximately two days until starvation when the populations achieved a maximum value. When the plate reached starvation the worms were washed off into separate microcentrifuge tubes with 0.5 ml of ice-cold TEEN solution (200 mM Tris-Cl pH 7.5, 100 mM EDTA, 400 mM NaCl). The worms were then spun down, the supernatant removed, and the plates re-washed to ensure that the maximum number of worms was retrieved. This procedure was repeated for both agarose plates. When the extraction was not immediate, the worm pack was frozen away at -75$^{o}$ Celsius. When ready to perform the extraction, 0.5 ml of lysis buffer (100 mM NaCl, 100 mM Tris-Cl pH 8.5, 50 mM

EDTA, 1% SDS and 1% beta-mercaptoethanol) heated to 60$^{o}$ Celsius was added to each of the microcentrifuge tubes containing the worm packs. Additionally 2.5 µl proteinase K (25 mg/ml) was added to each tube. The microcentrifuge tubes were placed in a 60$^{o}$ Celsius water bath for one hour and mixed by inversion every 30 seconds for the first 10 minutes. To extract the DNA, 0.5 ml of water-saturated phenol was added to each tube and shaken vigorously. The tubes were centrifuged for one minute and the upper aqueous phase was removed and placed into another microcentrifuge tube containing 0.5 ml of water-saturated phenol/chloroform/isoamyl alcohol (25:24:1). The tubes were once again shaken vigorously, centrifuged for one minute, and the upper aqueous phases were added to separate tubes containing water-saturated phenol/chloroform/isoamyl alcohol as mentioned above. The extraction was repeated once again making for a total of three times. The aqueous layer of the final extraction was then added to a separate microcentrifuge tube and an equal volume of isopropanol was added to precipitate the DNA. This tube was spun down for 10 minutes in the microcentrifuge at 13,000 RPM. The isopropanol was then decanted and the pellet was washed twice with 70% ethanol. The pellet was left out and allowed to dry overnight. The next day 30 µl TE (10 mM Tris, 0.1 mM EDTA pH 8.0) was added to each tube along with 1µl RNase A (1 ug/ml), resuspended, and placed in the 50$^{o}$ Celsius incubator for one hour to degrade any residual RNA. This DNA was then analyzed using the NanoDrop program to analyze both the purity and concentration. The concentrations were then adjusted to approximately 50 ng/µL through the addition of TE to ensure maximum yields for PCR.

**Polymerase Chain Reaction (PCR)**

Following DNA extraction polymerase chain reaction (PCR) was utilized to amplify the *unc-58* gene to a concentration that could be read by the sequencing analyzer. The reaction contents consisted of 14 µl $MqH_2O$, 2 µl 10X Buffer, 1 µl homemade Taq Polymerase, 1 µl dNTPs, and 1 µl primer pair combined in a master mix with 1µl of extracted DNA added separately to each tube. The primers were designed by Whitney Finstad and Dr. Phil Hartman to divide the *unc*-58 gene into 6 segments (Finstad, 2011). The tubes were mixed and placed in a thermocycler and allowed to undergo the following steps as part of the PCR reaction:

**Denaturation**
One 1-minute cycle at 94 ˚C
*Note: tubes were not inserted until block was at 94 ˚C

**PCR amplification**
Forty cycles:
Denaturation: 94 ˚C for 30 seconds
Annealing: 55 ˚C for 30 seconds
Extension: 72 ˚C for 1 minute
**Final extension**
One 5-minute cycle at 72 ˚C

**Holding temperature:** 4 ˚C

Following the PCR 5 µl of each sample was combined with 1 µl of a tracking dye solution containing gel red and loaded into a 1% agarose gel in TAE buffer to check for the presence of pure DNA. The gel was run at 150 V for approximately 15 min to allow for the DNA bands to make significant migration. A single band per lane signified a pure sequence of DNA and thus a successful PCR. These successful runs were then allowed to move on to the next step of the procedure, sequencing.

**Sequencing**

Following the successful PCR reaction, sequencing reactions were run on the samples to obtain the sequences of the DNA fragments of the *unc-58* gene. Before the sequencing reaction could take place, the unincorporated primers and dNTPs had to be removed. This was accomplished through the creation of a master mix containing 1.19 µl $MqH_2O$, 0.01µl Exo I (which recognizes single stranded DNA/unincorporated primers), 0.1 µl Antarctic Phosphatase (which removes phosphates from the unincorporated dNTPs to render them useless), and 0.7 µl Antarctic Phosphatase Buffer 10X per tube. This master mix was kept on ice due to the temperature sensitivity of the enzymes. 2 µl of the master mix was then added to 5 µl of each individual PCR sample making a total reaction volume of 7 µl. The samples were then placed in a thermocycler and run the ExoSap® program (37 ˚C for 15 minutes and 80 ˚C for 15 minutes).

Following the cleaning of the PCR product, the actual sequencing reaction was set up. A master mix was made that consisted of 5.5 µl $MqH_2O$, 1.0 µl primer (left or right), 2.0 µl Buffer 5X, and 0.5 µl Big Dye per tube. For each sample two reactions were run such that the sequence of both stands could be determined. The master mix was partitioned with 9 µl per PCR tube and 1 µl of ExoSap/PCR product from above was added to each tube making a total reaction volume of 10 µl. The tubes were micropipetted up and down approximately 10 times to mix and then spun down. These tubes were placed in the microcentrifuge and run on the BigDye® program which is outlined below:

**Denaturation:**
One 1-minute cycle at 95 ˚C

**Cycle Sequencing:**
Twenty-five cycles:
Denaturation: 95 ˚C for 30 seconds
Annealing: 50 ˚C for 20 seconds
Extension: 60 ˚C for 4 minutes

**Holding temperature:** 4 ˚C

After completion of the sequencing reaction the tubes were cleaned of unincorporated fluorescently labeled dNTPs to avoid the formation of messy dye blobs on the ABI 3130 Genetic Analyzer. Twenty µl of BET (64 ml 100% ethanol, 7 ml $dH_2O$, 6.4 ml Tetra Ethylene Glycol, and 1 ml Compel beads (previously washed three times with 10 mM Tris pH 8.5)) was added to each tube and mixed by pipetting up and down approximately 10 times. The tubes were then placed on a magnetic plate in order to isolate the beads which contained the pure DNA. The liquid which contained the unicorporated dNTPs was removed. The beads were washed twice with 100 µl 70% ethanol without removing the tubes from the magnetic plate. After removal of all ethanol the DNA sequences were eluted from the beads through the addition of 40 µl of 0.1 mM EDTA and mixed up and down approximately 10 times after being removed from the plate. The beads were then magnetized for approximately 30 seconds and the liquid containing the DNA was removed and placed in a clean semi-skirted plate. The plate was spun to remove air bubbles and was placed in the ABI 3130 Genetic Analyzer.

**Sequence Analysis**

To read the sequence, the files from the ABI 3130 Genetic Analyzer were transferred and analyzed using to Wormbase program, (http://www.wormbase.org). Text files of the sequences were input into the BLAST program and compared to *C. elegans* strains in the database. The location of the *unc-58* gene, the X chromosome, was used as

the standard. Any sequence differences were then recorded by both mutation type and the number of nucleotides on the X chromosome."

## RESULTS

### DNA extractions

For the original 17 embryonic mutants, DNA previously extracted by former researchers were used. For the classically mutated strands, 5 of the strands isolated by Numan Khan were put through the protocol for extracting DNA. After the protocol was completed, the strands were spun down into a pellet, where the DNA could be visually observed. It appeared as an off white pellet below the supernatant, and was very viscous and stringy. All of the strands were successfully extracted and used in future parts of the experiment.

### Nano Drop

For each of the 5 strands extracted, a Nano Drop test was performed to confirm the presence of DNA, the concentration of the DNA in the sample, and to ensure minimal to no presence of proteins. For all samples, the purity was verified by the 260/280 nm ratio, and the samples were considered pure DNA with a ratio value of 1.8 to 2.0. All samples extracted fell within this range. In addition, Nano Drop analyzed the concentration. For all samples, their initial concentrations were upwards of 1000ng/ml, increasing to about 5500 ng/ml, signifying very concentrated samples with plenty of DNA present for analysis. These samples were then diluted down to 50-100 ng/ml, testing them again with the Nano Drop for concentration, so that they could be more effectively utilized for the PCR and then the sequencing reactions.

**Polymerase Chain Reactions**

After the DNA was extracted and diluted to the correct concentration through use of the NanoDrop, the DNA was used in the PCR protocol in order to amplitude the amount of DNA. In order to determine if the PCR was successful, gel electrophoresis was run on the PCR product, and a successful result is illustrated by figure 1. Many PCR reactions did not work, especially with primers 1, 2, and 6. For these gels, there were typically smears. Even with careful mixing of the master mixes, smears were still common for these primers. Creating new primers and using new taq polymererase was attemted to fix this issue, but many smears were still roduced for unknown reason. However, even with many smears still produced, many succesful PCR's were also produced, giving many results ready to sequence. The results that showed good PCR products were taken to the sequencing protocol in order to determine if any mutations existed in that region of the unc-58 gene.

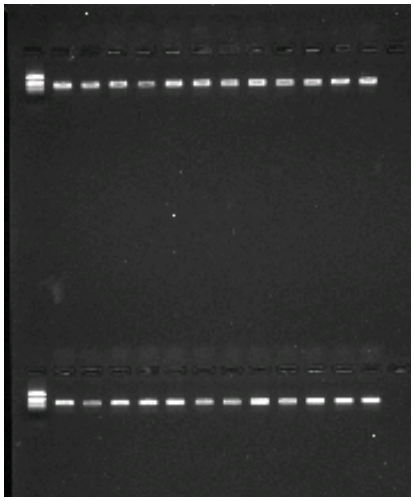

**Figure 1:** Example of a successful PCR product verified by gel electrophoresis.

**Sequencing**

The PCR products that were successful as illustrated by the gel electrophoresis were used in the sequencing protocol. For the sequencing analysis, the ABI 3130 genetic analyzer was used. After sequencing, using the sequinter program, the sequences were paired together to form contigs from the sense and anti-sense sequencing primers. Once this was completed, they were converted to text files and analyzed for mutations using

the Wormbase BLAST analysis, which compared the sequences to the wild type *C. elegans*. Table 1 below lists the results of the genetic analysis.

| DNA sequence | Primer 3 | Primer 4 | Primer 5 | Primer 6 |
|---|---|---|---|---|
| **JB3** | | | | 100% match 167 Bp: 10105224-10105058 |
| **JB5** | 100% match 459 Bp: 10107206-10106748 | 99% match 367 bp: 10106303-10105937 Mutation: 10106178 G to A | 99% match 346 bp: 10105619-10105274 Mutation: 10105601 C to T | |
| **JB6** | | 100% match 313 bp: 10105991-10106303 | 99% match 349 bp: 10105618-10105270 Mutation: 10105601 C to T | |
| **JB7** | 99% match 357 bp: 10107192-10106839 Mutations: 10107106 G to A 10106877 C to G | 100% match 272 bp: 10106082-10106353 | 99% match 367 bp: 10105261-10105627 Mutation: 10105639 G to A | |
| **JB8** | 99% match 459 bp: 10106740-10107198 Mutation: 10107106 C to T | 100% Match 368 bp: 10105936-10106303 | | |
| **JB9** | | 100% match 365 bp: 10105939-10106303 | 99% match 351 bp: 10105269-10105619 Mutation: 10105601 A to G | |
| **JB10** | 100% match 456 bp: 10106742-10107197 | 100% match 346 bp: 10105958-10106303 | 99% match 359 bp: 10105261-10105619 Mutation: 10105601 G to A | |

| | | | | |
|---|---|---|---|---|
| **JB11** | 99% match 456 bp: 10106745-10107200 Mutations: 10107101 C to T 10107132 C deletion | 100% match 304 bp: 10105930-10106233 | | |
| **JB13** | 100% match 459 Bp: 10107265-10106807 | | | |
| **JB14** | | 100% match 316 bp: 10106054-10106369 | | |
| **JB15** | | | 99% match 346 Bp: 10105678-10105333 Mutation: 10105696 C to T substitution | |
| **JB16** | | | 99% match 210 Bp: 10105592-10105383 Mutation: 10106010 A deletion | |
| **JB17** | | 100% match 313 Bp: 10106050-10106362 | | |
| **JB19** | | | 99% match 349 Bp: 10105677-10105329 Mutation: 10105660 C to T substitution | |

| | | | | |
|---|---|---|---|---|
| **JB20** | 99% match 357 Bp: 10106895-10107251 mutations: 10106936 G to C substitution 10107166 C to T substitution | | | |
| **NK1** | | | 99% match 358 Bp: 10105685-10105328 mutation: 10105660 C to T substitution | |
| **NK2** | | | 99% match 360 Bp: 10105691-10105329 mutation: 10105660 C to T substitution | 100% match 157 Bp: 10105137-10105293 |
| **NK3** | | 100% match 262 Bp: 10106107-10106368 | | |
| **NK4** | | 100% match 352 Bp: 10106369-10106017 | | |
| **NK5** | | 99% match 378 Bp: 10106369-10105992 Mutation: 10106178 C to T substitution | | 100% match 221 Bp: 10105278-10105058 |

**Table 1:** Sequencing results of successful PCR products. Numbers correspond to the nucleotide number on the X chromosome and where the mutation, if any, occurs.

## DISCUSSION

The goal of this project was to sequence as many of the mutant strains of *C. elegans* as possible and compare the mutation rate of the classically and embryologically mutated strands, as well as to check the sequencing done by David Neal. The EMS

mutagen was applied by James Barry and Numan Khan, giving rise to the JB strands and the NK strands. The 21 JB mutants were obtained from approximately 6,400 $P_0$ embryos, giving a mutation frequency of $3.3 \times 10^{-3}$. This rate is statistically significantly different than the reported $10^{-4}$reversion frequencies for classical EMS mutagenesis protocols, as well as the spontaneous *e655* $10^{-6}$ reversion rate (Barry, 2010).

After successful extraction of the JB strands from David Neal, as well as the successful extraction of the NK strands, they were tested for purity by using the NanoDrop. The NanoDrop illustrated that the extractions performed were successful in isolating pure DNA at very high concentrations. Because they were at such high concentrations, it was necessary to dilute the strains down to about 50 ng/ml for the PCR reactions. After successful dilutions, the strains were then run through the PCR protocol with 6 different primers of which covered all of the exons of the *unc-58* gene. This was where the most difficulty was encountered. Initially, a tested by gel electrophoresis, many of the PCRs worked fine, and there were many successful sequences gathered. However, after winter break, the PCRs seemed to only intermittently work, which slowed the progress down to almost a stop. Many solutions to resolve this were attempted, but none seemed to work. New *taq* polymerase was tried, the primers were recreated in case they had degraded, the PCR reactions were immediately sequenced, and the samples were more thoroughly mixed to ensure proper reactions. However, after attempting these, the PCRs still only intermittently worked. This could have been due to a few issues. The biggest possibility could have been that DNA itself could have degraded over time since some of it had been extracted over a year ago from when it was being used for PCRs. However, this was still not believed to be very likely, as the samples were always stored

at -80$^0$C. Another possibility for the failure in PCRs could have been a problem in the PCR protocol or in the buffers utilized. A contaminant in the buffer or a missed step could have caused many unsuccessful reactions. Ultimately, however, the reason for the sudden PCR problems is unknown, as following the same procedure and using the same materials for the reactions gave great results initially. In addition, primers 1 and 2 seemed to not work at all, giving no results, and primer 6 only worked in a few cases. The majority of the results came from primers 3, 4, and 5. Again, this could be contributed to the possible problems mentioned above.

As expected, most of the mutations observed were C to T or G to A substitutions. However, what was not expected was the high mutation rate. One of the main purposes of re-sequencing what David Neal sequenced was to check for correctness. Although not as many mutations were observed as in David Neal's work, there was still a high mutation rate. In David Neal's work, roughly 55% of the sequences he sequenced had mutations in them. David's results can be seen in table 2.

| Mutant Strain | Primer Set 3 | Primer Set 4 | Primer Set 5 |
|---|---|---|---|
| JB 5 U | 61bp: 10107015-10106955<br>100% | 324bp: 10106258-10105936<br>99%<br>C Insertion between:<br>10106236&10106235 | 313bp: 10105585-10105273<br>100% |
| JB 5 L | 411bp : 10106796-10107206<br>100% | 317bp: 10105982-10106299<br>99%<br>10106179: C→T | 304bp: 10105322-10105625<br>99%<br>10105601: G→A |
| JB 6 U | 363bp: 10107103-10106741<br>99%<br>10106769: G→A subst.<br>10106767: T→A subst. | 318bp: 10106253-10105936<br>100% | 313bp: 10105585-10105273<br>100% |
| JB6 L | 386bp : 10106818-10107203<br>100% | 322bp: 10105988-10106310<br>99% | 319bp: 10105306-10105624<br>99% |

| | | | |
|---|---|---|---|
| | | 10106300: T→G<br>10106301: G→T | 10105601: G→A |
| JB 7 U | 385bp: 10107127-10106743<br>99%<br>10107106: G→A subst. | 321bp: 10106256-10105936<br>100% | 302bp: 10105574-10105275<br>100% |
| JB 7 L | 392bp : 10106805-10107196<br>99%<br>10107106: C→T subst. | 312bp: 10105988-10106299<br>99%<br>T Insertion between:<br>10106002 & 10106003 | 290bp: 10105331-10105620<br>99%<br>10105601: G→A |
| JB 8 U | 405bp: 10107146-10106742<br>99%<br>10107106: G→A subst. | 324bp: 10106258-10105936<br>99%<br>A Insertion between:<br>10106232&10106233<br>10106178: G→A subst. | 314bp: 10105574-10105261<br>99%<br>10105497: C deletion<br>10105500: C→A subst. |
| JB 8 L | 385bp : 10106816-10107200<br>99%<br>10107105: C→T subst. | 311bp: 10105988-10106299<br>100% | 326bp: 10105307-10105632<br>99%<br>10105601: G→A |
| JB 9 U | 404bp: 10107143-10106740<br>99%<br>10106769: G→A subst. | 318bp: 10106253-10105936<br>100% | 313bp: 10105585-10105273<br>100% |
| JB 9 L | 402bp : 10106805-10107206<br>99%<br>10107196: G→A | 322bp: 10105988-10106310<br>99%<br>10106300: G→T | 297bp: 10105330-10105626<br>99%<br>10105601: G→A |
| JB 10 U | 402bp: 10107143-10106742<br>99%<br>1017103: A→T subst.<br>10106768: G→A subst. | 326bp: 10106261-10105936<br>100% | 312bp: 10105584-10105273<br>99%<br>10105593: T deletion |
| JB 10 L | 393bp : 10106804-10107196<br>99%<br>10107103: T→A | 351bp: 10105988-10106299<br>100% | 297bp:10105330-10105626<br>99%<br>10105601: G→A |
| JB 11 U | 409bp: 10107150-10106742<br>99%<br>10107101: G→A subst. | 338bp: 10106267-10105930<br>99%<br>10106256: A deletion | 314bp: 10105586-10105273<br>100% |
| JB 11 L | N/A | 323bp: 10105977-10106299<br>99%<br>10105990: A deletion | 292bp:10105330-10105621<br>99%<br>10105601: G→A |
| JB 12 U | 389bp: 10107130-10106742<br>99%<br>10107100: G→A subst.<br>10106769: G→A subst.<br>10106767: T→A subst. | 319bp: 10106258-10105940<br>100% | 314bp: 10105586-10105273<br>100% |

| | | | |
|---|---|---|---|
| JB 12 L | 400bp: 10106807-10107206<br>100% | 312bp: 10105988-10106299<br>100% | 309bp: 10105317-10105625<br>99%<br>10105601: G→A |
| JB 13 U | 414bp: 10107155-10106742<br>100% | 319bp: 10106258-10105940<br>100% | 314bp: 10105586-10105273<br>100% |
| JB 13 L | 406bp: 10106795-10107200<br>100% | 322bp: 10105978-10106299<br>99%<br>10105990: A deletion | 291bp:10105330-10105620<br>99%<br>10105601: G→A |
| JB 14 U | 401bp: 10107142-10106742<br>100% | 319bp: 10106258-10105940<br>100% | 313bp: 10105585-10105273<br>100% |
| JB 14 L | N/A | 312bp: 10105988-10106299<br>100% | 298bp:10105331-10105629<br>99%<br>10105601: G→A |
| JB 16 U | 404bp: 10107143-10106740<br>99%<br>10107133: G deletion | 323: 10106258-10105936<br>100% | 302bp: 10105574-10105273<br>100% |
| JB16 L | 402bp: 10106805-10107206<br>100% | 322bp: 10105978-10106299<br>99%<br>10105990: A deletion | 290bp:10105331-10105620<br>99%<br>10105601: G→A |
| JB 17 U | 409bp: 10107150-10106742<br>100% | 317: 10106256-10105940<br>100% | 314bp: 10105586-10105273<br>100% |
| JB 17 L | 395bp: 10106812-10107206<br>100% | 312bp: 10105988-10106299<br>100% | 302bp: 10105331-1010560<br>99%<br>10105601: G→A |

**Table 2:** David Neal's sequencing results.

As opposed to David, table 1 is the contigs of the sequences. This means the program used to read the sequence combines the sense and anti-sense strains together to give one resultant strand. In essence, this gives the same results as splitting them up, just in a more readable way. In table 1, the mutation rate is 50%. Although not as many mutations were observed, the number of strands with mutations was comparable. It is interesting to note that although not many NK, or classically mutated strains, were analyzed, there was a lower mutation frequency of only 43%, whereas the JB, embryologically mutated strands, had a 55% mutation rate. This could help illustrate that there is a difference in the embryo mutation spectrum when compared with classically

mutated strains. However, it is most likely the case that these mutations are a result of error in the sequencing or PCR process, as these are still very high mutation rates.

In comparison to table 2, only a few of the mutations matched up with each other, and in many cases, where one table marks a mutation, the other table marks no mutation. This illustrates a possible problem with the actual sequencing steps, as it should be the case that two of the same strains sequenced at separate times should have the same mutation or non-mutations. This could also be attributed to the degradation of the DNA over time. Although results seem to slightly differ, it is still notable that the sequencing reactions are highly specific and error free. Therefore, in the case no mutation is observed on a sequence, then that sequence should be confidently decided as having no mutation. Because of this, it was worthwhile to combine the two tables to observe if this would illustrate better results, and possible eliminate some of the mutations caused by sequencing error. This can be observed in table 3 below, and it is done completely as contigs.

| DNA sequence | Primer 3 | Primer 4 | Primer 5 | Primer 6 |
|---|---|---|---|---|
| **JB3** | | | | 100% match 167 Bp: 10105224-10105058 |
| **JB5** | 100% match 459 Bp: 10107206-10106748 | 99% match 367 bp: 10106303-10105937 Mutation: 10106178 G to A | 99% match 346 bp: 10105619-10105274 Mutation: 10105601 C to T | |
| **JB6** | 99% match 363 Bp 10107103-10106741 Mutations: 10106769 G to A substitution | 100% match 313 bp: 10105991-10106303 | 99% match 349 bp: 10105618-10105270 Mutation: 10105601 C to T | |

| | | | | |
|---|---|---|---|---|
| | 10106767 T to A substitution | | | |
| **JB7** | 99% match 357 bp: 10107192-10106839 Mutations: 10107106 G to A 10106877 C to G | 100% match 272 bp: 10106082-10106353 | 99% match 367 bp: 10105261-10105627 Mutation: 10105639 G to A | |
| **JB8** | 99% match 459 bp: 10106740-10107198 Mutation: 10107106 C to T | 100% Match 368 bp: 10105936-10106303 | 99% match 326bp: 10105307-10105632 mutation 10105601 G to A substitution | |
| **JB9** | 99% match 404bp: 10107143-10106740 mutation 10106769 G to A substitution | 100% match 365 bp: 10105939-10106303 | 99% match 351 bp: 10105269-10105619 Mutation: 10105601 A to G | |
| **JB10** | 100% match 456 bp: 10106742-10107197 | 100% match 346 bp: 10105958-10106303 | 99% match 359 bp: 10105261-10105619 Mutation: 10105601 G to A | |
| **JB11** | 99% match 456 bp: 10106745-10107200 Mutations: 10107101 C to T substitution | 100% match 304 bp: 10105930-10106233 | 99% match 292bp: 10105330-10105621 mutation 10105601 G to A substitution | |
| **JB12** | 99% match 389bp: 10107130-10106742 mutations: 10106769 G to A Substitution 10106767 T to A substitution | 100% match 319bp: 10106258-10105940 | 100% match 314bp: 10105586-10105273 | |
| **JB13** | 100% match 459 Bp: 10107265-10106807 | 100% match 319bp: 10106258-10105940 | 99 % match 291bp:10105330-10105620 mutation 10105601 G to A substitution | |

| | | | | |
|---|---|---|---|---|
| **JB14** | 100% match 401bp: 10107142-10106742 | 100% match 316 bp: 10106054-10106369 | 99% match 298bp:10105331-10105629 mutation 10105601 G to A substitution | |
| **JB15** | | | 99% match 346 Bp: 10105678-10105333 Mutation: 10105696 C to T substitution | |
| **JB16** | 100% match 402bp: 10106805-10107206 | 100% match 323 bp: 10106258-10105936 | 99% match 210 Bp: 10105592-10105383 Mutation: 10106010 A deletion | |
| **JB17** | 100% match 409bp: 10107150-10106742 | 100% match 313 Bp: 10106050-10106362 | 99% match 302bp: 10105331-1010560 mutation 10105601 G to A substitution | |
| **JB19** | | | 99% match 349 Bp: 10105677-10105329 Mutation: 10105660 C to T substitution | |
| **JB20** | 99% match 357 Bp: 10106895-10107251 mutations: 10106936 G to C substitution 10107166 C to T substitution | | | |
| **NK1** | | | 99% match 358 Bp: 10105685-10105328 mutation: 10105660 C to T substitution | |
| **NK2** | | | 99% match 360 Bp: 10105691-10105329 mutation: 10105660 C to T substitution | 100% match 157 Bp: 10105137-10105293 |

| | | | | |
|---|---|---|---|---|
| **NK3** | | 100% match 262 Bp: 10106107-10106368 | | |
| **NK4** | | 100% match 352 Bp: 10106369-10106017 | | |
| **NK5** | | 99% match 378 Bp: 10106369-10105992 Mutation: 10106178 C to T substitution | | 100% match 221 Bp: 10105278-10105058 |

**Table 3:** Combination of table 1 and table 2 to create overall results from the sequences performed.

With the combined table, the overall results are much more complete. The overall mutation rate is 51%. For the JB strands, the mutation rate is now 53 percent, which is lower than in both table 1 and table 2. Although not a major change, it does illustrate some sequences that had no mutation of which were initially recorded as a mutation. This demonstrates more evidence for an error in the PCR or sequencing steps. The NK mutation rate is still 43%, as David did not sequence any of these strands. However, since the mutation rate was lowered slightly from combining the tables, it seems as though it is necessary to carefully look over the PCR and sequencing protocol to see if there is a way to obtain more consistent results. The combined table still illustrates the possibility that the emryologically mutated strands did have a higher mutation rate, possibly inferring that the embryo mutation spectrum is different.

For future work from this research, it is necessary to finish sequencing all of the primers for each strand and to recheck the sequences already done once a more consistent

protocol can be established. Once all of the strains are sequenced, it could lower the mutation rate, even if all of the current mutations exist, as not even half of the total data is present, as primers 1, 2, and most of 6 are missing data. Once all of the data is acquired for both the JB stains and the NK strains, then the mutation rates must be analyzed again to calculate if there is any statistically significant difference between the two rates. If so, then this could mean the embryos have a different mutation spectrum. If this is the case, then the next step would be to start attempting to figure out what causes this difference between embryos and adults, and whether or not this difference could be extrapolated to humans. Overall, this part of the project has been able to gain much insight into the possible differences between embryos and adults when mutagens are applied to them, as seen by the difference in mutation rates, and it has verified and corrected previous sequence work done to ensure accurate data. The final step will be to continue sequencing, and eventually draw conclusions on reasons for the difference, if any, in mutation rates between the JB and NK strains.

REFERENCES

Anderson, P.; (1995). Mutagenesis, pp. 31–58 in Caenorhabditis elegans- Modern Biological Analysis of an Organism, edited by H. F. Epstein and D. Shakes. Academic Press, New York/London/San Diego.

Brenner, S.; (1974). “THE GENETICS OF CAENORHABDITIS ELEGANS”. *Genetics*. 77(1): 71–94.

The C. elegans Sequencing Consortium, Consortium (Dec 1998). "Genome sequence of the nematode C. elegans: a platform for investigating biology". Science 282 (5396): 2012–2018. doi:10.1126/science.282.5396.2012. ISSN 0036-8075. PMID 9851916.

Cutter, A. D.; (2010). Molecular evolution inferences from the C. elegans genome (March 5, 2010), WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.149.1, http://www.wormbook.org.

Edgar L.; McGhee J.; (1988). “DNA synthesis and the control of embryonic gene expression in *C. elegans*”. *Cell* 53(4): 589−599.

Fields S.; Johnston M.; (2005). "Cell biology. Whither model organism research?". *Science* 307 (5717): 1885–6.

Fox, Michael Allen; (1986). *The Case for Animal Experimention: An Evolutionary and Ethical Perspective*. Berkeley and Los Angeles, California: University of California Press. ISBN 0-520-05501-2.

Harris, Jasper; Lowden, Mia; Clejan, Iuval; Tzoneva, Monika; Thomas, James H.; Hodgkin, Jonathan; Ahmed, Shawn; (2006). “Mutator Phenotype of Caenorhabditis elegans DNA Damage Checkpoint Mutants”. *Genetics*.

174:601-616

Hodgkin J.; Introduction to genetics and genomics (September 6, 2005), WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.17.1, http://www.wormbook.org.

Holway, Antonia H.; Kim, Seung-Hwan; La Volpe, Adriana; Michael, W. Matthew; (2006). "Checkpoint silencing during the DNA damage response in Caenorhabditis elegans embryos". *Journal of Cell Biology*. 172:999-1008.

Kindler C; Yost C; Gray A (1999). "Local anesthetic inhibition of baseline potassium channels with two pore domains in tandem". *Anesthesiology* 90: 1092–1102.

Koreth, John; van den Heuvel, Sander; (2005). "Cell-cycle control in Caenorhabditis elegans: how the worm moves from G1 to S". *Oncogene*. (24) 2756–2764

Kunkel, Mary T; Johnstone, Duncan B.; Thomas, James H.; Salkoff, Lawrence. (2000). "Mutants of a Temperature-Sensitive Two-P Domain Potassium Channel". *Journal of Neuroscience*. 20 (20):7517–7524.

Lans, Hannes; Vermeulen, Wim; (2011). "Nucleotide Excision Repair in Caenorhabditis elegans". *Molecular Biology International*, vol. 2011, Article ID 542795, 12 pages, 2011. doi:10.4061/2011/542795

Neal D; (2012). "Genetic Analysis of Embryonically Mutagenized *C. Elegans*" Texas Christian University

O'Farrell, PH; Stumpff, J; Su, TT; (2004). "Embryonic cleavage cycles: how is a mouse like a fly?". *Current Biology*. 14:R35–45

Park, Eun-Chung; Horvitz, H. Robert; (1986). "Mutations with Dominant Effects on the Behavior and Morphology of the Nematode *Caenorhabditis elegans". Genetics.* 113: 821-825.

Reboul J.; Vaglio P; (2001) "Open-reading-frame sequence tags (OSTs) support the existence of at least 17,300 genes in *C. elegans*". *Nat. Genet.* 27(3):332-6

Riddle DL, Blumenthal T, Meyer BJ, et al., editors. C. elegans II. 2nd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1997. Induced Mutations.

Salkoff L.; Kunkel M.; Wang Z.; Butler A.; Nonet M.; Wei A.; (1999). "The impact of the *C. elegans* genome sequencing project on $K^+$ channel biology". *Potassium ion channels: molecular structure, and diseases* edited by Y Kurachi, LY Jan, M Lazdunski (Academic, San Diego), pp 9–27.

Sarin S.; Bertrand V.; Bigelow H.; Boyanov A.; Doitsidou M.; et al., (2010). "Analysis of multiple ethyl methanesulfonate-mutagenized Caenorhabditis elegans strains by whole-genome sequencing". *Genetics* 185: 417–430.

Stoeckius, et al.; (2009). "Large-scale sorting of embryos reveals the dynamics of small RNA expression". *Nature Methods*. 6(10): 745-751.

Sulston J, Horvitz H; (1977). "Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*". *Dev. Biol.* 56 (1): 110–56. doi:10.1016/0012 606(77)90158-0. PMID 838129.

Sulston J.; Schierenberg E.; White J.; Thomson J.; (1983). "The embryonic cell lineage of the nematode *Caenorhabditis elegans*" *Dev. Biol.* 100: 64−119.

Wei A.; Jegla T.; Salkoff L.; (1996). “Eight potassium channel families revealed by the *C. elegans* genome project”. *Neuropharmacology* 35: 805–829.

ABSTRACT

Both embryonic and classical mutagenesis of *C. elegans* were performed to analyze the difference in mutation rate and spectrum (i.e., types of mutations) observed upon genetic analysis using the chemical mutagen ethyl methane sulfonate (EMS). With embryonic mutagenesis, embryos were exposed to EMS at the one to four cell stage. With classical mutagenesis, the mutations are typically observed in the F2 generation; however, with embryonic mutagenesis, these mutations were seen in the F1 generation, indicating these mutations occurred early in development. For this project, 21 mutants were obtained, and 17 of these were analyzed and found containing mutations intragenic to unc-58 confirmed by previous genetic crosses. These strains are being reanalyzed from a previous, similar experiment to check for correctness and completeness, as a higher mutation rate was recorded than what was expected. In addition, 5 classically mutated strains were analyzed containing similar mutations. All of these strains were used for DNA extractions, polymerase chain reactions for the unc-58 sequence, and then genetically analyzed for mutations in the gene. Because of the difference in cell cycle checkpoints in embryos and adults, we expect to observe a difference in mutation rate between the two populations sequenced. From this study, we hope to gain scientifically significant knowledge with respect to the lack of homologous genes in human embryos.