Huntington's Disease and *C. elegans*

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Works Cited

Introduction

HUNTINGTON'S DISEASE OVERVIEW:

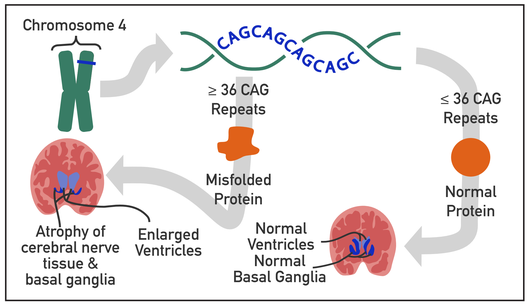


Figure 1: Diagram of Huntington's Disease

Figure 2: Graphic of Huntington's Disease Symptoms[1]

- Affects more than 30,000 Americans[2]
- No currently known cure[3]
- Destroys neurons, resulting in death 10-30 years after disease emerges[4]

C. elegans BACKGROUND:

- *Caenorhabditis elegans* - A nematode worm
- Easier to study compared to vertabrates (behavioral assay)
- Complex nervous system - ideal for studying neurodegenerative disease
- *Escherichia coli* as a food source
- Frequently used in transgenic disease studies

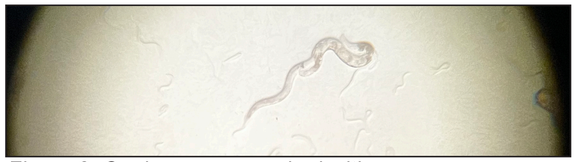


Figure 3: C. elegans we worked with

IMPORTANCE AND STEPS TAKEN:

We acquired and cultured C. elegan nematodes that were normal (i.e. "wildtype") and ones that contained an insert for the Huntington's disease gene. To understand and identify the differences between these strains, we created a behavioral assay, and also amplified the Huntington's fragment using Polymerase Chain Reaction (PCR). Our project thus sets up further studies using CRISPR to genetically engineer and remove the Huntington's gene fragments and then test for genotype and phenotype difference using PCR and the behavioral assay.

Methods

C. elegans PREPARATION:

- Make plates by melting agar and pouring it into plates
- Grow *E. coli* on the new plates as food for *C. elegans*
- Transfer the *C. elegans* from one plate to another by cutting some agar from a previous plate and placing it face down on a newly prepared plate
- Transfer to a new plate every week for fresh food

BEHAVIORAL ASSAY:

- Created a behavioral assay to understand functional differences between Htt's and Wildtype *C. elegans* strains, also used as a check to make sure Htt's disease is removed if we were to perform CRISPR
- Observe the speed, general movement, and ability to move through the agar
- Quantify the behavior (tail wags per second) to compare to the Huntingtons *C. elegans*
- Collect data in an excel sheet for later analyzation

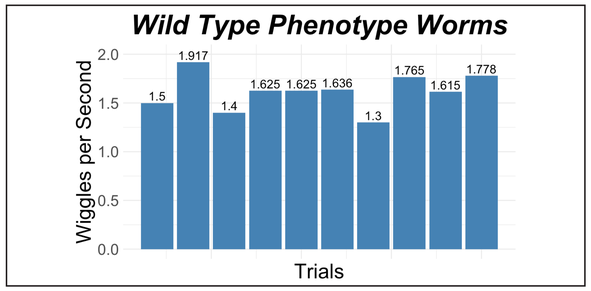
DNA PREPARATION:

- Scrape *C. elegans* from the plate, add proteinase K, an enzyme, and heat bath the reagents to break down the tissue
- Elute out the DNA with a centrifuge
- 3 primers: Punc54 (htt insert primer), blaTEM (plasmid insert primer), Unc93a (General *C. elegan* primer)
- Visualise the 3 primers with both Huntington's and Wild type *C. elegans*

Figure 4: Some tools and materials we worked with

Results

BEHAVIORAL ASSAY AND GELS:



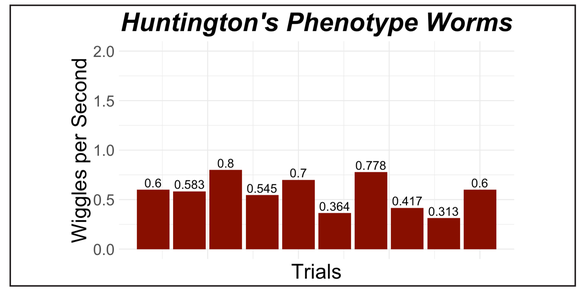


Figure 5: C. elegans Behavioral Assay looking at number of times the head or tail of one C. elegan crosses the midline

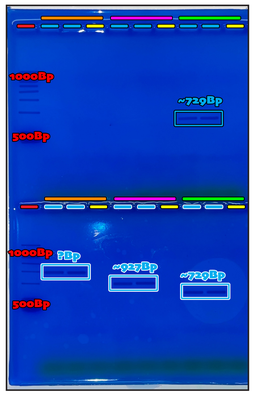


Figure 6: Predicted gel indicating the length of DNA

- Punc-54 primer → HTT DNA
- blaTEM primer → Plasmid DNA (~927 base pairs)
- Unc93a primer → general *C. elegan* DNA (~729 base pairs)
- For Punc-54 primer, see bands → Htt *C. elegan* only
- For the blaTEM primer, see bands → Htt *C. elegan* only
- For the Unc93a primer see bands → both the Htt and Wt *C. elegan*
- DNA Sequence Size: blaTEM>Unc93a
- Punc-54 DNA Sequence Size was unknown

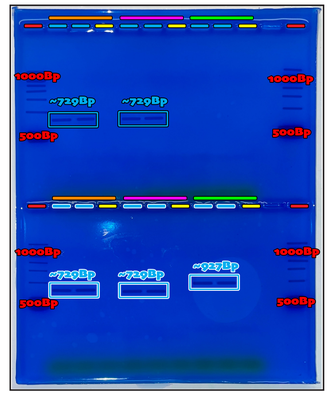


Figure 7: Actual gel indicating the length of DNA

- For Punc-54 primer, there was a band in the Wt and Htt *C. elegan* at 729 base pairs
- For the blaTEM primer, there was a band in both the Wt and Htt *C. elegan* at 729 base pairs
- For the Unc93a primer, there was a band in only the Htt *C. elegan* at 927 base pairs

Conclusions & Future Directions

CONCLUSIONS:

- Successfully performed DNA Extraction, PCR, and Gel Electrophoresis on the mutant htt gene of *C. elegans*
- Fragments in gel were of the right size, indicating that methods and primers were correct
- Created a procedure for acquiring mutant and wild-type *C. elegans*, along with cultivating them for weeks
- Created a procedure for conducting a Behavioral Assay

FUTURE DIRECTIONS:

- Optimize DNA Extraction, PCR, Optimize cultivation and behavioral assay of *C. elegans*
- Validate knockout of mutant htt gene with PCR and gel

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POST-CRISPR EXPECTATIONS:

- No bands on Punc-54 primer because removed from Htt *C. elegan*
- Band for blaTEM primer on only Htt *C. elegan* because primer insert
- Band for Unc93a on Wt and Htt *C. elegan* because general *C. elegan* primer
- blaTEM primer amplified larger DNA sequence

Figure 8: Predicted gel if CRISPR edits are succesful