Effects of Green Tea Extract on the Expression of *DAF-16* gene in *Caenorhabditis elegans*

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Background

Caenorhabditis elegans **are microscopic, transparent roundworms that are found in temperate environment soils. These microscopic organisms contain many genes, but in this experiment, *DAF-16* gene is our focus. Expression of *DAF-16* in the intestines of *Caenorhabditis elegans* seems to increase longevity and stress resistance in these worms (Warnhoff et al.2014). *DAF-16* gene is an ortholog of *FOXO-1* gene in *Caenorhabditis elegans*. *DAF-16* is alternatively spliced to produce multiple isoforms with different functions, one of which is longevity. In this experiment, we are going to determine if treating the worms with green tea would cause DAF-16 to become alternatively sliced, but further studies are needed to verify the effect of green tea extract on *C.elegans*' lifespan.**

Hypothesis

When *Caenorhabditis elegans* are treated with 0.24 g/ml of green tea, RNA from DAF-16 gene would be alternatively spliced resulting in different isoforms with different functions.

Methods

- **Chose an organism of study (*C.elegans).* Picked a gene (*DAF-16*) and treatment (green tea extract). Designed primers using "Primer3plus".**
- **Cultured bacteria (OP50 *E.coli*) for feeding Caenorhabditis elegans. Placed the worms in the cultured bacteria and incubated in 23˚C. Divided the *C.elegans* into 2 tubes: experimental and control.**
- **Treated the experimental group with 0.24g/ml concentration of brewed green tea. Isolated RNAs from the *C.elegans* cells. Conducted PCR (Polymerase chain reaction). Ran the product on agarose gel and analyzed the results.**
- **Chose the best annealing temperature. Reconstructed full length Reference seq and alternative RNA. Translated RNA into protein.**
- **Set up PCR for experiment and control cDNA and compared protein isoforms using SMART domain. Ran agarose gel for experimental and control**

Results

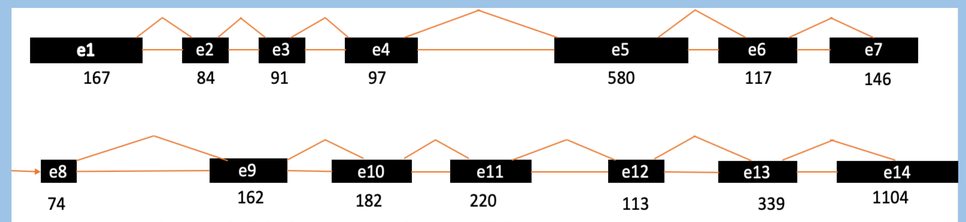

Figure 1. Map drawing of all the fourteen exons found in REFSEQ.
This map is a depiction of DAF-16 gene exon lengths. The black boxes represent exons, and the numbers are the length of each exon measured in base pairs (bp). Box size roughly corresponds to exon size. Lines connecting the exons roughly represent the number of base pairs (introns) between exons. This figure is made with information from Ensemblmetazoa (Kersey et al. 2019).

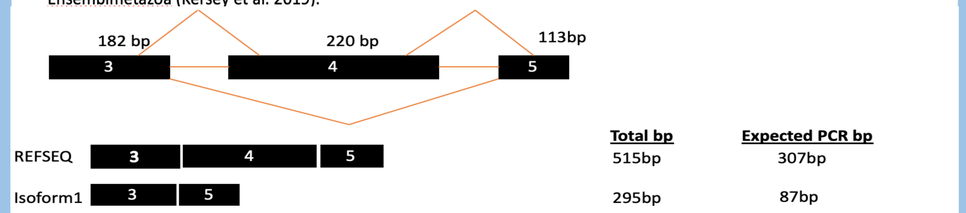

Figure 2. DAF-16 Polymerase chain reaction (PCR) sizes.
The figure above shows the RNA sizes obtained from PCR. The first diagram shows the chosen exons including the middle exon (REFSEQ), while the second one shows chosen exons with the middle exon skipped (Isoform). Numbers represent the sizes of the total base pairs and the expected PCR base pairs of each set ie the REFSEQ and the Isoform. The figure is created using information from Primer3Plus. (Untergasser et, el. 2007).

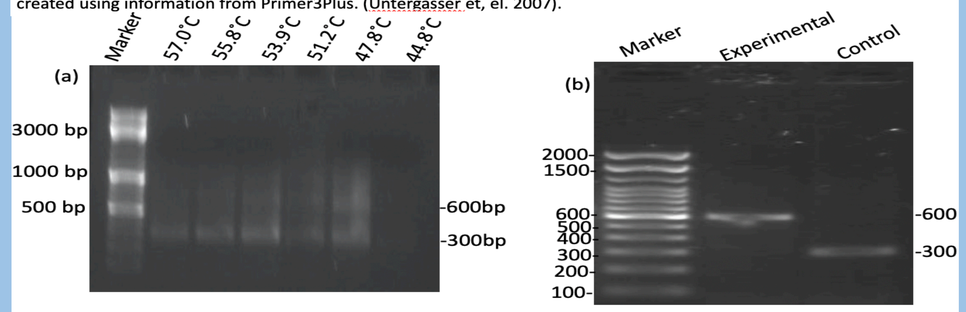

Figure 3. Reverse transcription-Polymerase chain reaction (RT-PCR) results show DAF-16 Primers anneal best at 53.9˚C.
The image (a) above shows an agarose gel of RT-PCR products from different annealing temperatures. The different annealing temperatures (in degrees Celsius) are indicated by the numbers at the top. Numbers on the left show size markers in base pair (bp), and those at the right indicate our observed PCR sizes. We picked 53.9˚C as the best annealing temperature because it has the most visible PCR product. Image (b) is the product of control and experimental PCR. The numbers on the left indicate the size markers while those on the right are the observed PCR sizes in bp. The marker used is from thermo Science (Alder and Bela 2017)

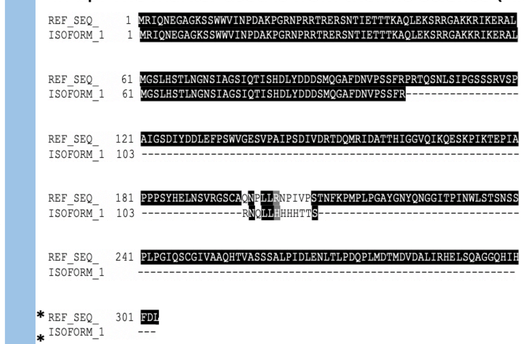

Figure 4. Translation comparison.
Above is the reference sequence and isoform1 translation results obtained from and aligned using ExPasy software (Cathy et al. 2003). The dark (highlighted) regions show amino acids that are the same in the isoform and REFSEQ while the dashes indicate regions of the isoform that are missing.

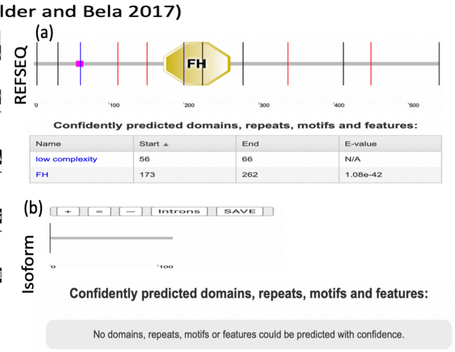

Figure 5. Domain structure for REFSEQ and Isoform.
The above structures (a) and (b) represents the reference and isoform sequences respectively, for alternative splicing of *DAF-16.* The numbers at the bottom indicate the length of the structure and the lines in (a) show different parts of the structure. There is no result for (b). These structure are made with SMART domain (Letunic and Bork 2017)

Result

- In figure 3. (b), The experimental product travelled a longer distance than the control.
- PCR product of 300bp band size is seen in figures 3 (a&b) which is close to our expected PCR size. 600bp is also observed in both figure 3 PCR products., but 600bp product is not what we expected. This could mean that introns that increased the product size were included in the sequence.
- Multiple Reference sequence structures with similar E values are generated when the reference sequence is copied to SMART domain, but there were no result for the isoform (Figure 5).

Conclusion

- **The best annealing temperature was 53.9˚C because it produced the most visible agarose gel product.**
- **The control nematodes produced a functional protein, but the nematodes that were exposed to green tea extract did not form the predicted isoforms rather, they produced other isoforms that seemed to be nonfunction.**
- **According to a previous study, "lifespan extension [in *C.elegans*] requires *DAF-16*, a forkhead/winged-helix transcription factor," and it does so by blocking and channeling some pathways that affect lifespan (Lin et al. 2001). Our experiment suggests a change in function which could suggest this finding to be true, but further study is needed to prove the validity of this conclusion.**
- **Finally, this research enabled us to test the prediction we made at the beginning. It may be helpful to this field of study by opening paths for questions, thereby furthering evaluations and critiques about the validity of our findings.**

Future Directions

- **Further research could be done to prove if green tea extract would result in increased lifespan among nematodes.**
- **If someone else would continue this experiment they would need to do PCR again for precision purposes.**
- **They would also need to be careful on which exons they would go with since *DAF-16* is a slightly complicated gene with multiple exon sets.**
- **Comparing the results obtained from different sets of the available exons when designing primers would also be a task that may be considered.**

Literature Cited

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