Over the summer of 2014, I spent two months working with Dr. Elise Kikis in the Biology Department. I provided assistance on projects involving gene modification, RNAi, and temperature sensitive proteins in the context of poly-Q ataxin-3 proteins in C. elegans. Because of previous lab experience with Dr. Mindy Farris, I had basic knowledge, such as the correct technical jargon and protocol that is involved with experimenting with C. elegans. Working with Dr. Kikis, I was able to learn more about the field and what can be accomplished with C. elegans to help cure diseases.

Dr. Kikis's projects focus on a disease called Machado Josephs Disease, or MJD, a neurodegenerative disorder which involves the incorrect folding and accumulation (aggregation) of proteins, specifically ataxin-3. Increased aggregation of ataxin-3 has been found to be directly proportional to an increase in the proteotoxicity of cells. The aggregation of these proteins is also influenced by the number of CAG repeats in the gene sequence, also called poly-Q's or polyglutamines. Our lab was able to document that an increase of polyglutamine shows an increase in aggregation of these proteins in C. elegans, *confirming* the findings of other labs. We expressed the ataxin protein along with different poly-Q lengths in the body-wall muscle cells of C. elegans along with YFP (fluorescent marker) to see the change in aggregation. We have successfully generated 5 lines of poly-Q C. elegans with and without the ataxin protein: Q0, Q35, Q40, atx-3 Q45, and atx-3 Q63. We compare these worms with wild type, N2, worms to see the change in aggregation due to the polyglutamine.

There were three projects that Dr. Kikis, Sheanna Algama, and I were working on this summer involving ataxin-3 poly-Q C. elegans. My main project involved feeding RNAi bacteria to the polyQ C. elegans in order to lessen their expression of certain genes. This would allow us to learn which genes are involved in aggregation of ataxin-3. Once certain genes were less expressed, we were able to see if aggregation increased, decreased, or remained the same for all 5 lines through the YFP protein. In order to perform this task, I had to read multiple papers involving screenings for genes that affect the aggregation of certain polyglutamine proteins when underexpressed in order to form a list of genes to test. Once I had my gene list, I chose a gene to test and grew up bacteria containing that specific RNAi. I then fed that RNAi to the C. elegans which causes those C. elegans

to have significantly less expression of those genes and documented differences in aggregation. I was only able to test one gene from my list due to contamination and other factors. I did, however, find that with under expression of HSF-1, aggregation in C. elegans, with a Q length greater than 35, increases in body wall muscle cells. This tells us that the HSF-1 protein, when normally expressed in C. elegans, decreases aggregation.

Along with my project, I also assisted in generating a plasmid to express ataxin-3 in the intestines of the C. elegans. This means we ligated the ataxin-3 gene into a plasmid and amplified it using PCR and ran a gel to ensure the size of the plasmid was correct. We introduced the plasmid to E. coli bacteria and grew them on a plate containing kanamysin (an antibiotic). If the plasmid had the correct segment of DNA and the bacteria took up the plasmid, they should have immunity to this antibiotic. Once the bacteria grew up on the plates, we picked colonies to grow in LB broth. We extracted the DNA from these bacteria using a mini prep kit and sent our product to Yale for sequencing. The results showed that the plasmid did have the ataxin-3 DNA. Unfortunately, this entire process was slowed and repeated multiple times over the summer, but we did finish with the needed result. The DNA was sent to Chicago to be injected into C. elegans, and this project will continue over this semester and hopefully lead to another publication.

I became involved in the temperature sensitive project during my final week. I was able to phenotype plates of worms; however, due to a contamination of mold on the plates, this task was more difficult than expected. Despite these setbacks, I was able to help the temperature sensitivity project progress with the help of Dr. Kikis. I had to single (place one worm on its own plate) as many uncontaminated worms as I could find and observe its phenotype and the phenotype of its progeny and their progeny. I found this part of the project challenging but enlightening because I had never phenotyped C. elegans before. I completed phenotyping before I left for the summer and to my knowledge, the experiment yielded positive results.

Over the summer, I learned how to look at a project and find the best way to design an experiment to test a question. Reading so many scientific papers also taught me how to quickly find important information and how to improve an experiment already performed. Every project will have set backs and these experiments were no different. This internship allowed me to face these challenges and look at the experiment from a different

prospective to solve the problem. With my project, I also realized that even with a flawless execution of a well practiced procedure, science takes time, effort, and self awareness. We experienced a mold contamination that kept me from completing part of my experiment. While I could have done nothing to prevent it, I learned how to keep moving forward and save the remainder of the project. A single mistake could also cost you time in the lab; for example, I used the wrong LB to inoculate bacteria and it costs us an entire week because we had no usable plates to move worms. Luckily these inexpensive mistakes could be fixed.

I intend to continue these projects in the same lab with Dr. Kikis and Sheanna Algama, along with others using the techniques and problem solving skills I learned over the summer for my final year here at Sewanee. It was a privilege to work with Dr. Kikis and Sheanna during my internship and a great opportunity to be apart of something so important in the medical field. I plan to continue on into medical school and be apart of finding cures and hopefully distributing them to the public. The internship showed me how much time and effort goes into the beginnings of a treatment or cure for a disease like MJD. I have a new found respect for all the researchers who spend so many hours asking questions, finding protocols, executing the procedures, and getting results that will put us one step closer to making medicines for people in need and I highly recommend that any pre-med student complete a Sewanee research internship.