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This summer I participated in an internship here at Sewanee under Dr. Alyssa Summers in an attempt to elucidate the function of HDAC3 in a variety of situations. My lab partner was Sarah Brown, and each day we performed a variety of tasks for the lab. Work usually started around 9:00 am, and would last until the mid-afternoon. Our schedule was very relaxed; Dr. Summers would allow us to work at our own pace and determine our own timetable for getting our projects done. This was very nice because it allowed for us to have flexibility in our schedule and permitted us to take time off as needed. We would meet with Dr. Summers when necessary, but she was a very hands-off instructor and wanted us to do the majority of the work to help us learn. While at times this made working difficult, I definitely feel that this helped me to become a better lab technician. Her hands-off process allowed me to do most of the work, and this meant a lot of mistakes in the lab, but after going through the summer and the learning process of figuring out what I did wrong when I didn't get results has definitely increased my critical thinking capabilities and made me more aware of the places one could mess up in a lab setting. This process taught me the value in being so organized that it hurt, and taking your time around the lab is the best way to do it. I learned that even if you think that you can rush through an assay and still get the same results, you probably can't and you will make small, mindless mistakes along the way that will be an impediment to getting results.

My work consisted of two separate parts: one set of projects focused on the analysis of HDAC3 in a mouse model and the other in a cultured tissue model. Analyzing HDAC3 in the mouse model required, as one may presume, that we had to take care of mice to do this. Weekly, Sarah and I would have to clean the mouse cages, and daily we had to check on the mice to make sure that they were in good health, look for new mouse pups, feed and water them, etc. I have never worked with mice before, and I will admit that in the beginning of the summer, I was a

little nervous around them. Treating the mice with respect, but also realizing that they are a scientific tool is definitely a skill one must know when working in the lab.

Before we could use the mice for any experiments, the mice had to be genotyped. This entailed putting the mice to sleep, cutting off a tiny portion of their ear, isolating the mice DNA, running a PCR (Polymerase Chain Reaction) to amplify target genes, and then running that DNA on an electrophoresis gel. This chore took up our first couple weeks of work and then we had to continue doing this throughout the summer as new pups were born.

There were three different types of mice that we worked with: PMT, MMTV, and LCK mice. All of these mice had genetic variations that affected the phenotype of the animal, and these different phenotypes told us different things about the effect of HDAC3 in the different models. One assay that I attempted to perform with the mice was a DP thymocyte enrichment assay that would tell us the effect of HDAC3 on thymocyte progression by analyzing the phosphorylation status of a different protein. This was a rather difficult procedure, and unfortunately, I did not get any results, but it was the thought that counts and I will try it again this coming semester!

The analysis of HDAC3 in a cellular model required that we grew different cells in the tissue culture lab. This required that we learned the specific ways to take care of the different cell lines. This entailed learning the different growth medias that the different cell lines needed, how to transfer the cells, when to transfer them, and how to determine if they are confluent. Another lab technique that we learned how to do was to count cells. I think that this is a very interesting procedure because it allows you to quantify cells, which are usually in monumental abstract amounts. I would say that taking care of the cells was one of the most enjoyable things that I did in the lab this summer. When I had my cells, it was like having a child that I needed to take care of. The cells never slept or allowed for any personal mistakes in their handling. They required diligence to work with and were unforgiving when you messed up. This presented a great challenge for Sarah and me, but it was a challenge that I loved facing.

One of the main things that we did with the cells was adding different HDAC3 inhibitors to the different cells lines and then analyzing the expression levels of different proteins. To do this, we would add the different inhibitors to the cells for different durations of time, usually twelve and 24 hours, and then we would isolate the protein using a RIPA lysis buffer. After the protein is isolated, we would run a Western blot to analyze the protein expression in the different cell lines for the different protein of interest. This was a very long and complicated process that takes multiple days, and learning this assay was a very big step for me. A Western blot is a canonical assay used by scientists everywhere, so learning how to do it is a big advance to my scientific knowledge.

Another thing that I did in the lab was design primers used to amplify the promoter region of different GIMAP proteins: GIMAP 8, 4, 6, and 7. This semester, we will use these primers to amplify the promoter region of these proteins and then we will put this promoter region into circular vector DNA, and then transfect cells with those same vectors. We will then perform a luciferase assay to investigate promoter analysis.

I would say that this summer has definitely helped me clarify my career goals. I have learned that I do want to work in a lab, but I also want to do something with more personal contact. What I do like about working in the lab is how stringent one must be; science is all about being perfect at what you do. I learned extensively about following protocols diligently, because if you do not then you will not get results. I really like the feel of a lab and actually getting results is one of the best feelings ever. After visiting Vanderbilt this summer and working in that lab for a couple of days and learning what a true researcher does, I can say that I do want to do that as some part of my profession, but I want to do something else as well. What I do not want is for when I become older to lose the scientific aspect of being a researcher and solely writing grants in an attempt to get money for the lab. While I know that at some point that is going to be necessary and is a part of every scientist's life, I do not want it to be the only thing that I do. I am thinking about doing an MD/ PhD program after Sewanee, and this experience has helped me

choose that path. Overall, I think that this internship this summer was a great experience and I can't wait to continue working throughout the year!