Undergraduate Research: A Student’s Perspective

An Honor Thesis (HONRS 499)

By

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Abstract

Undergraduate freshman are encouraged to get involved with extracurricular activities and undergraduate research nonstop from day they arrive on campus. However, these students often only hear this encouragement from their professors and advisors. The purpose of this thesis is to tell my journey through my experience as an undergraduate researcher in hopes of encouraging freshman and upper classmen to find ways to get involved. Under the supervision of Dr. John McKillip, I investigated the sequence of a fragment of DNA that appears to be associated with disease-causing bacteria called *Bacillus cereus*. The goal of our research has been to determine the sequence of the fragment so that it can be used in rapid detection of *B. cereus*. *B. cereus* has been demonstrated in many local and systemic infections. Rapid identification of these bacteria can ensure that the patient will receive correct and effective diagnoses and treatment quickly. Even though, this research was largely unsuccessful, the experience I gained and the friends I made have been well worth all the failures and frustrations. I truly believe that getting involved is the best way to get the most out of the undergraduate experience.

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Undergraduate Research: A Student's Perspective

I began my honors thesis as a sophomore when I started doing scientific research with Dr. John McKillip, Associate Professor of Biology. I had just changed my major concentration from Genetics to Microbiology so that I could find more research opportunities. Under Dr. McKillip’s supervision, I started to work on determining the sequence of a DNA fragment that appears in some disease-causing bacteria of the *Bacillus* genus. In identifying the sequence, we hope to determine if this diagnostic fragment can be used in rapid identification of pathogenic *Bacillus* species. These species have been found in many local and systemic infections, such as infections of the eye, skin, liver, heart, central nervous system, gastrointestinal tract, and urinary tract. Notably infections of the eye can cause blindness if not treated within eight hours, making rapid identification paramount.

Originally, my honors thesis was going to be an academic manuscript to be submitted to the *Journal of Food Protection*. But despite the time I have spent on the project, the sequence of this fragment still remains unknown. Instead, I have decided to go through my journey in this project and through it, make a case for doing undergraduate research even if the project does not succeed.

When I began this project in August 2011, I was nervous and unsure of myself. As a freshman and sophomore, I had been a part of Dr. C Ann Blakey’s project: Breeds of Rare and Endangered Equines Database System (BREEDS). The goal of BREEDS is to create a database that will help prevent further inbreeding and improve the health of endangered breeds internationally by providing professionals with complete pedigrees of any registered horse. I had only done research as a part of a team and I felt that my part in the BREEDS project was rather insignificant. For this project I was responsible for gathering concrete data about the social awareness of rare and endangered horse breeds. The result of my work was one pie chart. So,
switching to an individual project from a team effort was a big change. I lacked confidence in myself and my abilities.

My knowledge of biotechnology was limited and I had little experience doing the experiments this project required. I had to learn how to do PCR (Polymerase Chain Reaction). This technique is used to copy DNA using a heat-stable DNA polymerase, an enzyme that creates DNA using a DNA template. In this project we use rep-PCR, or repetitive element palindromic PCR, to isolate our fragment of interest. When doing rep-PCR, many small fragments of DNA are created. Our fragment is interesting because most rep-fragments are smaller than 1,000 base pairs (bp), but our fragment is 1,240 bp. This means that our fragment could be more than a rep-fragment and might be involved in causing disease.

It is required to use a sterile hood to prepare PCR reactions. This hood prevents contaminants, such as DNA, RNA, proteins, and enzymes that degrade DNA, from accidentally being incorporated into the reaction. I also wore gloves to protect the samples from the DNA-degrading enzymes on my skin. If these precautions are not taken, the resulting PCR will not yield reliable results.

To separate the fragment of interest from the rest of the rep-fragments, I ran gel electrophoresis, which uses a gel-like medium to separate DNA by size. The gel creates friction that acts against the DNA as it is pulled through. Since DNA is negatively charged, it can be pulled through the gel using an electric current toward the positively charged cathode. Larger DNA molecules will move slower through the gel since more friction is acting on them than the smaller molecules. In this way, the size of the fragments can be determined using a standard DNA ladder that has several different fragment sizes to compare to the sample (Figure 1).
Interpreting the gel results is not the hard part though. Setting up the gel and running it can be difficult. There are three parts to making a gel: Mixing the correct amount of agarose (a thickening agent for the gel) and buffer, sealing the gel case to hold it, and pouring the gel with the comb in place. For most of my experiments, I made a one percent agarose gel. This means that my agarose is one part agarose and 99 parts buffer. The amount of agarose added to the gel determines the density of the gel. If you add in too much agarose, your gel could be too dense and prevent your DNA from moving through it. If you add too little, the DNA will get pulled through the gel so fast that it will run off of the gel. In both cases, you get unreliable data, if any. Once it has been mixed correctly, the gel mixture has to be heated in order to dissolve the agarose. If the agarose is not properly dissolved, two things can occur: The gel will not set correctly or the DNA will not run through the gel properly. Basically, technique is very important in gel electrophoresis. Mixing and preparing the gel must be precise; otherwise the data is unreliable and therefore unusable.

Sealing the gel case with tape was the most difficult part of the process for me. The gel case looks like a box that is missing two of its sides. This is so that when the gel is ran the current can flow freely between the two missing sides. However, the gel case also serves as a cast for the gel. So these sides have to be sealed before the molten gel mixture is poured. The edges are sealed with tape to prevent the hot gel from leaking out. If the tape is not stuck tightly

Figure 1. The dark bands represent different sizes of DNA fragments. The first lane is the standard DNA ladder. Using this, the size of the sample DNA fragments can be determined.
enough to the edges of the gel box, the gel mixture will leak. The first time I made a gel independently, I did not properly seal the edges.

Previously, I had made a gel under Dr. McKillip's supervision. The gel turned out fine because Dr. McKillip was there to make sure the edges were tightly sealed. However, when I did it by myself, I was sure that Dr. McKillip was being a little too careful. How wrong I was. So, I did not spend as much time sealing the tape edges. When I poured the molten gel medium into the gel case, its heat caused my poorly sealed tape to lose its adhesive. As a result, molten gel slowly leaked all over the lab bench. Since the gel should not be moved while it is hardening, I just had to wait and hope that there was still enough gel in the case to use in electrophoresis. Alas, there was not enough.

It was my first big mistake in research. Because I had not been willing to spend the extra couple minutes to ensure that my gel case was sealed, I had wasted reagents (materials required for an experiment) and thirty minutes of my time. As my first mistake, it was the first lesson I learned in research: Always be willing to double-check that your set-up is correct and orderly. Though it may seem tedious, double-checking can save you a lot of time in the long run. This is especially true when you get results that are unexpected. If you double-checked your work, you will be able to confidently say that the results are different because of something in your experiment, not because you messed up.

**Lesson No. 1. Always double-check to ensure your experimental setup is correct and orderly.**

I spent the first part of the semester learning how to do PCR and gel electrophoresis. For the rest of the semester, I tried to isolate the fragment and create more of it. By the time December rolled around, I was confident in these two techniques. In fact, Dr. McKillip was so
impressed with my work he decided to let me mentor the new lab assistant, Kyla Adamson, the next semester in January 2012.

The second semester of research began and I started working with Kyla. We were both a bit shy and awkward at first. I later found out that Kyla was really intimidated by me since I had been doing research so long. On the other hand, I was just nervous because this was my first formal teaching experience. In my lab courses, I had helped other students with experiments and explained procedures. But, that was after the instructor had taught them the basics of the experiment. With Kyla, I had to be the actual teacher. I was unsure how to start teaching her the methods involved in PCR, so I started by showing her how I do PCR. In my experience, the best way to instruct someone in the intricacies of a scientific experiment is by demonstrating the process. Since Kyla had watched me do the experiment, she had a more concrete idea of the steps involved in it. Then, she was able to do PCR under my supervision with few corrections.

After doing PCR so many times, I had developed my own idiosyncrasies. Because I performed basically the same PCR reaction each time, I was able to memorize the protocol. I kept track of the approximate amount of time each step in the process takes: Twenty minutes for set up, thirty minutes for mixing reagents and setting up the reaction, five minutes to set up the PCR machine, and two to five hours for PCR run to finish. I found it helpful to keep track of this because it allowed me to become more efficient through improving my reaction time. It also helped tremendously when I came in on weekends to do an experiment because I could estimate how much time I needed to set aside for it. This information was extremely helpful in teaching Kyla because I could compare her progress with mine.

**Lesson No. 2.** Keep track of how long you take to set up experiments. This allows you to become more efficient and helps you decide how much time you need to run an experiment.
After Kyla had mastered PCR and gel electrophoresis, we moved on to do bacterial transformations. Basically a transformation involves putting a foreign piece of DNA into a bacterium after exposing it to harsh conditions that make it more receptive to the foreign DNA. In this case, the foreign DNA was a plasmid, or a small, circular piece of DNA, that contains our PCR fragment of interest. Before we can do the transformation, we have to insert the fragment into the plasmid. So, the PCR fragment and the linearized plasmid are placed in a solution together to allow them to combine. The hope is that when the plasmid is made circular again, the fragment will also be inside it. The process is basically random and we could not confirm the presence of the fragment until after the transformation.

In transformations, the bacteria, usually *Escherichia coli*, and the plasmid with the fragment are combined. Then the *E. coli* are exposed to a heat shock that creates pores in the cell membrane. The plasmid will then rush into the cell. The *E. coli* have to be rescued in a nutrient rich medium for about an hour. This allows the bacteria to repair their cell membrane. Then, the bacteria are put on agar plates that have an antibiotic. Since the plasmid contains an antibiotic resistant gene, only bacteria cells with the plasmid will be able to grow on the media. After the bacteria incubate for 24 hours, we can assess the success of the transformation based on how many blue and white colonies are present. Blue colonies result from the breakdown of X-gal, a chemical that turns blue when degraded. On the plasmid, a gene for the enzyme that breaks down X-gal is located at the same point where the fragment inserts. So, if the fragment is present, the bacteria will not have the ability to degrade X-gal and will appear white, and vice versa.

Kyla and I experienced a lot of problems with the bacterial transformation. We spent most of the spring semester running multiple transformations, all of which yielded no white colonies or no colonies at all. This basically means we spent the whole semester troubleshooting
one experiment. We would tweak small parts of the experimental protocol and try it again. Perhaps allowing the cells more time to recover before they are placed on selective, stressful media would produce more white colonies. Perhaps increasing the amount of plasmid and DNA fragment mixed with the cells would increase the number of white colonies, and so on.

It was frustrating and discouraging. It made me question my abilities as a research assistant. I can vividly remember going over each step of the process and assessing my performance. I would remember minor errors like accidentally leaving the cells in the water bath a couple seconds too long or possibly leaving the tube cap open too long. It worried me, because I knew it was impossible to perform each experiment in exactly the same way. As a junior, I had not had very much experience with failure. In fact, I was a perfectionist. I avoided failure as much as possible. I even feared it. However, I was able to push my fears aside and continue working largely due to Dr. McKillip’s encouragement. He always had a plan of action. I have never seen Dr. McKillip wallow in failure. He would not let failure have power over him. That is the state of mind that I strive for in my daily life.

**Lesson No. 3. Don’t let fear of failure have power over you. Failure is as much a part of science as success.**

The weeks rolled by with little success with our clones. Suddenly it seemed, March arrived and the annual Spring Meeting of Indiana Branch of the American Society for Microbiology (IBASM) was fast approaching. One of Dr. McKillip’s requirements for working in his lab was to present the result of the project at the Spring Meeting. But our project was unfinished! We had no results and we had made little progress. About all we discovered was that the commercial transformation kit, or cloning kit, we were using was ineffective. Fortunately, Dr. McKillip was fine with having us present an incomplete project. In fact, it was more common than we thought.
Kyla and I worked to put together a poster for me to present at the meeting. Our poster had to have the following sections: Abstract, introduction, methods, results, and discussion. We focused on the introduction and the methods first since these two sections could be completed without any results. I even made a graphic that outlined the basic premise of our methods (Figure 2). The methods section included an explanation of PCR and the cloning process. For the results section, we could use two figures: a gel electrophoresis image showing the fragment of interest and a PCR analysis from previous work. Needless to say, the result section was very short. In the discussion section, we focused on our future endeavors rather than the lack of data in our current work. We did our best to make the poster look as complete and professional as possible. However, we did not have much to work with and the poster will always seem incomplete to me.

**Figure 2.** An outline of our cloning procedure. The vector is the plasmid that was used to clone the DNA. The Multiple Cloning Site is the point on the plasmid where the DNA will insert. The vector and the insert are ligated, or connected, together and mixed with competent cells, or cells that are transformation-ready. A heat shock allows the DNA to enter. Then cells are plated onto an agar plate with antibiotic and X-gal. After incubation, white colonies are selected for analysis.
I was very nervous about presenting my poster at the IBASM meeting. I had attended the meeting as an observer the previous spring in 2010, so I had an idea of how the conference would be. Posters were set up around the conference room and the judges moved from poster to poster, listening to each presenter give a brief synopsis of their project. The judges then ranked the presenters based on professionalism and the quality of their project. The prospect of judges assessing my performance made me uneasy. Before the IBASM 2011 meeting, I had had no experience in presenting scientific research to a professional audience. In high school, I had not even participated in a science fair. I was also worried that the judges would think I had been slacking since I did not have any results. Again, my fear of failure was creeping up on me.

As luck would have it, I was the first poster in line for judging. The judge, Dr. Dominique Galli of the Indiana University School of Dentistry, listened patiently to my two minute spiel on my research (Figure 3). Since I was anxious, I stumbled over my words. But, I am proud to say that I got my point across. When Dr. Galli started asking me questions about the project, I froze. She asked me why we had chosen to use a specific cloning kit to do transformations, instead of how the technique was done. My knowledge of how to perform experiences was extensive, but I only had a rudimentary knowledge of why we chose one technique over another. So, doing a poster presentation without complete data turned out to be a challenge, but with practice and preparation, I was able to overcome my fears and present my research with confidence.
be very beneficial because Dr. Galli offered a unique perspective on my project that I could have never thought of on my own. Her questions taught me that it is better to understand the reason for doing an experiment rather than the procedure for that technique. Had I understood the reasons behind my experiment, I would have been able to defend our choice in cloning kit.

**Lesson No. 4. Knowing why you are doing an experiment is better than memorizing the protocol.**

In the summer of 2012, I had been accepted into a Research Experience for Undergraduate (REU) internship funded by the National Science Foundation at Western Kentucky University (WKU). This internship was a huge boost for my confidence. I was one of eleven interns who had been selected from hundreds of applications. Plus, I was going to get paid for doing research eight hours a day! It gave me a taste of what my life could be like as a full-time lab assistant. In addition, I would be able to start a new project, instead of troubleshooting the same experiments over and over. After the past semester of unsuccessful attempts at research, I needed a change of scenery.

I worked with Dr. Ajay Srivastava, Assistant Professor of Biology at WKU, on a *Drosophila melanogaster* (fruit fly) model of a mammalian protein called Cathepsin L that has been found to be involved in tumor invasion and growth. Cathepsin L is responsible for degrading proteins in the cell. So, my job was to assess the function of a similar protein in *Drosophila*: Cysteine protease 1 (CP1). I did this by using interference RNA, which can be used to specifically silence gene expression. I used it to silence the expression of CP1 in the flies and observe the resulting physical traits it produced. I was very excited to start this project because it involved cancer research, which is the area I would like to specialize in.
At the REU, I found out that my experience working in Dr. McKillip's lab was actually quite substantial. During the week of training at WKU, I found out that I understood most biotechnology techniques quite well. The purpose of the training week was to acquaint interns with the methods used by the WKU Biotechnology Center and to ensure that each intern could perform different laboratory techniques. I discovered that every lab will have slightly different protocols for each technique. For example, the Center has very strict rules about biosafety hoods. No materials are allowed to be stored in the hoods because they can block the flow of the sterile air in the hood. When the flow is interrupted, the hood will not be able to properly filter the air and samples could easily get contaminated. I know I will use the same strict protocols when I am in charge of my own laboratory.

The REU also gave me something more important: An experience with successful research. I was surprised when my experiments with the fruit flies worked right away. It was a huge boost to my self-esteem. The issues with my project at Ball State were not just because I could not handle doing research. Without constant failure hanging over me, I realized how much I loved research. For two months, I was in the lab every day and enjoyed every minute of it. Still, I did have some issues with my project. But after working on the Bacillus project, I thought they were very minor. I no longer viewed problems and failures in research as the end of the world. This change allowed me to maintain a positive attitude and eventually achieve great results.

**Lesson No. 5. The obstacles and setbacks encountered in past research can train you to be tenacious and to think positivity in future endeavors.**

I returned to Ball State with another poster presentation and a first-author manuscript under my belt (Figure 4). I felt refreshed and ready to tackle the Bacillus project. We knew that the previous kit was not efficient enough for our purposes, so I choose a new cloning kit that had
better commercial ratings for transformations. Kyla and I started doing transformations again and after several tries, we were successful. Finally in October 2012, we found a colony that we were able to confirm via PCR that the fragment was indeed present in the plasmid. Now, all we had to do was sequence it!

Before we could start sequencing, we had to learn how to use the sequencer. Ball State has a GenePro Sequencer that uses a similar idea to gel electrophoresis to determine the sequence of DNA. However, the process of setting up a sequencing reaction with the GenePro is more complicated. Kyla and I spent the rest of the semester learning the intricacies of this process and performing practice runs with water instead of using a gel medium. Instead of filling a gel case with the agarose, the GenePro requires that an acrylamide gel be made in the thin space in between two specialized glass panes called gel plates (Figure 5). We were running practicing runs for three reasons. Firstly, acrylamide is a neurotoxin and carcinogen and must be handled with care. Secondly, the gel cannot have any air bubbles in it, so pouring the gel must be constant and fluid. Thirdly, once the acrylamide gel mixture has been prepared, it solidifies in three to five minutes. So, Kyla and I spent a month and a half perfecting the technique of pouring the gel.
One of my favorite memories from doing these practice runs was from the first time we attempted a water run. We had to make the water go between the two glass plates using a syringe. I had been having issues keeping the water in the syringes. I kept accidentally squirting a little water out of it as I tried to minimize the amount of air bubbles in the syringe. Dr. McKillip decided to help us and accidentally pulled the syringe back too far, releasing the water all over himself. There was a pause and then we busted into laughter. It was the funniest thing. We laughed and laughed as we cleaned up the mess. That is one thing I loved about doing research with a lab partner. Kyla was always ready to laugh with me.

**Lesson No. 6. Laughter is the best way to recover from a mistake with a good attitude.**

In January 2013, we started attempting to sequence the fragment. Previously, I had set aside two days or more per week in my schedule to do research. However, this semester, Friday was the only weekday I was free to do research. Since we were doing GenePro sequencing, this arrangement turned out to be best. With PCR and gel electrophoresis, only about three hours are needed to set up and complete most gel and PCR runs. However, GenePro acrylamide gels are very time consuming. The GenePro requires six hours for set up alone. The actual run takes thirteen hours. So, Kyla and I would spend six hours meticulously cleaning plates, preparing reagents and samples, pouring the gel, and loading the samples every Friday. Then we would

![Figure 5. Example of one of the acrylamide gels we ran. The two gel plates are held together with the black rails until the gel solidifies. The gel is 0.2 millimeters in thickness and is very delicate.](image)
come in Saturday morning to read the results and clean up the plates again, a process which took another hour and half.

We ran into several obstacles on the way to doing a successful GenePro run. The first issue we encountered was expired gel reagents. Usually, when an acrylamide gel is poured, it takes approximately an hour and a half for it to solidify. However, the first couple times we tried setting up a sequencing reaction, our gel did not solidify even after three hours. When we moved the gel in order to set up the reaction, the liquefied gel slid out from the plates. Unlike gel electrophoresis, redoing a gel was not feasible. Repouring a gel in electrophoresis takes approximately thirty minutes. Repouring a gel in the GenePro takes about four hours. Since we only had one set of usable gel plates, they must be thoroughly cleaned and the reagents prepared again. This means that instead of the set up taking six hours, it would ultimately take ten hours. Our other commitments interfered with doing this. After much troubleshooting, we determined that the solution to this issue was to buy new reagents.

Once the new reagents arrived, we finally succeeded in getting the gel to solidify. Since very few people at Ball State know how to use the GenePro sequencer, we had to figure out how to interpret the data on our own. However, the sequencer never yielded usable data. The machine calculates the probability that the results gained from the GenePro run are simply due to randomness and it will indicate one way or another. If the machine indicates that the results are due to randomness, then the data is inaccurate and unusable. All the sequence data we generated were due to randomness. It was incredibly disheartening after spending twenty hours or more preparing and waiting for the run to finish. It was like working with a defective cloning kit all over again. With each failure, we would change some detail and come back the next week to try
it again. All the while, time was ticking away. Since I had planned on finishing this project and using it as my honors thesis that semester, each subsequent failure added to my anxiety.

In March, we decided to stop attempting to use the GenePro and to have our fragment sequenced professionally after many unsuccessful attempts. We wanted to have some concrete data to present at the IBASM 2013 Spring Meeting. Professional sequencing companies generally send results after two days. However, this is where disaster struck. The sequencing company could not find our fragment inserted in the plasmid, meaning that the samples we had been using all semester could have lost the fragment somewhere along the way. Not willing to give up hope, we began double checking our samples.

We grew up some more bacteria that allegedly had the fragment and plasmid and extracted DNA from them. We checked for the presence of the fragment using PCR. No results. We checked again. No results. We pulled our long term storage samples frozen at -80 degrees Celsius from the ultra-low freezer. We systematically screened all ten samples for evidence of the fragment. No results. Then it hit me that all the work I had done for the past two years was for nothing. We had no fragment or data. We had to start back at the beginning. I was going to have to give a talk on my incomplete project at the IBASM Spring Meeting in one week. I was so upset I remember trying to hold back tears when Dr. McKillip told me that we had no fragment. All of my plans connected with this project were moot. I could not use it for my thesis, I probably would not get to be first author of a paper for it, and I would have to start back at the beginning. I had very little hope for this project.
I made a snap decision to dye my hair just to make a change that I could control. When things go wrong in my life, I always want to change my hair in some way. My eldest sister, Amber, convinced me to dye my hair instead of cutting it short because she knew I would regret it. It was a good choice. Then, I decided to make a much needed trip to my hometown in Goshen, Indiana to meet my newborn nephew instead of staying in Muncie to work on homework (Figure 6). I am so glad I did. It was good to be home in a safe place that had no laboratories or science equipment. I was surrounded by my loving and supportive family. I had time to think about the project and prepare myself to start again.

I returned to Ball State refreshed. Before going to Goshen, I had been working on my presentation for the IBASM meeting. Thankfully, Dr. McKillip had decided to suspend any lab work until after the spring semester finished, so I had time to work on it. I had already finished the introduction and methods sections and I was just waiting on data to finish it. Instead, I had to go more in-depth with the introduction and methods sections, while minimizing the results and discussion sections. For example, my power point had ten slides of introduction and methods, but only two slides of results and discussion. Kyla had to do much of the same with her poster presentation (Figure 7).
Needless to say, Kyla and I were both nervous about presenting incomplete data. I had experience doing it the year before, but that was with a poster presentation. A talk is completely different than a poster. Instead of talking to one person about my research, I would be in front of everyone at the meeting. I was very anxious about that. I had given several presentations in my classes and been alright with that. However, this presentation was within my field and about my own research. I was afraid that the professors I had talked to the previous year would think I was slacking off since I still had no data. I learned that when writing an academic presentation, I could not include all of the failed attempts at sequencing. In a talk, the audience wants to hear about experiments that yield results, whether or not they match with the hypothesis. With our project, we had trouble getting any yield at all. As a result, I had virtually nothing to talk about in my results and discussion sections.

Nonetheless, I prepared to give my presentation. I waited anxiously for my turn to come. I did not feel very prepared. I had not sufficiently practiced my speech to calm my nerves. When I got up to the podium, I basically blanked out. I was aware that I was speaking, but to this day I cannot remember what I said. It was a strange experience. In any case, I think it went well, but I am not sure. After I finished my talk, I answered the audience's questions. This part was the most nerve-wracking part because I had imagined that I would freeze up and not be able to answer their questions. However, this was not the case. Each question put to me I answered with
minimal difficulty. I realized that I had become an expert with this project. Instead of deferring questions to Dr. McKillip, I could answer questions accurately and confidently. It was a great experience.

**Lesson No. 7. You become an expert on your project. No one else will know it the way you do. Use that to your advantage in presentations.**

Since I was returning to Ball State in the fall of 2013 to finish up my biology degree, I decided to stay at Ball State to do research over the summer with Kyla. My goal was to finish this project before I graduated. However, we ran into a lot of obstacles along the way. Our first step was to find the fragment again by screening *Bacillus* species for it. We screened nineteen species with no results. It turned out that the DNA we were using was too old to be used. So, we made fresh DNA samples and tried again. After a month of research, we finally found two species with the fragment. However, when we tried to do a bacterial transformation, we found out that the media we made with antibiotic was not working. The antibiotic did not create a selective environment because it had expired, so we had no way of knowing if the bacteria actually had the plasmid. We redid the transformation with new media. It appeared to be successful, but Kyla and I were skeptical.

By the time July arrived, we were ready to start screening the resulting bacteria for the fragment. Alas, we found that our clones had the plasmid, but not the fragment. Then another tragedy struck. The ultra-low freezer we were using to store our reagents for cloning suddenly stopped working. Usually, when the freezer temperature deviates from -80 degrees Celsius an alarm will sound so that the items stored within it can be rescued. This time no alarm sounded. When I went to the freezer to get something, I was surprised to find that the freezer temperature felt warm even though the thermometer said it was the correct temperature. There were no ice
crystals in the freezer, nor was there a pool of water on the floor, indicating that the freezer had shut down days ago. Thousands of dollars’ worth of reagents and samples were lost as a result.

At this point Kyla and I were feeling like nothing with this experiment would ever go smoothly. We had started comparing this project to a bad boyfriend. Our project “boyfriend” was a jerk most of the time, forgetting important dates and refusing to cooperate. But on occasion, he would bring us flowers, in the form of a successful experiment, and we would decide to keep him around for a while longer. August arrived and Kyla and I were contemplating breaking up with the Project Boyfriend. But, Dr. McKillip convinced us to do one last try. So we analyzed the issues we had had with the project and searched for solutions. We tweaked the PCR, extracted new DNA, etc. However, all of our efforts were for naught. We could not even find the fragment in samples that had previously contained it. So, Dr. McKillip decided to give Kyla and I a much needed break from doing research.

I was pretty sure this project would never be finished and I had mostly given up on it. Until suddenly in late October, Dr. McKillip told us that his lab assistant, Yinling, had finally found the fragment for us and it was ready to send to IUPUI for sequencing. We were so excited (Figure 8)! Finally, we might know what this troublesome piece of DNA is! However, there was some delay in sending out the fragment, so we did not get it sent out until right before Thanksgiving break. We have yet to hear back from IUPUI, but I am confident that this project will result in an academic paper eventually.

Despite all of the strife, participating in this project has been very beneficial for me. I have grown as a scientist and as a person. As a perfectionist, failing and letting other people
down were my worst fears. Each failed experiment forced me to confront these fears. In fact, the
near constant contact I had with failures helped me get over my perfectionism. Instead of striving
for perfection, I now strive for excellence. Nothing I do will ever be perfect, but I can make sure
everything I do will be excellent. I learned that beating myself up over failure is one of the
biggest wastes of time because it does not help you solve a problem. It just adds to it.

Last spring’s failures forced me to confront my need to please other people. Instead of
worrying about what others thought of my accomplishments and failures, I decided to let that go
and replace it with trying to meet my own standards. I am positive that I will not be successful if
I am constantly comparing myself to other people. I want to make myself proud of my work and
as a result, other people will be proud of me, too. Subsequently, I have stopped working myself
ragged over insignificant details. I am doing better in classes this semester than my previous ones
surprisingly. This project has helped me grow personally as well as professionally.

In addition to being extremely confident in the lab, I am now more confident with
networking and socializing. Because of this project, I was able to put two and a half years of
experience in research on my graduate school applications. I actually have something to talk
about at interviews and conferences. I have never felt so confident about my skills and merit. I
owe it all to this project. Additionally, I am confident that I will be able to commit myself to any
research project that comes my way. If I can be committed to an unsuccessful project for two
years, I can do anything.

The greatest gift this project gave me was an extensive knowledge of biotechniques, such
as PCR, gel electrophoresis, and GenePro sequencing. This past semester, I took a biochemistry
laboratory course that dealt with some of these techniques. I actually ended up correcting my
professor on occasion. Before this project, I had never felt confident enough to even dream of
correcting a PhD! In addition, the presentation I gave at the IBASM meeting that I had thought would be one of my greatest embarrassments turned out to be one of my greatest accomplishments. Even though I do not remember how the actual speech went, the question and answer session showed me that my experience in research was actually worthwhile. I had no data to show for it, but I had my knowledge of biotechniques to recommend me.

Another beneficial aspect of my research was that I learned how to read and review scientific articles. Dr. McKillip offered a "journal club" for his lab assistants, where we would read scientific articles and critique them. This was extremely helpful because I became skilled at reviewing articles. A skill which I used this semester as a founding member of Fine Focus, an international undergraduate research journal dedicated to showcasing the research of undergraduates in all fields of microbiology. Other than our review board, this journal is entirely run by undergraduates from production to print. My role was to formulate editorial policies for manuscript submission, establish guidelines for the peer review process, and assist in the publication and launch of the journal via web and print. With research teams around the world already planning to submit manuscripts, this journal will impact undergraduates and research mentors internationally. The first of its kind, Fine Focus will provide a unique forum for undergraduates to publish their research in a peer-reviewed journal, resulting in increased dissemination of information in all fields of microbiology. Without my experience in Dr. McKillip's lab, I probably would not have been a part of this amazing journal.

I am confident that I can reach my goal of being a university professor and conducting my own research on pediatric cancer. This research has definitely prepared me for graduate school. I am used to working long hours in the lab and giving up my weekends to finish experiments. I can easily handle doing research and class work simultaneously. I have
experience as a leader and mentor for other students. I have experience failing, which I think is more valuable than succeeding. Failure taught me so much more than success could have. I no longer view failure as a taboo or something to be avoided. Instead, I think of it simply as a step along the journey to success.

Even though my research was unsuccessful, I benefited greatly from it. I think that every university student should get involved in some type of research project or extracurricular activity. Taking classes alone does not provide the real world experience necessary to succeed in life after college. Immersing yourself in your chosen field will give you an advantage over other job applicants. It shows potential employers and graduate schools that you have the initiative to seek out opportunities and improve yourself. In today’s economy, employers are looking for applicants who have experience in their field. Undergraduate research provides students with the opportunity to gain experience before graduating.

Being involved in research will also help students make friends that will last a lifetime. Going through so many struggles with Kyla has created a bond between us. I would not trade the friendships I have made working in Dr. McKillip’s lab for anything, even for successful research (Figure 9).

Undergraduate freshman hear “Get involved” from advisors and professors over and over again. I sincerely hope that my story will give them concrete evidence that getting involved is the best way to make the most of their college experience.