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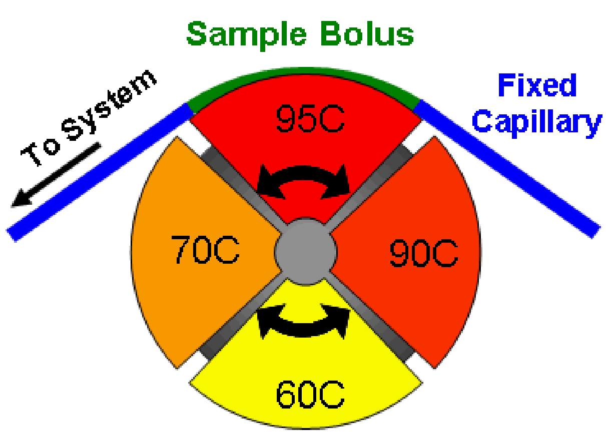
Biology in Engineering: the Rotary Zone Thermal Cycler and rzPCR

When I arrived at Sandia in mid-May, I expected to be a biology lab monkey for the summer. I had been told that my project involved polymerase chain reaction (PCR) and saw myself running samples day after day, more concerned with data mining than the device itself. I'd taken no engineering or programming classes in college and counted my blessings when I finished my undergraduate Physics courses with passing grades. I had no way of knowing that within ten short weeks I would have accomplished so much more than running countless DNA amplifications (though I did that too) and would leave Sandia feeling like I really had learned something more useful and pertinent to my future career than how to speed up my pipetting skills. Under the tutelage of Dr. Michael Bartsch and Dr. Kamlesh Patel, I learned more about the hardware of scientific endeavors and was taught to have a questioning attitude about whether a certain protocol really is the "right way" to do something, just because that's how it has always been done.

The project I was assigned to, called the Rotary Zone Thermal Cycler, is a novel device for performing more rapid PCR. PCR is a cornerstone technique in molecular biology and forensics, and is performed to amplify genetic material (DNA or RNA) from organisms to a readily detectable level so that other assays can be used to identify the organism of origin. This is of concern to the government and our lab in particular because faster amplification of genetic material allows for faster identification of biological warfare agents or the causative disease of an outbreak. Original PCR technology involved the heating and cooling of a large metal block with tubes of reagents placed inside drilled out holes, and this method is still widely used in labs today. However, the cycling of temperatures necessary for PCR often takes more time than the actual time that the sample must remain at a certain temperature and results in amplification times of 1-3 hours. Additionally, after amplification, the contents of the tubes must be pipetted to a different device in order to continue analysis of the sample.

In order to combat this, several groups have come up with various ways to modify the heat source and the sample format in order to cut down the cycling times. The Rotary Zone Thermal Cycler accomplishes both amplification and integration by using a wheel with four different metal faces that can be heating to any required temperature. A piece of Teflon capillary tubing is pulled tight against a groove in the wheel faces so that the heater surrounds the sample inside and heats it quickly and efficiently due to a higher surface-to-volume ratio than is found in a traditional bench top PCR machine that uses plastic PCR tubes. The wheel itself rotates while the tubing stays stationary, meaning that the temperature can change in the short (less than a second) time it takes for the wheel to rotate from one face to the next. The capillary tubing flows to other parts of a larger machine to create an integrated PCR wheel that can pull sample from a module that extracts DNA or RNA from cells (in our case, isolated from blood spots) and then



This image depicts the four different thermal faces of the Rotary Zone wheel and shows how the capillary nests into the grooves. The sample bolus is the approximately 5μl PCR sample to be amplified.

push the amplified DNA downstream to a system for analysis.

When I arrived at Sandia, I picked up where several interns left off last summer. They had been working on an older version of the wheel that was very similar but had since gone through a revision process. I worked with Karen Tew, another DHS STEM intern and fellow microbiologist. Together, we looked through old data and began to create schemes with which would could test the new iteration of the PCR wheel with the goal of publishing a paper outlining the system and its potential for field deployment. In our first weeks at Sandia, we received a crash course in microfluidics, java scripting, and the overall operation of the device itself. We eventually created many of our own scripts for running the machine, something neither of us had done in our college careers. The time we spent working with microfluidics allowed us to be confident when handling the machinery and assisted us in debugging any problems as they arose during routine operation. Five weeks into the internship, we were ready to run our schemes to put the wheel through its paces and compare the results to a traditional bench top device.

As with any novel technology, the device had some interesting challenges that we had to overcome. Because of the large amount of tubing that runs from where the sample is introduced through to where it is amplified and dispensed, we had to thoroughly bleach the system to prevent cross-contamination. For the first several tests, we were getting inconsistent results until we realized that residual bleach was denaturing the DNA. Even after fixing this problem, we had to optimize the protocol for amplification differently because of the nature of microfluidic systems. The polymerase, an enzyme necessary for PCR to function properly, seemed to get stuck to the side of the tubing and required us to use a much larger amount than typically required. We also found ourselves running more heating cycles than traditional machines to get similar amplification. This entire process provided valuable experience in the challenges of lab work and the importance of a strong experimental method in generating good data that is publishable. In the end, we had a few paper-quality images and were well on our way to optimizing a protocol for the operation of the rzPCR wheel.

Ms. Tew and I worked on different papers near the end of the internship experience. While she focused on the automation of the process of human identification, I focused on combining the results of our experiments with those of the previous interns and researching other rapid PCR technologies with the end goal of publishing a mini-review that presents the Rotary Zone Thermal Cycler as a novel technology. In this process, I used online journal databases provided by Sandia to compile over 30 papers discussing the enzymes involved in rapid PCR, the different technologies that have come into play within the last 5-10 years, and the importance of integration in a field-deployable PCR system. I made a complete list of references and a summary of each for the next person who works on the paper because it was not completed within the time frame of my internship. I also plan to continue working on it after I leave Sandia if at all possible. At the end of our internship, we were expected to present a 20 to 30 minute PowerPoint to our department outlining our research successes and future goals for the project.

In addition to working on the machine itself and the paper and presentation, we were also expected to participate in a journal club weekly. Some weeks we were instructed to follow a particular theme or research a particular topic while occasionally we were allowed to choose journals that were of interest in our own fields. For each journal, we gave a 10-15 minute long PowerPoint presentation and answered questions from our peers, as well as Dr. Bartsch and Dr. Patel. I presented on the papers concerning digital droplet PCR, Sanger sequencing, multidrug resistant tuberculosis, environmental monitoring of fecal coliforms and anthrax. These presentations helped shape our oration skills and made the final presentation much less stressful.

I have never been a particularly confident personal speaker, but the in-depth critique and low-stress environment helped me immensely and I feel more prepared to present projects that I am a part of in the future.

Sandia was a wonderful host institution and made a great effort to keep its interns engaged and part of the community. Sandia generously allowed interns to use their gym, library, and medical facilities. Frequent invitations were sent out for interesting events, including a weekly farmers market and a "meet the staff" brown bag lunch. Additionally, the Student Intern Program coordinators planned an excellent speaker series that included talks on persuasive psychology, nuclear weapon threats, and getting a job at Sandia in the future. Many of these were complimented with meals after the lecture, further encouraging interns to attend. Sandia also plans a large poster and presentation session at the end of each summer which interns are encouraged to participate in or at least attend in order to network and glean inspiration from the endeavors of other groups. Each of these lectures and events provided an idea of the kind of community I would like to seek in my future employment and inspired me to be productive and active in the community as a whole.

Perhaps the most beneficial aspect of the entire experience of this internship was the networking opportunity it provided. While connecting with fellow interns was enjoyable and provided a strong community, I most appreciated the chance to talk to professionals in biological careers. While my mentor and manager were both more involved in the engineering side of biotechnology, many people in our department were more oriented towards careers in microbiology. Dr. Steve Branda was incredibly influential and discussed with me, at length, careers in public health and things I should be thinking about as I apply to grad school in the coming year. Everyone I worked with, however, was more than willing to give me advice about career options, important aspects of grad school to consider, and how best to proceed forward as I exit my undergraduate career and enter the professional world.

An important aspect of my experience was the choice to attend a program across the country from my home state of North Carolina. Where once I might not have considered attending a university across the country for graduate school, it is now a very real possibility. Several of the people I worked under also encouraged me to pursue the next stage of my education in a different part of the country than I am used to in order to become a better-rounded individual. It also opened my eyes to the two very different potential career paths I could follow depending on where I attend graduate school. Pursuing a career in public health or epidemiology in California holds a much larger chance of continuing in research for at least part of my career. Pursuing the same path in the DC area could likely result in a career more inclined towards policy making. Both are appealing options, but it has been very beneficial to become aware of the opportunities different schools might provide.

My experience at Sandia has left me surer of my presentation skills and given me a start in networking within government research positions and the scientific community as a whole. It has also reinforced a suspicion I've had within the last year that I do not want to continue in research as a large part of my career. As much as I appreciate the advances provided by researchers to the scope of human knowledge, the often lonely and tedious work is not inspiring or very engaging for me. The most rewarding parts of my experience were giving presentations on existing articles, discussing topics with coworkers and the staff and Sandia, and constantly learning about the changing climate of the interface between science and politics. Many of my journal club topics gravitated towards bioterrorism research and I was also led to apply for an internship position with the EPA concerning water quality analysis and, presumably, fecal coliform testing protocols. Through my experience here, I have found that I am passionate about bringing important information together into concise patterns and researching and presenting on topics that are important to human health. This has encouraged me to pursue a career in epidemiology and potentially in bioterrorism monitoring more than any other experience in my career as a student.

The DHS STEM program has been an amazing, rewarding opportunity. The stipend is generous, especially considering that the site pays for our housing and our transportation was provided by the program. However, there are a few aspects of the process that could be taken into consideration in the future. In the initial stages of choosing a site, it would be beneficial to be able to have a more complete description of the project in order to make a more informed decision about preferred locations and projects. I ended up somewhat blindsided by what I would actually be doing, even when I contacted my mentor as soon as I found out where I would be working. It would also be nice to have a disclaimer somewhere (it's possible that I didn't see it if it's already on the site) that it is likely your position will challenge you to explore different fields than those that are within your major. I was told when I arrived here that this occurs frequently in order to give students a novel experience and it is a very valuable experience, but some previous knowledge could have helped readjust my thinking about the project before I began.

Additionally, I have heard other interns (including many DHS interns) wish that they could have received more advice and assistance with finding housing near the site. I was very fortunate to have my housing arranged by someone that works for Lawrence Livermore National Lab, but upon moving out I realized that it was 20 minutes away from work on a busy highway and far away from most other interns. Many interns, including some DHS interns, did not even get this opportunity and had an incredibly stressful experience trying to find housing. The DHS program attracts people from all over the country who may want the experience of traveling like I did, but if I had known how daunting housing could have been I might have reconsidered attending what has been an amazing experience.

One thing that I wish could have been different about this experience was the difficulty I had in locating a position or even someone to talk to about careers in public health and epidemiology. While this may seem like a "softer" science, I wish that the DHS STEM program would look into finding internship position for people interested in the interplay between public health and policy making along with epidemiological research. This would provide an amazing opportunity to help a growing biology program, such as the one at Sandia, expand into a multi-disciplinary approach to bioterrorism prevention. It would also appeal to a wider audience of driven, intellectual people who may see that public health policy is becoming more and more dictated by research and should include precautions for bioterrorism attacks. I believe that it would be beneficial to have more scientists involved in public policy who understand both the research and the political aspects of issues in order to make more informed decisions. I think that this would fit soundly into the mission of the DHS of terrorism prevention.

I also believe that providing more internship opportunities that allow biology, microbiology, or biochemistry students to learn and work with programming languages, including java script like we were required to, would strengthen the future workforce and could foster a new generation of researchers who were more aware of the dangers of cyberspace as well as the incredible strength and importance of programming in laboratory settings. This also ties into disease progression modeling and other positions that the DHS and other government organizations value in their potential employees.