SAND2014-17420R

Description of my internship project and my specific role:

This summer I worked on projects that involved RNA sequencing of pathogens after an infection of host cells. The goal of these projects was to continue developing pathogen enrichment strategies for transcriptomic analysis, and also to perform host-pathogen interaction studies. One of the pathogens I worked with was Yersinia *enterocolitica* biovar 1B, a virulent strain of bacteria that causes gastrointestinal disease. It is classified as an extracellular pathogen, but previous transcriptomic studies showed that some of the bacteria were actually able to persist within the host during murine macrophage infection. There was also up-regulation of certain genes by the internalized bacteria, which led us to believe that these genes were important for virulence of the bacteria, and played a part in the bacteria's ability to survive within the host cells. Based on these findings a model was created to explain Y. *enterocolitica's* mechanism of intracellular pathogenesis and a goal of this summer was to test this model.

In order to evaluate the model we worked on two experiments this summer. First, we put GFP promoters in front of the systems we saw were up-regulated in the previous transcriptomic analyses. GFP is a protein that, when exposed to a certain wavelength of light, will fluoresce green and is used as a tag. If these genes were being expressed within the host cells we would also see GFP expression. Murine macrophages are infected with the bacteria with GFP promoters and using microscopy we can observe if fluorescence is occurring within the host cells after infection. This would allow us to conclude that the genes are being expressed within the host. I helped with the molecular cloning for this experiment.

Second, we created mutants for each of the three up-regulated systems we were interested in studying. The mutants have the gene essentially deleted; this knockout experiment allows us to get a better idea of the functions of the genes that were found to be up-regulated. These genes are thought to be essential for survival within the host cells, so when knocked out the mutants should not be as fit as the wild type bacteria. To test their fitness we set up competition assays where we plated wild type bacteria and the mutants together on media with different antibiotics. We then compare the ratio of wild type colonies to mutant colonies by colony counting to see the effect this gene had on fitness. We expected to see reduced competitiveness in the mutants when compared to the wild types if these genes are important for survival within the host as we believe.

We created the mutants by using molecular cloning techniques, inserting genes into plasmids by using restriction enzymes and getting these vectors into our bacteria by mating or electroporation. Once the mutants were made we then did a competition assay to compare the amount of colonies formed by wild type bacteria compared to our mutant strains. Tissue cultures of murine macrophages were made and then infected with both wild type and mutant bacteria and allowed to infect for 24 hours. I helped with these infections as well as with the plating of the bacteria after infection for our competition assays; I also helped with colony counts and with making graphs with the resulting data. Although data is still being collected on these experiments, so far the competition assays show that, in at least two of the three up-regulated genes we were interested in, the genes have an impact on fitness as expected. There is reduced fitness seen in those mutants when in competition with the wild type bacteria. In my last

week of my internship we infected more murine macrophages with our mutants and wild type bacteria and then plated them to obtain colony counts. These last competition assays will give us more information on the affects of the genes on virulence for the publication that is being worked on.

The second project I worked on was a couple of different methods to enrich pathogenic nucleic acid from infections for RNA sequencing. When analyzing the transcriptome of a pathogen during an infection, the amount of host nucleic acids greatly outnumbers the amount of pathogen nucleic acids making it difficult to obtain an effective analysis. In order to get greater depth and coverage of the pathogen transcriptome a technique to enrich the pathogen nucleic acid is needed. During the summer we worked on a couple of different enrichment techniques.

One technique we worked on, with the pathogen Klebsiella *pneumoniae*, was called spin capture. Spin capture is a novel pathogen enrichment technique that will be published later this year. One of the goals of this summer was to obtain data on the spin capture technique and compare it to untreated transcripts for the publication. We set up an infection of murine macrophages, infecting them with K. *pneumoniae* and then obtaining samples at certain time points of infection. The pathogen transcripts then went through enrichment via the novel spin capture technique and were to be sequenced for the various time points using RNA sequencing. We also compared our enriched transcripts to transcripts that had not been enriched to determine the ability spin capture has to enrich transcripts. In my last week of the internship we were just beginning to sequence our enriched product so data is still being collected on this technique and the results should be published.

The second novel enrichment technique we worked on was one that involves a vacuum column system that one can run many more samples on simultaneously than was previously available. It is an easy to use, low cost, and user-friendly technique that will make enrichment much quicker. I helped put together some of the newly made columns for this procedure and then ran many tests to see if they would work. This technique is still in development but looks to be promising for the enrichment of pathogen transcripts.

Achievements during the internship, including contributions to publications, presentations, and accomplishment of project milestones:

Two papers are being written that include some of what I worked on this summer. One of the publications is on the spin columns as mentioned above. The publication is on the technique and how well it enriches the bacteria compared brute force methods of sequencing and prior enrichment techniques. The other paper is on Y.*entercolitica* host-pathogen interaction and will include information about the genes we tested. I contributed to obtaining the data that was needed for both of these publications.

I also presented a poster at an intern symposium that Sandia National Laboratories had available for their interns. It was the first poster I worked on individually and presented on my own, as in previous research I had worked in pairs or groups for presentations and posters. The poster session was a three hour session where I could present and answer questions on my poster to Sandia employees as well as other interested interns. I was able to share a little bit of what I worked on this summer, as well as get experience in presenting and creating a poster so it was a very valuable experience.

New skills and knowledge gained:

I gained knowledge and skills in sequencing this summer. Illumina's Nextera kit was one kit I learned to work with which is then put on a MiSeq sequencer both of which I had never worked with before. Nextera has a lengthy protocol that I was able to complete myself with the goal of preparing a library ready for sequencing. It fragments and tags DNA with sequence adaptors so your sample is ready to be sequenced. I also was able to see how the MiSeq worked and the data that it gives you, a cDNA library.

I also learned a technique in making and amplifying biotinylated-DNA probes for the use in the detection of DNA and RNA. I followed a protocol to produce and clean up probes that were needed in later experiments. The probes are synthesized from the genomic DNA of the pathogen randomly and this enables us to enrich the transcripts from infections because the probes will bind to the pathogen transcripts and we can then capture and wash the sample so we can separate and enrich the pathogen transcripts that are mixed with host transcripts. I was able to make probes that would be used for the different novel enrichment techniques we were testing.

One new tool I learned to use was a Qubit Fluorometer, a process where you can quantify the amount of RNA or DNA you have in a sample by comparing it to standards. I learned how to set up the samples and standards and how to use the Qubit in order to see how many milligrams per microliter of DNA or RNA we had which was crucial for some of the other steps in our procedures. Also for quantitation I learned to use the Nanodrop to quantify the Nucleic acid concentration in a sample. I also learned how to set up and run a Bioanalyzer, which is able to separate, fragments of DNA or RNA by size and is used to quantify and analyze the quality of DNA or RNA in a sample.

I also did a lot of PCR this summer. I set up PCR reactions as well as restriction digests for our experiments. Though I had done PCR before, I had never had the chance to set the programs to run on the thermocycler and I learned how to do that this summer. I also saw qPCR for the first time this summer. It allows us to see if our DNA is within a certain range and we can use the information from qPCR as a cycle threshold number for the PCR amplification we later run.

I also learned about tissue culturing which is something my school does not teach us about or have lab classes available to learn how it is done. I got to watch the steps to preparing tissue cultures, cell splitting, and how the cells are then infected. A lot of the technology and tools that Sandia has my school does not have available so it was very valuable to learn about these, and even get to work with them, to gain experience that I would not be able to get at school.

***How the internship experience impacted your academic and/or career* planning**:

The host site, Sandia National Laboratories, offered many lectures to help students with their future education and careers in science. First, they offered a lecture on resume writing where they had hiring managers and other HR representatives talk about what the resume should look like and what they look for in a resume, explaining your resume will likely only be looked at for a few seconds. They also reviewed your resume and offered tips on how to improve it. They also had a talk on interview techniques, focusing more on behavioral type interviews. They had us act out mock interview questions and answers so we could see how one should answer behavioral type interview questions. They also were able to answer any questions we had on interviewing for a job, this talk was very helpful to learn what we could expect in an

interview and how to approach answering certain questions. Another talk that was given was on networking and how to reach out to people and remain in contact with them.

A very useful lecture was one given by HR that taught us how to negotiate an offer of employment appropriately. They also had a talk on getting a job at Sandia, or any other industry job. They included information on other government laboratories we could look for employment at, and how to be competitive about getting a job. The best tip from that talk was always having an "elevator speech" ready about your research so if you run into a recruiter, or any other person who has the ability to help get you a job, you can quickly tell them what you have been doing. The last intern event was how to get into grad school, which gave great advice on not only how to apply, but also how to select schools to apply to. They also talked about using the National Research Counsel's rankings on graduate programs to begin the search for programs to apply for, and also to look at the publications and the people in the program to see if it's the right fit for you. All of these talks were very beneficial to me as I near obtaining my bachelors degree and will be pursuing graduate school and then looking for a job in industry.

Another activity Sandia offered was a symposium event where people could give a poster presentation on the work they did this summer. It was a very good experience to have in being able to summarize and explain what I did and being able to answer peoples questions about my work. The internship experience also allowed me to meet a lot of Sandia employees including my mentor, who were able to give me advice on pursuing further education. I have learned that the field I would like to go into, molecular biology, is a good choice because molecular biologist are always needed. I

also learned more about how to pick a graduate program. I now know how to go about selecting a graduate program and even what to expect. I really appreciated this advice and found it very helpful to be able to ask people questions that have been through graduate school and are in the field I am interested in; their opinions and insights were very valuable to hear.

I was also able to attend department meetings as well as talks given by employees on their research. This helped me get to see what working in industry, and particularly at a government job is like. I really enjoyed being included in the meetings and being able to hear the talks so I could get a better understanding of what a career in my field of interest in biology is like. The technical talks I went to were part of people job interviews so it was also interesting to see that aspect of obtaining a job.

Ideas on areas of research that should be considered to help the Department of Homeland Security accomplish its mission and goals:

I believe that the Department of Homeland Security should consider devoting more resources to researching biological threats and countermeasures. The internship program had very few biology positions for students to apply for and I think more students would be interested in biology positions at government labs. I think more studies and funding for studies on pathogen detection and countermeasures would be beneficial for the mission and goals DHS has also.

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