Karen Tew
DHS-STEM Summer Internship 2014
Sandia National Laboratories, CA

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A description of your specific role

I spent the last ten weeks working in the Systems Biology department at Sandia National Laboratories in Livermore, CA. Under the direction of Zachary Bent, I helped do preliminary testing/optimization of a vacuum-driven, capture-based system for pathogen RNA transcript enrichment. I also worked on a project to create mutant *Yersinia enterocolitica* strains in order to test which genes are involved in intracellular pathogen virulence, as well as sequencing several *Klebsiella pneumoniae* samples for use by a bioinformaticist.

Throughout the lifecycle of a pathogen, it can experience many environments that present physiological challenges. To cope, a pathogen can up- or down-regulate certain genes that will help it survive. For instance - in a nutrient poor environment, an organism could increase its food uptake by up-regulating the genes that make the proteins that create food vacuoles. At a given time point, the collection of genes being transcribed is called the 'transcriptome'. We can see what genes are being produced and in what quantity by sequencing the pathogen's RNA transcripts and aligning them with the known genome.

Analyzing the transcriptome of a pathogen throughout the course of host infection can provide insight into complex host-pathogen interactions. Unfortunately, sequencing mixed host/pathogen RNA from an infected sample results in an overwhelming amount of host reads and a small minority of pathogen coding transcripts. Without enrichment, only 0.0075% of all sequencer reads are bacterial coding sequences; the rest are host RNA, pathogen ribosomal RNA or non-coding RNA.

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Because sequencing is expensive and time consuming, brute force tactics are not feasible for bacterial transcriptomics. Therefore, there is a need to develop a technique that can selectively enrich for bacterial coding sequences while maintaining an accurate representation of the range of genes present ("nonbiased").

Previously, a capture-based technique was developed at Sandia that uses a nonbiased, hybridization-based method to enrich for pathogen transcripts in infected samples and allows for greater depth and coverage when sequencing. This technique enriches pathogen transcripts in mixed host/bacterial infection samples by more than 100 fold at a much lower cost per sample than found in commercially available enrichment kits. By understanding gene expression throughout an infection, we can identify and understand potential virulence determinants so that we can develop methods to better counteract diseases.

Three devices have been tested to aid in this protocol with an ultimate goal of inexpensive, multiplexed and replicable pathogen capture (a syringe-pump driven system, a spin column system and a vacuum column system). Specifically, this summer I worked on optimizing the latest version of the capture device, the vacuum columns. Essentially they are plastic tubes with a filter and monomeric aviden coated beads in the middle that bind to pathogen cDNA and lets host cDNA get pulled through by a vacuum. When we prepare the pathogen transcriptome correctly, we can eliminate much of the uninformative ribosomal RNA and maximize the coding sequence reads. In this way, we can go from a sample with 0.0075% bacterial coding sequences to a sample with 15% bacterial coding sequences - a 120 fold increase.

The syringe pump driven system was the first model to be tested. It could only run one sample at a time (single-plex) and the protocol took more than two hours per sample. The procedure was complicated and used bulky, expensive and unique equipment, making it not feasible for use in other labs. Additionally, this system could only reach about 60 fold enrichment.

The second system developed, the spin column system, could handle up to 12 samples at a time and produced 120 fold enrichment of samples. The hands-on time took about an hour, and it used inexpensive, commonly available lab equipment. Unfortunately, the lab equipment needed to be designated and sacrificial, as the experiments all take place at relatively high temperatures.

The current system (vacuum column) allows us to multiplex up to 48 samples at a time. The protocol for all 48 samples together takes less than 20 minutes and costs very little per sample. The procedure is user-friendly and uses commonly available lab equipment that doesn't get damaged in the process, unlike the spin column system.

Our preliminary results indicate that the vacuum columns produce a significant amount of enrichment (13 fold). Using this system, samples can easily be multiplexed while maintaining high enrichment, minimal hands-on time and low cost per sample. We plan to increase yield by further optimizing the protocol through varying vacuum pressure, wash number, wash buffer concentration and ambient temperature. Eventually, we hope to get the vacuum columns to be as efficient as the spin column method (enrichment of at least 120 fold).

We also developed mutant models to explore what particular genes were involved with allowing *Yersinia enterocolitica* biovar 1B, typically characterized as an extracellular pathogen, to survive inside host cells. Previous research showed up-regulation of certain genes by internalized bacteria, including those that encode Ysa T3SS, Yts2 T2SS and Tad pilus. These systems are highly expressed and may contribute to the bacteria's survival within the host.

To confirm these observations in vitro, we designed two experiments using *Yersinia* mutants and murine macrophage host cells. In the first, we cloned a green fluorescing protein reporter plasmid into *Yersinia* using *E. coli* intermediaries. After infecting the host cells with the mutant pathogen, we washed the plate with gentamicin – a potent anti-bacterial agent that kills all the extracellular bacteria while leaving internalized bacteria intact. When viewed under a microscope, we were able to see some green glowing *Yersinia* inside the host cells, confirming our suspicions that some *Yersinia* can maintain viability within the host cells.

The second experiment involved creating *Yersinia* strains that had defective Ysa T3SS, Yts2 T2SS and Tad pilus genes. By knocking out these genes, we could see if the altered pathogens were as effective at infecting host cells than the non-altered wild type *Yersinia*. We set up a competition assay to compare the abundance of wild type vs mutant pathogen after an infection, and found that, as expected, the wild type mutants were consistently more abundant than each of the three mutant strains tested. Therefore we can conclude that the three genes that were previously found to be up-regulated are indeed important for intracellular *Yersinia* survival.

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Your achievements during the internship, including contributions to publications, presentations, and accomplishment of project milestones contributions you made to the research project

At the end of my internship, I participated in a poster symposium where I presented our progress on optimizing the vacuum columns for capture-based enrichment of pathogen cDNA transcripts. I did some of the lab work for several papers, including one that is in progress for JOVE. Using Nextera, we successfully prepared a DNA library of high enough quality to be sequenced and used by the bioinformatics folks that needed it. We built around fifty vacuum columns from parts and did some preliminary decontamination tests on them. In my spare time, I assisted in other intern's projects and helped run decontamination experiments on their digital microfluidic device.

New skills and knowledge gained

There is an interesting mix of disciplines that work in the systems biology laboratory here at Sandia. There are engineers, biologists, chemists, materials manufacturers and software designers all collaborating on the same projects. Because of this, I got to learn a wide variety of different skills and be exposed to many different career paths. The initial training required to work in the laboratory was also different than I had previously encountered as a biologist. I went through security training, counterintelligence training, computer safety training, chemical safety training, laser training, electrical safety training, environmental health and safety training and fire extinguisher training, all of which were new to me.

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I learned many new molecular biology techniques throughout my internship here, including cloning, cell culture, cell splitting, how to work with RNA, probe-making, cDNA capture, DNA clean-up, DNA extraction, plasmid prep, electroporation, making competent cells, creating sequencing libraries and DNA/transcriptome sequencing. My work this summer has been much different than my background in biotechnology, and it was good to get practice in some of the more 'traditional' biological techniques. I learned that growing cells takes a lot of work – you have to take care of them constantly; they don't care if it's the weekend or if it is midnight. It was interesting to see just how sensitive the cells were – we had an incubator malfunction and run a few degrees too hot. Because of this, our cells couldn't grow properly and we several weeks of set backs as we tried to figure out why the cells looked weird.

Some of the most valuable things I learned during this internship were related to career planning. I talked to a lot of the post-docs and full time employees at Sandia about why they picked the career they did, what they liked/didn't like about it, what they would do differently, etc… I learned that it is a really tough marked out there for people who want to get a doctorate in biology. A post-doc is essentially required to apply for any decent jobs, and PhDs are taking longer and longer. It is apparently valuable to broaden your skill set to include bioinformatics or statistics. I got some good tips about how to choose a grad school and developed some connections at some local universities that will be valuable resources in the future.

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How the internship experience impacted your academic and/or career planning. Include a description of lectures/activities that were sponsored by the hosting facility and that were of benefit to your personal and professional development.

Sandia organized several different intern activities, including talks about how to get jobs (in Sandia, or in general), how to manage your own career, how to make the most of your internship and how to get into graduate school. They also sponsored several special topic talks about the history of terrorist interest in nuclear weapons and the psychology of persuasion that were really interesting. I attended a Biosurveillance team meeting video-conference with Sandia, NM that got me caught up with what kinds of new biosecurity research is going on around the country. The few team meetings I went to for my department helped me get a handle on what kinds of administrative things have to go on behind the scenes in order for everyone to have the resources they need to do research at this site.

Aside from the official meetings, I got a lot of useful advice about graduate schools and finding a job from the people that I worked with. Most people recommended that I go out of state for graduate school, and go to a place that I know will fit me depending on what kind of environment I like (lots to do or fend for yourself, crowded or not). I personally took a few tours of the universities around the area and checked out the facilities where I would be spending my time if I decided to go there. I also learned that how well you work with your PhD adviser in graduate school is probably more important than what exact project you are working on. The speaker from

Berkeley mentioned that it's probably good to go at least a semester or two without funding from external grants, but that for a science major, grants are fairly easy to come by and paying for graduate school shouldn't be an issue.

Any ideas you may have of areas of research that should be considered to help the Department of Homeland Security accomplish its mission and goals

Because I am planning to get an MD/PhD, I would be interested to see more medicine-related opportunities. Research involving disaster cleanup, environmental safety, biological threats or preparing doctors for disasters in an administrative sense would be useful for attaining DHS goals. When I was applying, it seemed that many of the summer research opportunities were geared toward math and engineering or computer scientists - very few for biologists. Even the biology opportunities were more along the lines of engineering than life-science, and it would have been good to see more variety there.

I think it would also be interesting to get a sense of what some of the other summer DHS interns are researching. You might consider putting together a presentation about what work is being done at other sites and sending it out to the interns to keep them interested, particularly if you want them to reapply for different opportunities next summer.