THE MHIRT NEWSLETTER - STAYING CONNECTED

July 16 through July 22, 2015
Newsletter 8

THE PUSH IS ON!
ONLY ONE WEEK TO COMPLETE RESEARCH PROJECTS AND PREPARE FOR RETURNING HOME

MHIRT 2015 students are trying to finish their research projects. Here is a summary of their accomplishments:

Taneesha Asing is working at Mahidol University and is detecting flaviviruses in mosquitoes that can be transmitted to humans. She reports "This past week has been very busy for me in the lab. I managed to finish over 300+ mosquito dissections and now am down to my final box. There are only 81 more samples to dissect until I am finished with all dissections. This past week, I did RNA extraction and RT-PCR with 16 mosquito pools. Today, I was able to analyze these samples through gel electrophoresis. Fortunately, 10 positives were detected for flavivirus! These positive samples from the gel have now been cut and are ready to send for sequencing! Looking forward to these results!"

Sakaria Auelua-Toomey is working at SEARCH designing surveys to improve health care of transgender women. He writes "… this week, I did end up completing all the online surveys for both English and Thai. So next week, we will test the surveys out to make sure that they run smoothly. I believe IRB is still in the process of approving the study. So it looks like there will be no data collecting by the time I leave. So, I had to create a tutorial for my Thai colleagues on how to create and edit the surveys." Sai went to a bunny fair this week and made a new friend.

Robinson Bucaneg is working in the soft matter lab at University of Munich studying protein-DNA interactions. He says, "I mentioned last time that we were able to conduct some imaging of our protein. However, since DNA PAINT works on the idea of distinguishing between moving and non-moving fluorescence, specific binding of the protein to a particular target is needed. The idea is that our protein binds to DNA. However, between different controls we have conducted, the protein seems to be binding to things other than DNA. Biotin and streptavidin are also present in the slides and we suspect that there might be binding to the streptavidin. Another idea is that the His-Tag present on our protein could be causing unwanted binding. As I have been learning throughout the summer, in science, expect the unexpected." Welcome, Rob to the ups and downs of science.

McMillan Ching is conducting research at Chiang Mai on the human fungal pathogen *P. marneffei*. He wrote, "We are repeating our experiments and doing some modifications with the protocol, because we are encountering many problems. Yesterday I activated my THP-1 monocyte cells using PMA to transform them into Macrophages. I will then infect them with the conidia of *P. marneffei* to follow their infectivity and transition from the conidia of the mycelial form to its yeast form. On the other hand, I am almost done with the transformation studies on 1% Proteose Peptone broth culture studies of *P. marneffei*, but I have yet to analyze data."

Christian Dye is working at AFRIMS on immunity to HIV. Chris wrote, "This week I ran more ICS [intracellular staining] and dendritic cell phenotyping flow cytometry experiments. Unlike previous weeks where I looked mostly at peripheral blood mono-nuclear cells, this week, I learned how to extract cells from [human] lymph nodes and sigmoid colon. With the cells extracted from these tissues, it was possible to run ICS and phenotyping experiments. Furthermore, I was asked by my mentor at AFRIMS, Dr. Alexandra Schuetz, to present to AFRIMS faculty and staff at their quarterly meeting about what I have been doing and what our future collaborations entail. As such, I needed to learn

how to present the analyzed data from FlowJo in an appropriate form for others to view. With the help of Dr. Schuetz, I was taught how to extract FlowJo data and present it in a meaningful way."

Samantha Esperanza is studying human polyomaviruses in chronic kidney disease patients at Mahidol University: "This week in lab we finished with the JC screening. We have collected 45 samples so far and we are awaiting 5+ more. Of the 45 screened for JC, 12/45 were positive. This is quite interesting because most of the literature that I have read reported 40-80% of the study's population being positive for JC. For BK, we ran the 1st PCR of about 27 samples and will run the 2nd PCR tomorrow. Like I mentioned last week, we will screen first with the VP1 primers, then perform a PCR with the NCCR primers for the positive samples. We will sequence as many of the positive BK samples as we can before I leave. Overall, everything is running smoothly."

Chaewon Im is conducting research on HIV and HPV at SEARCH. She reported, "This past week in the lab was quite busy. Eleanore and I finished PCR, gel screening, and hybridization of our last sample set. We also began to run qPCR, and we have finished two sample sets so far. We plan to finish running qPCR on our remaining sample sets by the end of next week. We are doing some troubleshooting with our qPCR and gel screening at the moment; the standards for HPV 16 look a bit off and the screening is still not giving us the confidence we need to determine that a sample is HPV negative." Ah, science can be frustrating!

Sairel Labasan is researching immune responses to the bacterium, *Burkholderia pseudomallei* at Mahidol. She says, "This past week we were finally able to do mass spectrometry using MALDI-TOF (matrix-assisted laser desorption ionization-time of flight) for 6 of our samples, hooray! This involved learning about the theory behind MALDI-TOF and how to prepare samples. I also got to learn how to use the machine itself and the software, called FlexControl, which goes with it. I was pleasantly surprised because I never expected for there to be so much flexibility. For instance, there is a camera that allows the sample to be visualized and for you to move around and choose specifically which area you want ionized and analyzed. P'Phon also did a demo on ELISA using the LPS of *E.coli, P. aeruginosa*, and *B. pseudomallei* to stimulate whole blood. In the meantime, we learned the calculations necessary to carry out an ELISA (i.e. preparing all the reagents)."

Kellyan Nguyen is having success with her project on detection of Hepatitis E virus in pigs and pork. She reports, "The highlight of the week was definitely a very rewarding moment! I ran gel electrophoresis on 24 known positive samples in a large welled gel for band cutting and DNA extraction. Afterwards, the DNA will be sent off to Korea for sequencing. Also, I am currently extracting RNA from tissue (200 samples were collected on Nakphon Pathom province in two separate trips) and will eventually use that data to correlate with the data collected from pig feces. So far, I have roughly 56 positives from my 400+ test samples. Samples include: swine, human, dog, bird, cow, chicken, duck, and raw pork products (liver, intestine, ground pork, pork meatballs, fermented pork, and blood sausage)."

Maya Uemoto is conducting research on immune responses to dengue viruses at Mahidol. She writes, "This past week we received one sample. I feel very comfortable doing flow cytometry now. I hope to find a way to incorporate flow into my future doctorate research. I have started to analyze my data by making graphs in excel. Dr. Kelley has taught me how to gate dot plots and export the data to excel." So, Maya is now reached the data analysis process.

Raphael Raman returned early from Chiang Mai to begin the Imi Ho'ola Post-baccalaureate program. He reports: "I am back in Hawaii, safe and sound. Tomorrow is my first day of class at JABSOM. It's an exciting time for me and I'm glad to be home."

Almost all MHIRT students will be back in Hawai'i on July 29th. See you all on August 5th for the workshop and E Ho'oula Haumana on Thursday, August 13th.