Logistical Improvements

Logistically, there are several improvements to this strategy that can immediately be implemented for this or a more challenging scenario that may not have readily available electricity, refrigeration, or ice. At the swine exhibit, our power needs were met by a reliable 120V AC outlet; however, this would likely not be available in future efforts to deploy this technology. While all the power needs for this equipment are relatively low, this is still a major challenge and should employ a robust approach to ensure electricity is not an issue. Lithium ion batteries designed to charge cell phones have enough capacity to run our thermocyclers, microfuge, and Qubit, however charging the laptop and or providing multiple days' worth of power would require batteries too large to transport on a commercial air liner. A possible workaround for this would be to use a battery array that is not technically considered to be a single battery. Portable solar panels, while low in power, could be used to extend the life of these batteries. A second approach to electricity is to transport a small generator. Unopened generators can be checked on commercial airliners. This approach would be quite effective with the assumption that fuel can be located on site and the caveat that the generator cannot be transported back once fuel has been added. A simple strategy would be to bring a power inverter to draw power from the 12V lead acid battery of whatever vehicle was used for transportation.

There were two reagents that weren't transported on the commercial airliner: 95% ethanol and superscript IV. Ethanol is limited to 70% on commercial airliners and our workflow required 95% ethanol for the extraction and 80% ethanol for the bead washes. Since our samples are diluted with water before ethanol is added, we can simply combine these reagents ahead of time and add 555 µL of 65% ethanol to have the same effect. Lowering the concentration of ethanol for the bead washes would simply mean accepting a potentially lower yield and could be overcome by overloading the input material. Superscript IV is highly temperature sensitive and the manufacturer insisted it be transported on dry ice, despite recommending laboratory storage at -20°C. Dry ice can be transported on a commercial airliner, 3 or 5 pounds according to an airport ticket agent or the FAA respectively, and we can simply add a cooler to transport this reagent. Alternatively, since our cooler maintained -20°C well, we can also transport this in the cooler as designed. Continued improvements, such as these, will be implemented as *Mia* continues to be deployed to less forgiving environments.

Speed

While speed is a primary success of our work, there remains room for improvement. A major delay in this effort was an Oxford Nanopore Technologies' software controller (MinKNOW) issue that failed to recognize and connect to the sequencer. The fix for this took 34 minutes and required sequencing on the Windows partition of the laptop whereas the analysis software runs on the Linux partition. This resulted in our automated analysis pipeline not having access to the raw sequencing data

until we terminated the sequencing experiment after 6 hours and transferred the data across the partition. This issue has since been resolved and would now save us up to 6 hours on the entire workflow, depending on the read generation rate of a given sequencing run. We are also developing a containerized version of *Mia* that will be able to run on any operating system that supports Docker (Docker Inc, San Francisco, CA), including Windows and Mac. Moreover, we are now incorporating the use of Oxford Nanopore Technologies' (Oxford, UK) MinIT portable GPU base caller that will dramatically reduce the time of base calling, our analytical pipeline's tightest bottleneck.

The sequencing time required to achieve our desired 20x mean coverage across each segment is also dependent on factors which are poorly understood, though likely related to reagent stability, library quality, and the integrity of individual pores on the flowcell. While this in-field trial produced nearly 100k reads in 6 hours, we have commonly produced over 1M reads in this time frame in the laboratory. Whether the low yield found in this field trial is due to the field conditions or an outlier is the subject of further investigation as we prepare for future field deployments.

Reference Database for Portable Analysis

The success of *Mia* hinged on our ability to curate a small reference set of virus genomes with lineage annotations per segment. While the rapid growth of viral genome sequences in public databases has allowed fine-grained phylogenetic analyses of viral pathogens, analyzing the complete evolutionary history is not necessary to identify pathogens and guide a public health intervention during an outbreak. *Mia* demonstrates that curating a small and diverse reference database is sufficient for estimating the risk of an influenza outbreak. Importantly, the reference set can be adapted for other IAV outbreak targets, such as avian IAV in live bird markets. Furthermore, *Mia*'s analytics are 100% automated as reads originate on the file system of a machine connected to the MinION. This is a critical feature in our goal of decentralizing influenza virus surveillance and outbreak response around the world, as pertinent information is provided back to the user in real-time.

Diagnostics

To select pigs to sequence via *Mia*, we needed a screen beyond simply looking for ILI. To do this, we used Influenza A Virus Swine rapid tests. While these tests did confirm the presence of influenza A virus within the barn, they appeared to have a high rate of false negatives. For future studies, we are interested in exploring more sensitive screening methods and portable diagnostic PCR.