Tracking epidemics through genome sequencing

Emily Koehler, Ian Bogdanowicz, Carly Esteves and **Ian Portelli** explain why genome sequencing is so important in epidemic outbreaks, and how new portable equipment could help in remote and developing areas

Genome sequencing is an important factor in determining the biological properties that make any organic material unique. Simplistic nucleotides and the corresponding order of their base pairs can determine the specificity of a living organism, virus or any sort of entity worth studying. And this is important in the outbreak of disease, especially epidemics. When a virus infects a cell, it immediately begins to replicate rapidly; these copies of the virus mutate, generating genetic variations.

If two people carry viruses with the same genetic sequence, they are likely to have contracted it in the same place. Scientists can therefore use DNA or RNA sequencing to trace the 'family tree' of an outbreak, establishing that infections with similar sequences originated from the same location at the same time, providing insight into when an infection entered a particular area and how it may have passed from one group of people to another. This helps healthcare workers and public health authorities to diagnose new cases, estimate who has been exposed to the disease and then work out public health strategies. It also allows the development of new vaccines and antivirals that can be used to treat patients.

Indeed, it was DNA sequencing that helped to establish the source of the cholera outbreak in Haiti after the 2010 earthquake (*CRJ* 11:4). The disease was not documented in Haiti until October 2010; one DNA sequencing study traced the particular strain of cholera involved back to Nepal. A Nepali contingent of peacekeepers had been stationed at a Minustah base in Haiti. Improper sanitation practices at the base led to untreated sewage entering the Meye Tributary, flowing into Haiti's principal river system, which tens of thousands of Haitians rely upon as a primary source of water for drinking, washing and farming.

Sequencing has come a long way since the early 1970s and current research is revolving around portable sequencers that can be implemented in mobile labs, rural areas or environments of distress.

DNA sequencing in real time with large samples has always been an issue. Often, researchers have to wait until a test is fully finished to analyse results and draw a conclusion. This can be a costly and time consuming process, so sequencing techniques have moved to automated sequencing, or the use of sequencing devices to carry out the same processes, only at a faster and higher level, with user-friendly displays and data acquisition processes.

The majority of these sequencers employ a technique called capillary sequencing, where delicate, narrow pore openings separate molecules of varying size after they have been stimulated by high voltages. These high voltages initiate ionic flow based on the electrical properties of the molecules within the capillary openings.

A breakthrough for sequencing came about in 1985, with Applied Biosystems' release of the first widespread automatic DNA sequencer, the ABI 370. Since then, Applied Biosystems and other companies have improved upon the automated sequencer, releasing multiple different processing and handling techniques. Other major milestones, including the 2003 completion of the Human Genome Project, have paved the way for cheaper and more accountable automated sequencing devices.

The MinION, produced by Oxford Nanopore Technologies, has been attracting attention for its size, speed of analysis, and its help in understanding the progression of Ebola in West Africa. This device can analyse DNA, RNA, and even protein samples, from biological donors.

MinION is the first genome sequencer to use a nanopore specific device (small microscopic openings in a membrane). According to the company, the MinION can: "Bring easy biological analysis to anyone, whether in scientific research, education or a range of real world applications such as disease and ▶

pathogen surveillance, environmental monitoring, food chain surveillance, self-quantification or even microgravity biology…"

The novel aspect of the MinION is that it is small, portable – about the size of a stapler – and can sequence a large amount of data within a short amount of time. Long gone is the need for bulky, large equipment when operating in remote areas.

Data analysis and preparing the DNA samples are designed to be simplistic and straightforward so that those with less advanced medical backgrounds can still operate these devices.

The simplicity of the design is such that the MinION can easily be integrated into a personal computer or laptop via a USB drive. The sensor array can be used for multiple experiments, and is processed in real time to give a quick turnaround time for results. Data acquisition is completely dependent on the researcher conducting the test; analysis can be short, long, or halted once a trend has been reached.

Each nanopore has its own individual electrode via the sensor chip to generate an ionic potential through the pore's inlet. Many scaffolds that hold these components are all placed onto a sensor chip at a micron level. The sensor chip is directly exposed to the genetic fluid from a research sample via a pipette. This chip is directly connected to resulting circuitry, within a flow cell, all located inside the sequencer.

These nanopores are essentially biosensors; small biological holes have an ionic current passed through them as simultaneous resultant molecules pass through. The diffusion of these particles through the pore change the current signal, based on their size and charge-intrinsic properties, ultimately providing information on which base is passing through the pore at a time. Biological nanopores are currently most used, but the company has plans to start implementing synthetic nanopores, or 'solid state' nanopores that will cut down sequencing costs and improve techniques even further.

Because of the ease of changing these synthetic nanopores for research purposes, solid-state nanopores could be implemented for more trials to further these portable devices and help them to become established globally.

The MinION is a perfect example of how the progression of DNA sequencing can be implemented for research purposes in remote areas, or even for disaster sites. Third-world countries, such as those where the original outbreaks of Ebola and the Zika virus were discovered, often do not have direct routes for a high-traffic influx of medical personnel to reach an outbreak zone. More portable and less excessive equipment can help to combat the problem, without needing the same resources at the average medical clinic in the developed world.

Currently, there is no other portable genome sequencing company competing with Oxford Nanopore. In addition to the MinION, the company has other systems that process and sequence the biological samples the same way, in real time. The PromethION, a tabletop sequencer, can sequence more complex testing, like that of blood or serum.

An even smaller device is currently in production: a smartphone sequencer that can easily be inserted into a smartphone's charging port. The SmidgION has been designed for mobile field sequencing, allowing researchers to work in even more remote or less-equipped areas. Of course, a smaller more portable device means that experiments are less in-depth, but this is surely a step in the right direction for disaster medicine.

Multiple SmidgIONs and MinIONs incorporated into a mobile sequencing lab could allow researchers to set up medical bases in remote areas, and even take sequencing by foot to a population, instead of a population having to make its way to a medical site.

This technlology can allow researchers to track RNA sequences and see how they mutate from previous sequences, giving an insight on how a disease is manipulating its environment. Analysis of the genome can show mutations, which can then be correlated to a particular region. Mapping these mutations permits researchers to do their best to contain the outbreak from spreading geographically.

Mapping mutations allows researchers to do their best to contain an outbreak and stop it from spreading

The MinION is about the size of a stapler, can be plugged into a computer or laptop, and can sequence a large amount of data in a short period of time

Oxford Nanopore

In a recent study performed by researchers at the University of Birmingham, the MinION was used in a genomic surveillance system implemented in Guinea to track the progression and mutation of the Ebola virus in Guinea. From March 2015 to October of the same year, 142 samples were accumulated and the genetic makeup of the Ebola virus was analysed. The team proved the MinION was applicable to remote areas by packing the entire processing and data acquisition software into a carry-on luggage sized unit.

After samples were taken, public health officials were notified

▶ of progress and were able to make conclusions about the spread of the disease in the area. Researchers discovered that two larger divergences of the genome existed, and were able to isolate the mutations to Guinea, Sierra Leone and Conakry.

An article published by *CRJ* in 2014 discussed the discovery of the MuPIT Ebola Edition, an interactive Ebola model, made by Johns Hopkins biomedical engineers. The ability to manipulate the antibody binding sites gave researchers a better understanding of how to prepare vaccinations to combat the virus. This, in conjunction with the research performed in Guinea, can give medical professionals a head start in not only understanding the virus on a molecular level, but in creating the necessary medicine to halt or slow its spread.

The Ebola study paved the way for a novel discovery of understanding and tracking a virus over an eight-month period. Researchers and engineers collaborated to understand the basic structure of the virus, enough to manipulate a vaccine to combat its spread, proving it was possible to sequence high volumes of genetic information in remote areas. Although Zika is spread differently through mosquitoes, the next step is to build upon these techniques for the Zika virus and future viruses that pose a threat to any region and its inhabitants.

However, although this experiment with the Ebola virus proved successful and is a step in the right direction for integrating medical research in underdeveloped areas, there are

issues that will have to be considered before implementing these devices.

Power sources were spotty, so the team often had to rely on generators and switching back and forth to different modes of power, halting the fluidity of the experiments. Also, Internet capability – which is where all the data analysis took place – was hard to maintain.

So what does all this mean? We have various types of sequencing techniques that have been built upon over the years to improve mass amounts of sequencing samples analysed at a time, limit use of reagents, and lower DNA sampling costs-to-benefit analysis of important genomes for disease tracking and identification purposes. The MinION can not only be applied to analyse new diseases, but also to help track epidemics such as AIDS progression in impoverished areas. We already know its importance in analysing the human genome, but the benefit of this broad application allows researchers to utilise the tool for many types of genetic analysis. This device is a stepping stone in rural and mobile sequencing, which can leave researchers and scientists with less data acquisition time and therefore more time to understand the underlying mutations and spread of biological pathogens throughout a geographic region. CRJ

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