

## **Roundtable Discussion on Portable Sequencing for Infectious Disease Detection, Diagnosis, Discrimination, and Discovery**

**Background** - This paper reports on a February 28, 2017 Roundtable Discussion convened by B.Next, an IQT Lab.

Several companies are developing DNA sequencing devices that can enable users to sequence DNA outside the traditional laboratory setting. Among them, Oxford Nanopore is perhaps the most well-known. The advent of portable sequencing devices opens up a wide variety of potential use cases that range from point-of-care medical diagnostics to on-site agricultural pest analysis. It will soon be common for scientists to study animal and plant genetics and the structure of microbial communities close to where these species are found in nature. In the realm of managing epidemics, the current state of portable sequencing technology presents potential opportunities to accelerate the collection of pathogen genomic sequence data during an outbreak. Distributed sufficiently broadly, portable sequencers could function as “sensors” that help detect the spread and evolution of a pathogen.

**Purpose** – To explore this concept further, IQT hosted a one-day discussion on this topic, with the goal of learning at what stages in the development of an epidemic (see the illustration at <https://www.bnext.org/premise/>) portable sequencing may have the greatest immediate and longer-term impact on quenching an outbreak.

The Roundtable included experts from industry, academia, finance and several USG agencies who manufacture, consume, invest in, or develop use cases for sequencing applications as they relate to disease outbreaks. The discussion took place over a single day, included invited presentations from four participants plus prepared remarks from three others (see below), and was held on a not-for-attribution basis. (The participants agreed to allow IQT to publish a summary of key insights from the meeting. In addition, participants named below consented to allow us to use their names in this report.)

### **Summary of Discussion**

The discussion at this round table was organized to discuss three questions:

1. What might be specific applications of portable sequencers for infectious disease detection and management? This discussion was opened with presentations on potential use cases from Kevin Olival of the Eco-Health Alliance (on pathogen discovery prior to outbreaks), Trevor Bedford of the Fred Hutchinson Cancer Center (on sequencing during outbreaks to track the origin and evolution of pathogens during outbreaks), and Alan Rudolph of Colorado State University (on sequencing applications in food safety, agriculture and soil quality).

2. What operational characteristics, performance metrics, and supporting technology or infrastructure will make portable sequencers more applicable to the problem? This discussion began with an in-depth briefing from James Brayer of Oxford Nanopore (on the current capabilities of the MinION sequencer), and from Sterling Thomas of Noblis, Inc (on bioinformatics-related challenges to fieldable sequencing).
3. What are the market drivers and opportunities? Alex de Winter of GE Ventures and Mickey Urdea of Halteres Associates opened this section with discussions of the investment challenges associated with diagnostics technologies.

## Discussion Topics

Several key take-away messages emerged from the discussion:

- 1) **The technology.** Portable sequencing is here. The quality and quantity of data generated by MinION are substantially improved over just a year ago, and will continue to improve. Oxford Nanopore has a tremendous first-to-market advantage, but we know of and expect other vendors to enter the market. James Brayer of Oxford Nanopore gave an update on the current specifications of MinION sequencers. The devices have seen a significant jump in the accuracy in base-calling, which is now in the low 90%'s. This is still lower than the 99+% of Illumina systems, but the quality of sequence is ramping quickly, and attendees noted that there is value to being able to quickly sequence a sample on-site and transmit data, rather than transport a sample. MinION is beginning to realize that potential.
- 2) **The importance of accuracy and sensitivity.** The sequencing accuracy issue is part of a larger conversation on the problem of false positive and false negative results. Other contributors to false positive results include the incompleteness of reference data for comparative purposes and the presence of microorganisms that are “conditional” or “opportunistic” pathogens (e.g. *Staphylococcus aureus*, *Clostridium difficile*), meaning they may be present without causing a disease, but may become pathogenic upon a change in conditions (e.g. immune status, nutritional state). A false negative result may occur due to the throughput of the sequencer when processing samples in which the pathogen’s genome is a small fraction of the total DNA or RNA in a sample. For example, in a clinical sample, the vast majority of DNA molecules will be host DNA. Sequencing the pathogen will therefore require sequencing a large excess of host DNA molecules to accumulate enough pathogen sequence to assemble a genome, unless techniques are employed prior to sequencing to enrich the pathogen-specific nucleic acids. Another contributor to false negative results is the characteristic of some pathogens residing in anatomically inaccessible reservoirs, such as cryptosporidium that burrow into the intestinal wall. Sensitivity and accuracy thresholds,

- including tolerance for false negatives and/or false positive results, can vary depending on the desired application, which will ultimately dictate whether portable sequencing is the appropriate technology to use.
- 3) **The continuing drag of logistics.** There remain technical, logistical, and network challenges to making portable sequencing practical for field applications. While the sequencing instrument itself fits in a pocket, sequencing from a field sample also requires a laptop computer, small lab equipment and some chemicals to purify DNA (“sample prep”) and prepare it for sequencing (“library prep”) before the sequencing reaction. Sample prep remains to be a substantial challenge and the needs (and supporting equipment) vary by pathogen and sample type. An end-to-end solution still requires one or more Pelican cases to transport to the field, and a cold chain to support temperature sensitive components. The participants believed, however, that like the sequencers themselves, the size and cost of computational and preparatory equipment required are also going to shrink significantly.
  - 4) **A variety of opinions on where portable sequencing is the appropriate technology.** There are many potential applications for portable sequencing during outbreaks, but not all may be cost-effective. Also, for some applications (like triage) that require a simple yes/no answer, sequencing may yield much more data than are necessary. Some attendees stated the opinion that the best use for sequencing is still immediately after a pathogen is isolated, when discovery of the genome can yield a sequence that can then be used to make cheaper detection and diagnostic tests. Others felt that the rate of decrease in the cost of sequencing would, within a few years, make sequencing competitive with other nucleic acid-based tests (such as real-time PCR). However, many of those tests remain too expensive for needed field applications. Cost is, and will remain, an important limiting factor for many detection and diagnostic tests, and the acceptable threshold will change based on the application.
  - 5) **Regulated shipments of biological materials.** Shipping and handling samples containing certain pathogens that are designated “select agents” is challenging. There are a number of laws and security procedures (domestic and international) that limit the possession of select agents to specially vetted persons. The point was made that shipping uncharacterized samples is conceivably riskier, but presents a much lower regulatory barrier. This situation alone creates a strong argument in favor of accelerating the development of in-field sequencing.
  - 6) **Accessing clinical data for public health use requires sequencing to enter the clinic.** The use of portable sequencing on patient samples in a clinical setting is inseparable from the broader challenges confronting many

clinical diagnostic tests: determining who pays, how is privacy handled, and how are data stored and shared.

- 7) **Sequencing technology as a hypothesis-free diagnostic.** Clinician participants noted the presence in hospitals of what was termed “biological dark matter”: illness in patients who later recover whose infection is never diagnosed; this includes cancer and immuno-compromised patients that harbor a far greater variety of pathogens than most other patient types. This situation parallels the time during an outbreak that patients present with an illness before the etiological agent has been discovered. The roundtable participants could envision a “hypothesis-free” diagnostic role for portable sequencing in both in the clinic and in the field. (Here, “hypothesis-free” refers to sequencing DNA from a patient without a prior hypothesis that a patient is infected with a specific pathogen.) However, correct diagnoses would require access to accurate reference datasets of pathogen genomes; these datasets are only now beginning to be compiled. The cost of sequencing, establishing the route to reimbursement, and FDA clearance/approval issues are also challenges that need to be overcome.
- 8) **Market forces alone aren’t sufficient to get portable sequencing to usefulness during outbreaks.** There is a key role for USG in advancing sequencing in the fight against infectious disease, but fully exploiting the capability will require real visionaries (especially to advance the possibility of hypothesis-free diagnostics). The US National Institutes of Health Biomedical Advanced Research and Development Authority (BARDA) is pioneering this approach by funding a British company, DNAE, to develop rapid DNA sequencing technology influenza diagnosis and detecting antibiotic resistance in bacteria. The same technology could easily be extended to diagnosis of other pathogens, making sequencing more accessible to non-technical users. BARDA’s strong working relationships with FDA should streamline the eventual approval process for clinical application.
- 9) **Other use cases for portable sequencing.** Other use cases for portable sequencing outside of combatting infectious disease are evident and will contribute to a market that sustains the capability. They include soil characterization for agricultural purposes, forensics, authentication, supply chain verification, customs enforcement, routine clinical disease diagnosis (including the detection of antibiotic-resistant bacteria and bacteria/virus discrimination), food safety, IP protection and biometrics. We will be discussing some of these in additional detail in a paper (*in draft*) on portable sequencing applications.

## Conclusions –

1. Portable DNA sequencers have tremendous potential as 1) a discovery tool for new pathogens, 2) a source of near-real-time genomic evolution data during outbreaks, and 3) a potent diagnostic tool that could be used in clinical applications ranging from triage to confirmatory testing to assessing the clinical presence of the pathogen post-treatment. Some of these applications have already been explored in limited ways and show promise.
2. Nanopore devices bring practical fieldable sequencing close to reality. However, even though the sequencers fit in one's hand, they still require one or more suitcases of support technology to operate, and at least some technical expertise. However, the instruments required for sample and library preparation are also shrinking in both size and cost, giving hope that the complete "end-to-end" field sequencing capability will be hand-portable within a few years, at a price point that makes more applications practical.
3. Despite the precipitous drop in the cost of DNA sequencing that has taken place over the last 15 years, the cost of sequencing a sample in the field remains high relative to other diagnostic technologies (such as immunochromatographic test strips, which work like pregnancy tests). The use of sequencing in the field therefore will remain a challenge in resource-constrained areas (such as West Africa) until either technical advances further reduce costs, or private and public donors prioritize funding for cutting edge molecular diagnostics.
4. Regulatory issues remain, and behind them lurk all of the business hurdles inherent to new diagnostic technologies: low return on investment, uncertain reimbursement structures, and the need to educate users (from clinical labs to the bedside) in their operation and the interpretation of sequence data. Public health could leverage a data stream from in-clinic use of portable sequencers, but getting portable DNA sequencing into the clinical setting will require its approval as a diagnostic technology. The regulatory environment is slowly evolving to cover this technology; a recent example (Dec 2016) of a next generation sequencing test receiving FDA approval for use as a companion diagnostic is FoundationFocus™ CD<sub>X</sub>BRCA from Foundation Medicine for the qualitative detection of *BRCA 1/2* alterations for ovarian cancer therapeutics<sup>1</sup> and the recently approved Oncomine Dx Target Test from Thermo Fisher<sup>2</sup>.
5. The exploitation of portable sequencing in the field during epidemics urgently requires new tools for collaboration among operators at widely dispersed locations. One example of such a tool is Nextstrain (nextstrain.org), which is an effort to create a portal that can allow scientists to analyze and dynamically visualize new data as they are received from

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<sup>1</sup> <http://investors.foundationmedicine.com/releasedetail.cfm?ReleaseID=1004896>

<sup>2</sup> <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P160045>

fielded DNA sequencers. Such portals should also facilitate the distribution of updated information based on near-real-time genome evolution tracking.