

Abstract

Portable and reliable molecular epidemiology techniques and field approaches for assessing viral genomes are necessary to protect forward deployed forces, and this need has been highlighted by the recent Ebola epidemic. To determine if the MinION™ can act as a viral genome sequencer, cDNA from RNA virus infected cells will be sequenced using various concentrations of starting material and multiple sequencer run times. These experiments will test if the current and future versions of nanopore sequencing technology can be used to rapidly identify and characterize pathogens in samples that might be encountered in the field by forward deployed personnel. To accomplish the evaluation of nanopore sequencing for rapid, field deployable pathogen characterization, raw read data and statistics were collected for Ebola virus and Venezuelan Equine Encephalitis virus (VEE) sequence runs on the MinION™. These reads were then mapped to the genome of Ebola virus and VEE to determine the level of identification and the accuracy of genome characterization. The results obtained here represent novel methodology and evaluation, and will help ECBC to continue its work on fieldable technology for warfighter chem/bio protection.

MinION™ Nanopore Sequencer

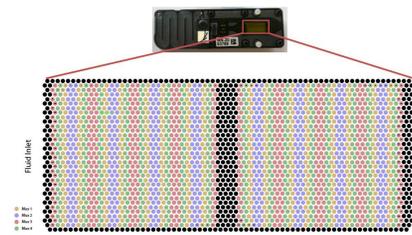
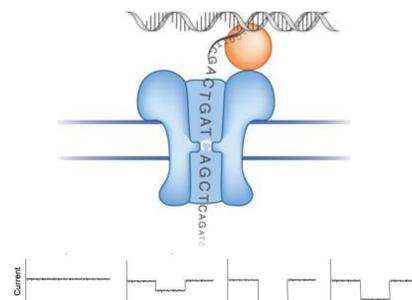


Figure 1. The MinION™ Nanopore Sequencer. Nanopore sequencing is the newest advance in next-generation sequencing technology. By utilizing protein nanopores that allow the passage of a single strand of DNA, the MinION™ from Oxford Nanopore reads the electronic signature generated from nucleotides passing through the nanopore. This technology does not require “sequencing by synthesis”, a technology relying on polymerases amplifying DNA using fluorescently tagged nucleotides. Nanopore sequencing significantly reduces the complexity of the sequencing device, with the palm-sized MinION™ being able to be plugged into a laptop via USB. Library preparation is easier, and the reagents necessary to sequence DNA are cheaper. Most importantly, nanopore sequencing can provide the sequences directly from the nucleotides within a sample. This removes amplification errors and bias that occur using other “sequencing by synthesis” technologies.

Approved for Public Release

Experimental Design

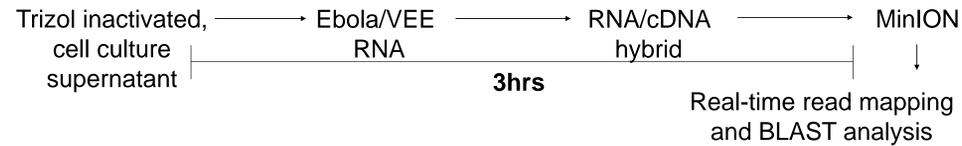


Figure 2. Sample Preparation Workflow for Rapid Nanopore Sequencing. Trizol inactivated cell culture supernatant (Ebola) or concentrated viral preparations (VEE) are subjected to total RNA purification. Ebola virus RNA must then be poly-A tailed to accommodate the cDNA synthesis primers. Reverse transcription of one strand occurs, yielding an RNA/cDNA hybrid. This hybrid can then be directly sequenced without further cDNA synthesis or PCR. The real-time data is then mapped to reference sequences and analyzed using BLAST for strain-level similarity matching.

Results

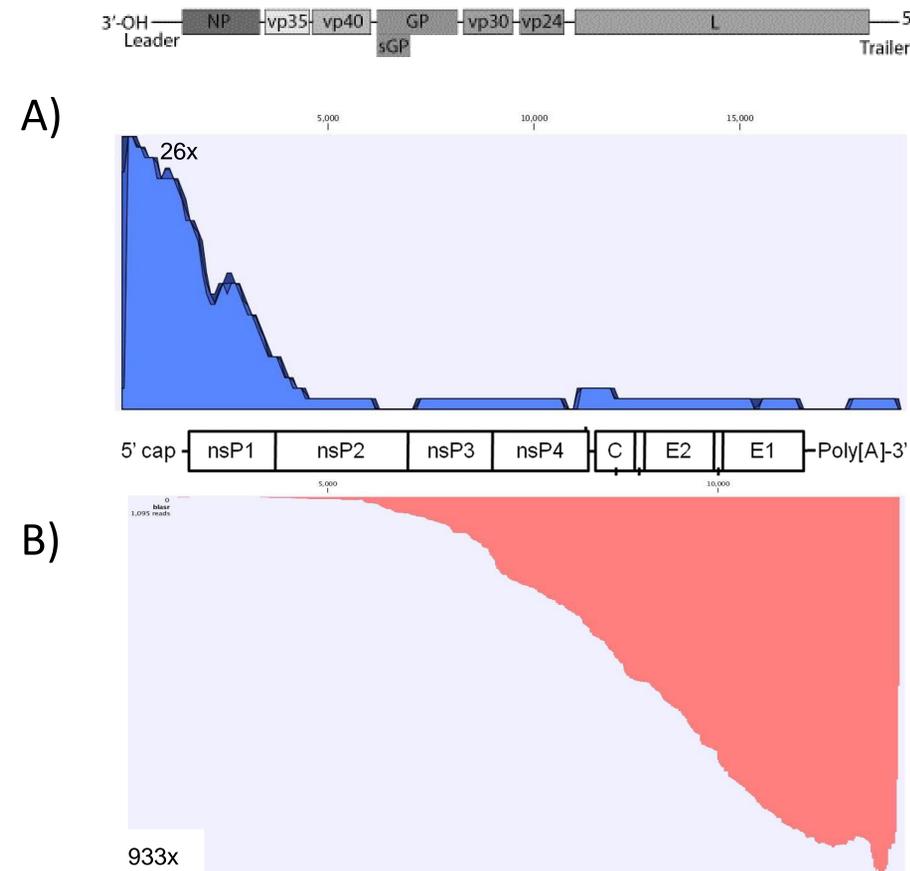


Figure 3. Genome Coverage of Ebola and VEE Virus Sequencing. A) Ebola virus RNA was first poly-A tailed on the 3' end of the genome, allowing for RT priming at that location. The RNA from the Trizol inactivated stocks was of poor quality, affecting the sequence yield from these preparations. However, there was still adequate coverage from the 3' end to identify the virus to strain-level. B) VEE sequencing did not require poly-A tailing, as the genome of VEE already contains a poly-A tail. These sequencing runs yielded excellent coverage depth and allowed for very accurate characterization. As sample prep methods improve, we predict full genome reads read by the MinION™.

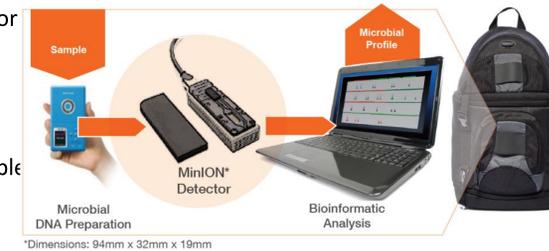
Conclusions

Nanopore sequencing continues to push the limits of fieldable sequencing technology, and ECBC has placed itself even with industry and academic laboratories in evaluating this technology for a variety of applications. Here we determined that the MinION™ nanopore sequencer is capable of viral genome sequencing from cultured samples. Our collective work has led for us to determine that:

- Nanopore sequencing has a variety of applications even in its current “alpha” TRL
- Limited library prep is attractive to fieldable applications
- Amplicon nanopore sequencing is a useful way to get coverage depth to accurately ID pathogens within complex sample backgrounds
- Unbiased, unamplified techniques can ID pathogens rapidly, and will only become more to be useful as technology matures
- Sequences are generated in real time, data can be analyzed in real time

Future Directions

These project funds have allowed for the generation of preliminary data to support a larger-scale proposal. ECBC is targeting SOCOM/DTRA as potential partners in this research with the aim of developing a fieldable platform that requires little input from the operator and does not require stringent cold-chain. DSTL in the UK has also expressed interest in working together, as they are developing software platforms that would be user-friendly for the operator in the field. The ability to rapidly characterize and ID pathogens in an unbiased manner does not exist within the DoD, so harnessing and evaluating emerging technology quickly is essential to ensuring the greatest level of chem/bio threat detection for our deployed forces.



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