Bioaerosol Surveillance via Untargeted Nanopore Sequencing

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Abstract

In the future, the ideal biological surveillance technology is a fully automated and untargeted sequencing device for continuous aerosol monitoring for both indoor and outdoor locations. This sequencing system would enable the identification of any biological threat (threat agnostic) including those that are emerging or genetically modified. Required modules for this system include an aerosol collector, a sample/library preparation module, the sequencer itself, and computational hardware. These modules could be upgraded based on continued technological advances and they could also be combined with other biological surveillance technologies, if desired, such as biological detectors/triggers or PCR technologies.

In addition to being small, inexpensive, and portable, nanopore sequencing technology lends itself to automation and recent progress has been made in this area. Nanopore sequencing workflows have also been developed for biothreat identification from aerosol samples. However, much more progress is needed, especially when it comes to understanding the natural microbial background in air. This can only be accomplished using large-scale, international collaboration since the microbial background varies with location, season, time of day, and fluctuations in weather among other factors. A proof-of-concept study was conducted to monitor the bioaerosol background in two locations: a mid-Atlantic site (Aberdeen Proving Ground, Maryland) and a desert site (Dugway Proving Ground, Utah). In collaboration with NATO, future studies will aim to characterize the background at many different locations around the world. Knowing the natural biological background will reduce false alarms and allow for the detection of true biological anomalies. Another focus area is on the bioinformatics analyses for metagenomic sequencing data generated from aerosol samples. How to effectively analyze, interpret, and present the data is a difficult challenge. Microbial standards are being developed and applied to effectively compare sequencing data that is generated by researchers across the globe. In addition, software tools are being developed to detect biological threats from nanopore sequencing data, irrespective of taxonomic identity.

Aerosol Background Pilot Study

- Proof-of-concept study for monitoring bioaerosol background via untargeted nanopore sequencing.
- Two samples locations: Aberdeen Proving Ground, MD and Dugway Proving Ground, UT.

Figure 1. Sample collection and monitoring equipment for aerosol background study.

- DFU and SASS 3100 air filter samples were eluted with PBS + 0.01% Triton via vortexing for 5 minutes in a 50 mL conical tube.
- Samples were lysed for 2 minutes using the OmniLyse device (Claremont BioSolutions).
- DNA was purified using the DNeasy PowerSoil Pro Kit (Qiagen) and DNA concentrations were determined by Qubit analysis.
- Library preparation was performed using the Rapid PCR Barcoding Kit (Oxford Nanopore Technologies).
- Samples were loaded onto a GridION sequencing instrument (Oxford Nanopore Technologies) and sequenced for 12–24 hours.
- Sequencing data was analyzed using the minimap2 aligner against the RefSeq database.
- For more details and results of this study, please see poster 287.

DHS BD21 Test Demo

- The BioDetection 21 (BD21) program is a DHS-sponsored investigation of potential improved capabilities to provide early and accurate detection of a biological attack and communicate the information to appropriate authorities enabling timely, well-informed decision making.
- A demo was conducted for the BD21 program to test the feasibility of utilizing untargeted nanopore sequencing technology for rapid bioaerosol surveillance.
- A probiotic solution was aerosolized via a nebulizer and aerosol samples were collected using an IBAC2. Samples were collected for 1, 3, and 7 minutes of exposure time.
- The air filter samples were cut into fifths and one of the filter sample pieces was eluted with PBS + 0.01% Triton via vortexing for 5 minutes in a 50 mL conical tube.
- Two different rapid sequencing protocols were tested.
- *Lacticaseibacillus rhamnosus* was correctly identified from the 1 minute air filter sample using both protocols.

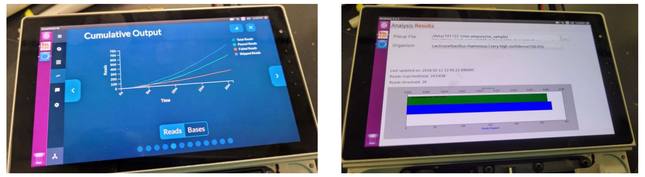

Figure 2. Results from highly rapid (< 90 min) probiotic air filter test displayed on MinION Mk1C. *Lacticaseibacillus rhamnosus* was identified from the 1 minute air filter sample in 31 minutes of sequencing time with very high confidence (100%).

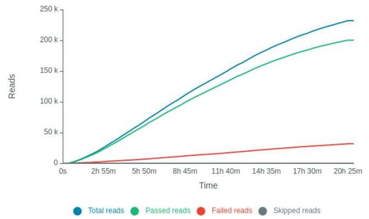

Figure 3. Sequencing results for probiotic air filter sample after ~20 hr of sequencing time. Sample was processed using OmniLyse for cell lysis, AMPure bead DNA purification, and the Rapid Sequencing Kit (Oxford Nanopore Technologies) for library preparation. Obtained 189,085 matching reads to *Lacticaseibacillus rhamnosus* using Centrifuge.

DARPA SIGMA+

- The DARPA SIGMA+ program aims to expand SIGMA's advance capability to detect illicit radioactive and nuclear materials by developing new sensors and networks that would alert authorities to chemical, biological, and explosives threats as well.
- SIGMA+ calls for the development of highly sensitive detectors and advanced intelligence analytics to detect minute traces of various substances related to weapons of mass destruction (WMD) threats. SIGMA+ uses a common network infrastructure and mobile sensing strategy.
- DEVCOM CBC has been serving as subject matter experts for the biological detection aspect of the DARPA SIGMA+ program, specifically the effort to develop a fully automated nanopore sequencing instrument for air samples.

IARPA Fun GCAT

- The Intelligence Advanced Research Projects Activity (IARPA) Functional Genomic and Computational Assessment of Threats (Fun GCAT) program developed software tools to rapidly assess the function of DNA sequences to determine if they pose a threat.
- DEVCOM CBC evaluated the Fun GCAT software tools for use with nanopore sequencing data.
- Downselected software was identified for future development and incorporation into bioinformatics workflows and nanopore sequencing systems.

NATO Exploratory Team

- Representatives from DEVCOM CBC are currently leading a NATO Exploratory Team on the topic of Nanopore Sequencing for Biological Identification.
- The Exploratory Team phase is a one year effort followed by a three year Research Task Group (RTG) phase. The focus of the RTG phase will be on biological aerosol background monitoring and metagenomic analyses.

Conclusions/Future Work

- The aerosol background pilot study provided a proof of concept and design which will be utilized for future larger-scale collections/analyses, including international environmental background studies with NATO collaborators.
- The DHS BD21 test demo showed that non-complex biological aerosol samples can be rapidly processed and analyzed via nanopore sequencing protocols/systems.
- A sustained collaborative and holistic DoD/DHS effort will be needed for the implementation of sequencing technology for environmental aerosol surveillance. There needs to be a focus on standards development and metagenomic analyses.

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