

Development of Untargeted Nanopore-Based Systems for Agnostic Biological Threat Identification in the Field

Adina Doyle¹, Maria Arévalo², Nicholas Rhodes¹, Mark Karavis², Pierce Roth³, Alvin Liem³, Rebecca Hutchins⁴, Michael Cato⁴, Jessica Hill³, Jacquelyn Harris², Sarah Katoski², Samir Deshpande², Meni Wanunu⁴, and R. Cory Bernhards¹

¹Precise Systems, Lexington Park, MD, ²U.S. Army Combat Capabilities Development Command Chemical Biological Center, Aberdeen Proving Ground, MD, ³DCS Corporation, Belcamp, MD, ⁴Georgia Tech Research Institute, Atlanta, GA, ⁵Northeastern University, Boston, MA

Abstract

Nanopore sequencing technology has enabled numerous applications for rapidly identifying and characterizing biological threats, including those that are emerging and/or genetically modified. Systems have been developed for use in far-forward and mobile laboratory environments. Military operators are being trained to execute DNA and RNA sequencing protocols, which will revolutionize biothreat identification in the field. Methods for sample and library preparation have been simplified and are being automated for use by non-laboratory trained operators, and bioinformatics software has been designed to automatically identify biothreats as the sequencer is running. Once the bioinformatics software reports the result to the operator, additional software has been designed to immediately send the result to the command center and for integrating into various command and control networks and architectures to enable situational awareness and informed decision making. An additional benefit with these systems is that they can be used on-the-move, which broadens the concept of operations (CONOPS) aperture. In addition, recent advancements have enabled the use of nanopore technology for untargeted protein identification, which can be applied to DNA-based threats, RNA-based threats, and toxins, which would serve as an all-in-one agnostic biothreat identifier.

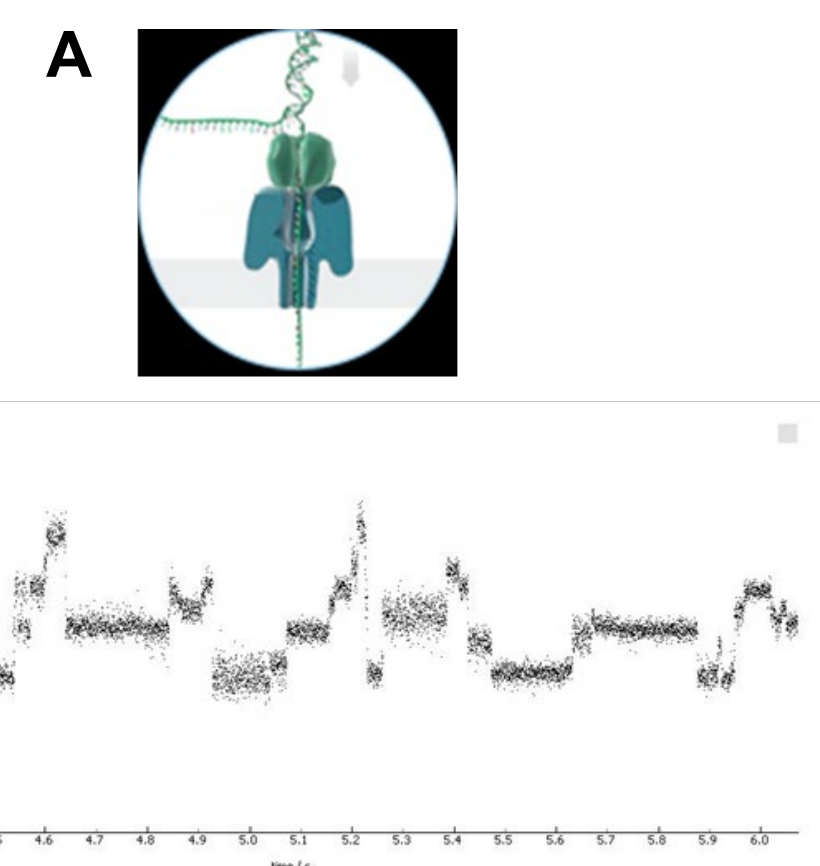


Figure 1. Nanopore Sequencing. **A)** Single-stranded DNA molecule passing through a biological nanopore embedded in an artificial membrane with an applied current. The current disruption is measured to determine what DNA bases (A, C, T, G) are passing through the nanopore in real time.



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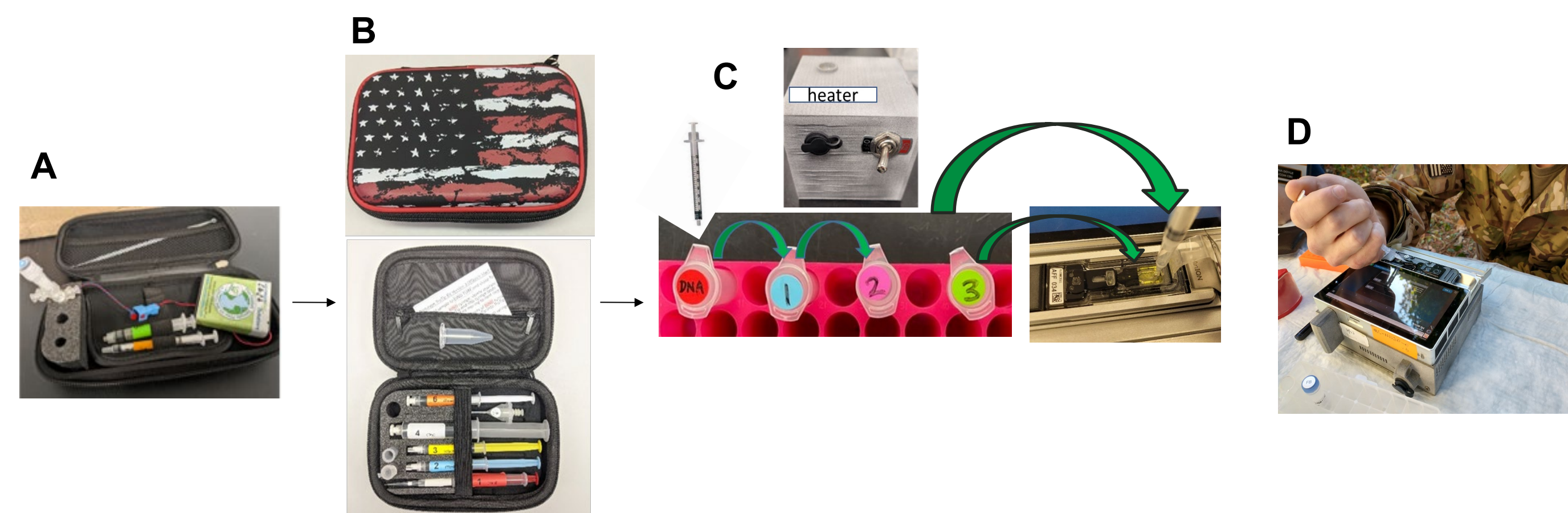


Figure 2. Rapid, Far-Forward DNA Sequencing System. For high biomass, low background sample types (bacteria and DNA viruses). Uses a syringe-based system for liquid movement to remove the need for pipettes. Sample to answer time < 30 minutes. **A)** Sample is physically lysed using modified OmniLyse device (Claremont BioSolutions). **B)** Syringe-based rapid DNA purification via custom TruTip Kit (Akonni Biosystems). **C)** DNA is prepared for sequencing using the pre-prepared, streamlined Rapid Sequencing Kit (Oxford Nanopore Technologies) and portable heater. **D)** Sequencing is performed on a modified MinION Mk1C (Oxford Nanopore Technologies) with an integrated battery. Data analysis for organism ID is performed while the sequencer is running using custom Realtime software on the Mk1C. An ISA message can be sent to command once the organism is identified via an MPU5 radio.



Figure 3. Extreme Hot Weather Testing. The DNA sequencing system was field tested by military operators at Dugway Proving Ground, Utah in July 2022 at an outdoor temperature of 100°F.

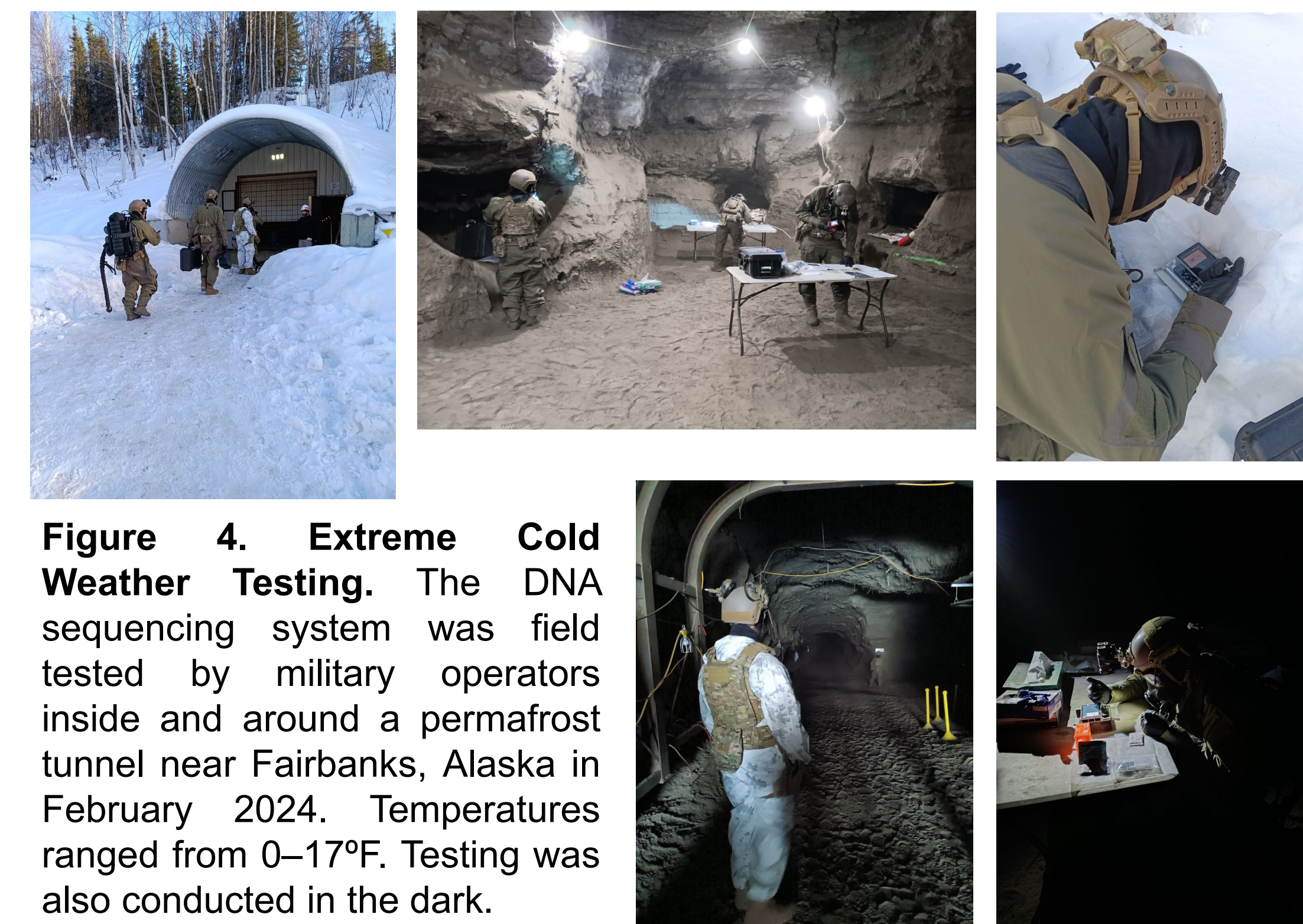


Figure 4. Extreme Cold Weather Testing. The DNA sequencing system was field tested by military operators inside and around a permafrost tunnel near Fairbanks, Alaska in February 2024. Temperatures ranged from 0–17°F. Testing was also conducted in the dark.



Figure 5. Automated Library Preparation Device. Automated library preparation prototype developed by Georgia Tech Research Institute (GTRI). The operator loads the purified DNA sample into a cartridge then places the cartridge into the slot and presses the start button. After the automated library preparation process is complete, the sample is removed and loaded onto the MinION sequencer.

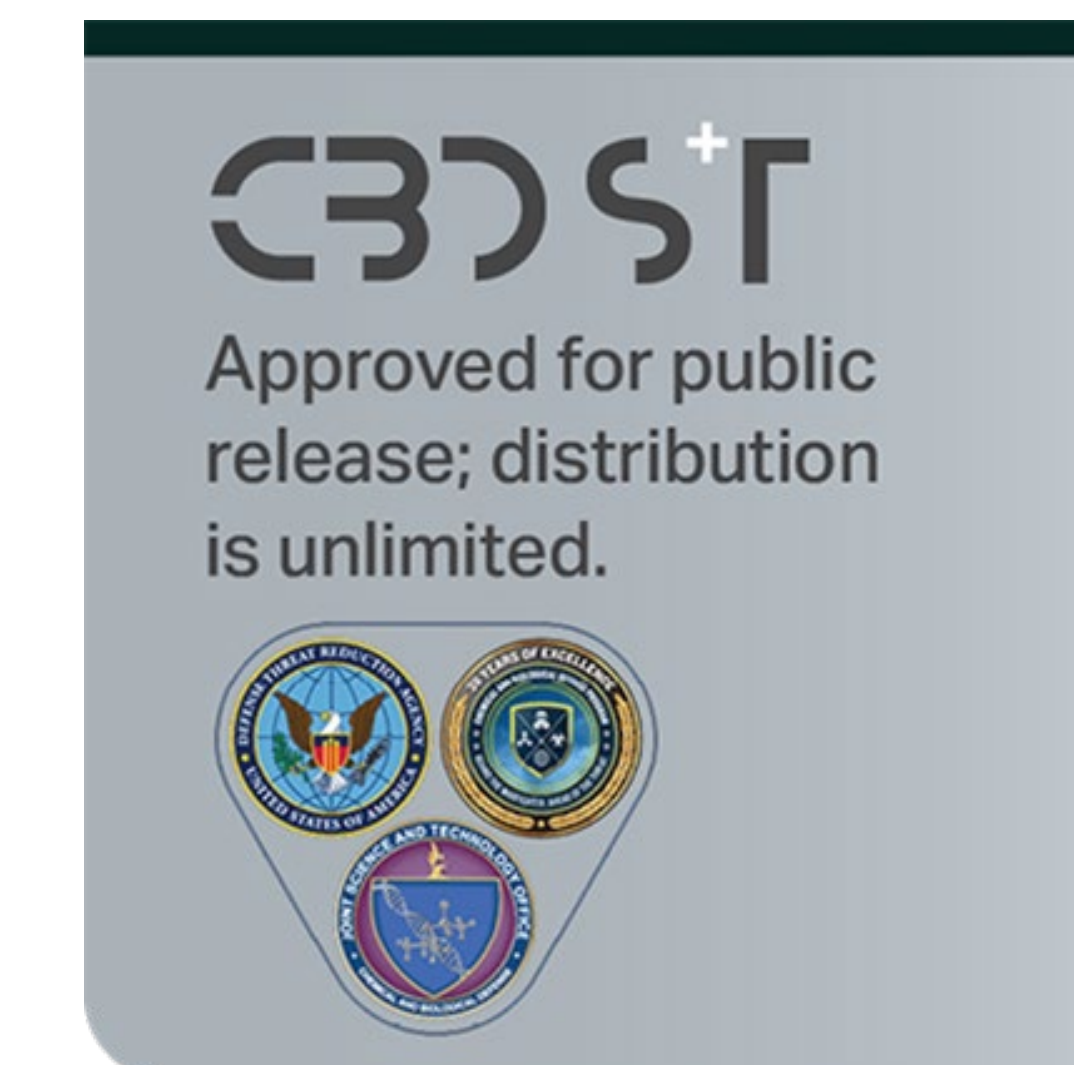


Figure 6. MinION Mk1D. DEVCBC CBC beta tested the new MinION Mk1D sequencer (Oxford Nanopore Technologies) paired with a ruggedized tablet.

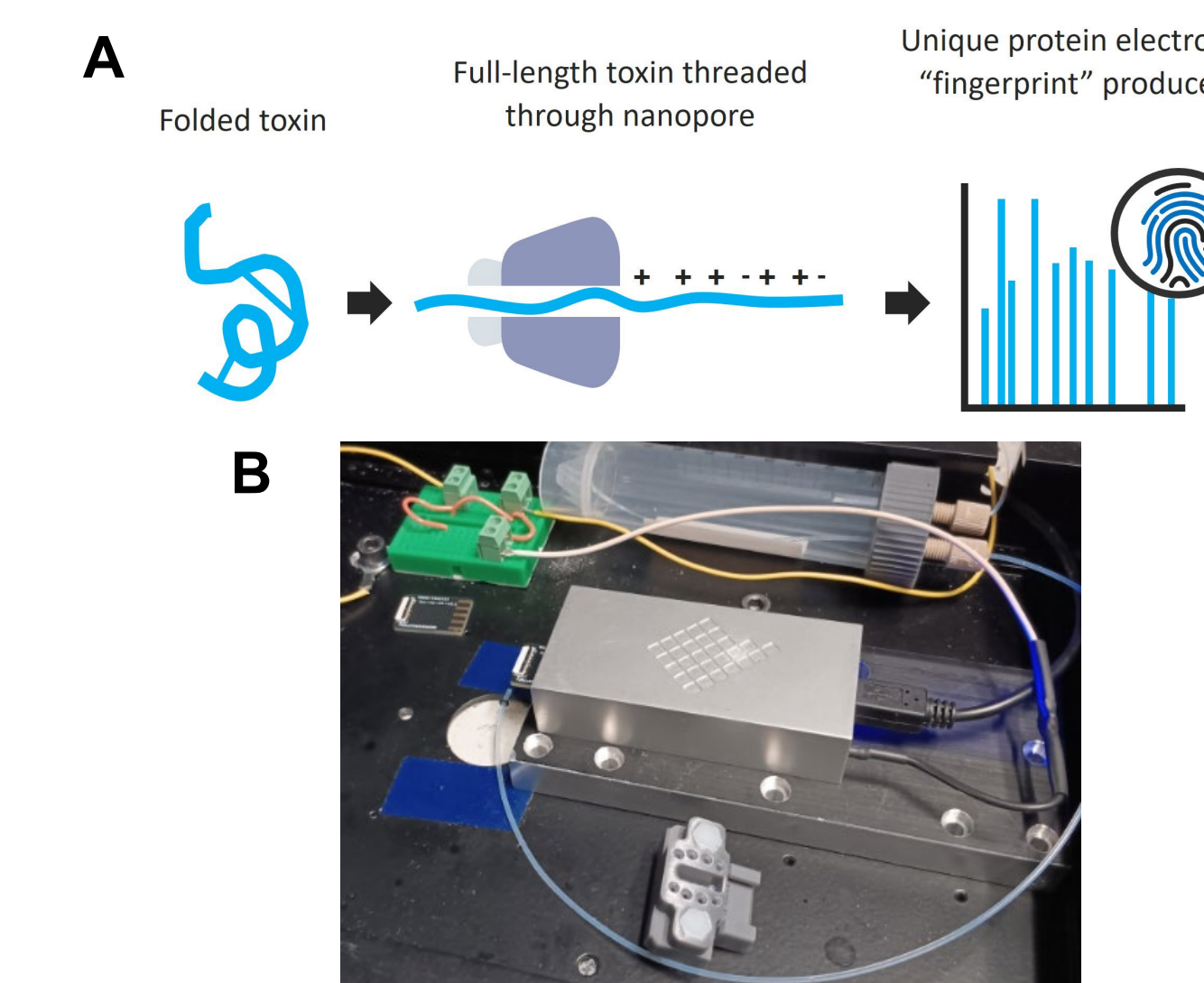


Figure 7. Protein Toxin ID via Nanopore Technology. DEVCBC CBC is partnering with Northeastern University to develop a fieldable nanopore-based device for agnostic protein toxin identification. **A)** A protein toxin is denatured and threaded through a biological nanopore to generate an electronic fingerprint, which can be matched to a toxin signature database. **B)** Small, nanopore-based prototype for protein identification developed by Northeastern University.

Conclusions/Next Steps

- The DNA sequencing system/protocol has been transitioned to JPEO-CBRND, JPM CBRN SOF for advanced development.
- Sample prep automation for sequencing is being continued by the SOFWERX G-NOMES program.
- For protein toxin ID, DEVCBC CBC will be setting up a testing platform and begin analyzing large protein toxins, followed by small protein/peptide toxins.

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