National Guard Civil Support Team (CST) Training for Nanopore Sequencing Workflows

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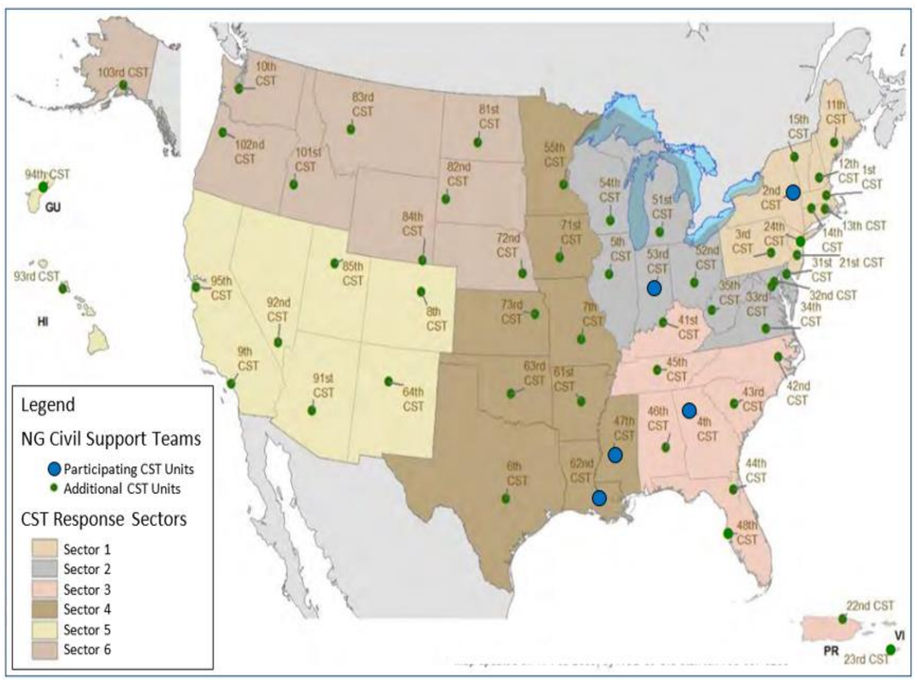
Introduction

Rapid and accurate biological identification technologies are critically needed in the field especially for unknown, emerging, and genetically modified biothreats. Next-generation sequencing (NGS) technologies are superior to other molecular-based identification methods because entire genomes can be analyzed. This allows for unbiased, conclusive identification of biological agents, including novel and synthetically modified threats. DEVCOM CBC is developing rapid and simplified workflows using MinION nanopore sequencing systems from Oxford Nanopore Technologies (ONT) for use by first responders and warfighters.

Abstract

In October of 2021, DEVCOM CBC hosted a five-day Sequencing Experiment Training Event for the National Guard Civil Support Teams (CSTs). The overall goal of this event was to gauge the impact nanopore sequencing technologies would have on the CST's mission and determine how the ability to sequence samples in the field would expand their current capabilities. Equipping them with a sequencing platform would exponentially increase their ability to identify emerging threats, determine antibiotic resistant biothreats, and identify genetically modified organisms with enhanced virulence. For this training event, five CST units were selected by the National Guard Bureau (NGB) to participate. Each CST unit consisted of one science officer and one enlisted technician for a total of ten participants. The CST members received hands-on training on nucleic acid extraction, RNA reverse transcription and library preparation protocols. They also learned how to prime and load a MinION flow cell and use REALTIME analysis software to identify organisms in the sample in near real-time. Participants were first trained on our DNA workflow to familiarize them with basic protocols. After mastering the DNA workflow, participants were then trained on our RNA workflow and finally our combined workflow, which can be used to analyze unknown samples. After workflow training, participants were given a blinded sample containing DNA bacteria and RNA virus to demonstrate their independent mastery of the workflows. After the event, DEVCOM CBC sent the CSTs all the materials, reagents and instruments needed to perform our workflows in their labs. The CSTs were also sent monthly blinded proficiency samples to sequence and report their results back to DEVCOM CBC. Having a field sequencing capability could potentially distinguish CSTs as the most capable biothreat identification personnel in the entire DoD.

National Guard CST Locations



2nd CST - New York
4th CST – Georgia
47th CST – Mississippi
53nd CST – Indiana
62nd CST - Louisiana

Combined Workflow for Bacterial and Viral Samples

OmniLyse Cell Lysis Kit

DNA Sample → OmniLyse – Cell/spore Lysis → AMPure XP Bead Clean Up

RNA Sample → Direct-zol RNA MicroPrep → LunaScript RT SuperMix Reverse Transcription → AMPure XP Bead Clean Up

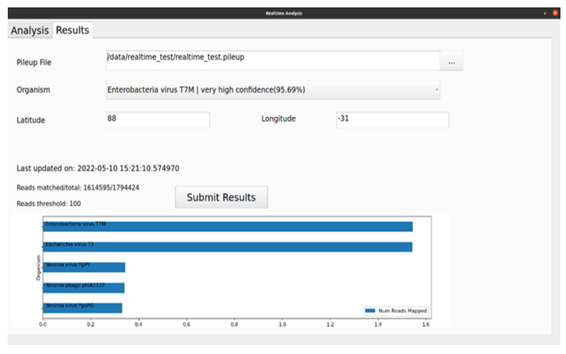
→ Rapid Sequencing Kit (SQK-RAD004) → MinION Sequencing Using a R9 Flow Cell on a Mk1B → REALTIME Data Analysis

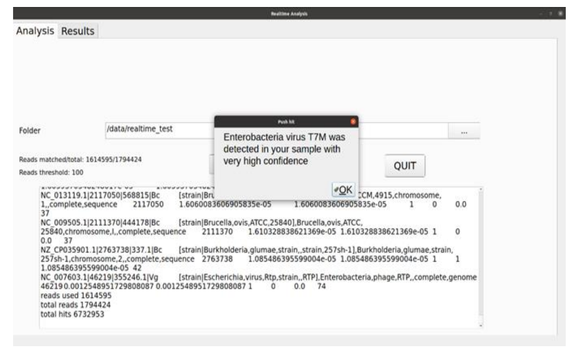
MinION R9 Flow Cell

MinION Mk1B

REALTIME Analysis Software

- After the MinKNOW software basecalls a predetermined number of reads, a FASTQ file is made.
- The REALTIME software then uses minimap2 with a modified RefSeq database to map the reads.
- As the run continues, more FASTQ files are generated and reads are mapped in near real-time.
- Once the number of mapped reads crosses a threshold, a pop-up window identifying the organism will appear.





Initial Proficiency Sample Set Results

CST	Sample	Organism	REALTIME Software Identification	Correct ID ?
2nd CST New York	Sample 1	BTK	*Bacillus thuringiensis*	✓
	Sample 2	MS2	*Escherichia* phage MS2	✓
	Sample 3	T3	*Enterobacteria* phage T3	✓
	Sample 4	B. subtilis	Laptop Failure	NA
4th CST Georgia	Sample 1	BTK	*Bacillus thuringiensis*	✓
	Sample 2	MS2	*Escherichia* phage MS2	✓
	Sample 3	T3	T7M (T3 2nd hit)	✗
	Sample 4	B. subtilis	Out of Reagents	NA
47th CST Mississippi	Sample 1	BTK	*Bacillus thuringiensis*	✓
	Sample 2	MS2	*Escherichia* phage MS2	✓
	Sample 3	T3	*Enterobacteria* phage T3	✓
	Sample 4	B. subtilis	*Bacillus intestinalis* *	✗
53rd CST Indiana	Sample 1	BTK	*Bacillus thuringiensis*	✓
	Sample 2	MS2	*Escherichia* phage MS2	✓
	Sample 3	T3	No Data	NA
	Sample 4	B. subtilis	*Bacillus intestinalis* *	✗
62nd CST Louisiana	Sample 1	BTK	*Bacillus thuringiensis*	✓
	Sample 2	MS2	*Escherichia* phage MS2	✓
	Sample 3	T3	*Enterobacteria* phage T3	✓
	Sample 4	B. subtilis	*Bacillus intestinalis* *	✗

* *Bacillus intestinalis* was the top hit but followed closely by 3 *Bacillus subtilis* strains.

CST Training Summary & Lessons Learned

- By the end of the training, CST personnel were able to independently use the workflows to successfully identify blinded samples including samples containing both bacteria and viruses.
- Participants made suggestions to help improve our protocols and training methods, such as an addition of a positive control and an in-depth explanation of the science of nanopore sequencing.
- CST personnel stated the training event was very informative and mission-relevant.

Future Exercises and Objectives

- Next year, the original ten participants will return for training on more complex samples.
- Five new CST units will receive the initial training and analyze proficiency samples.
- DEVCOM CBC will continue to develop their workflows to incorporate new technologies.

Acknowledgements

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