091-E

Ultra-Portable Technologies for On-Site Arbovirus Surveillance From Mosquitoes: Bringing the Lab to the Sample

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Introduction

Recently developed molecular analysis platforms offer the promise of bringing comprehensive detection, diagnostics, and biosurveillance of emerging infectious diseases to extremely remote and austere locations, completely detached from a traditional brick and mortar laboratory. For example, Biomeme's two3 qPCR machine and the MinION from Oxford Nanopore Technologies (ONT) (Figure 1) offer target amplification and long-read sequencing capability, respectively, in an ultracompact form factor that is amenable to transport and use by a single operator. Additionally, Biomeme offers nucleic acid extraction kits that can be adapted to different matrices, do not require pipettes, and can be completed in approximately ten minutes. We tested a combination of these technologies in an arbovirus surveillance project monitoring *Culex cedecei* mosquitoes captured at several sites near Everglades National Park. This work is the first demonstration, to our knowledge, of direct, non-PCR-based, metatranscriptomic detection of an RNA virus from mosquitoes using nanopore sequencing. Additionally, this work shows the feasibility of a backpack-enabled workflow for field-able, genomics-based biosurveillance.

Methods

C. cedecei mosquitoes were collected via CO2/light traps and sorted into pools of 25 females. Twenty pools (500 mosquitoes) were processed through Biomeme's bulk nucleic acid extraction developer kit with a modified protocol. Extracted RNA was processed through the GeneReads rRNA depletion kit in an attempt to reduce host mosquito signal in sequencing reads. Extracted RNA was queried with pan-*Flavivirus* and Everglades Encephalitis Virus (EVEV) qPCR assays on the Biomeme two3 device and the BioRad CFX96 Touch Realtime PCR system. Whole transcriptome amplification (WTA) of virus-positive samples was performed using the REPLI-g WTA single cell kit. Resulting cDNA was sequenced on the Oxford Nanopore (using Rapid 1D sequencing kit) and an Illumina MiSeq machine using a 150bp paired-end library.

Methods Cont'd

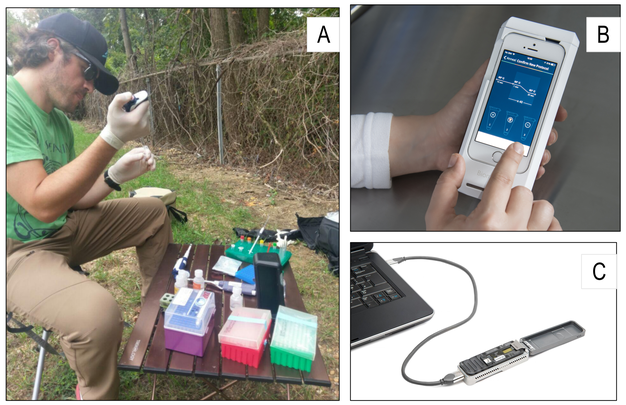


Figure 1: Extracting total RNA from mosquito pools in the field (A) and readying for analysis on the Biomeme two3 Thermocycler (B). Resulting amplicons can be sequenced on the MinION from ONT (C) for strain-typing of any detected viral pathogen. Alternatively, total RNA can be converted to cDNA and sequenced on the MinION for direct meta-transcriptomic detection of mosquito-born arbovirus. ***The direct meta-transcriptomic approach was used in this study.***

GeneRead-depleted, WTA'd cDNA sequenced on Nanopore:

- 1D SQK-LSK108 Library prep kit
- R9 (FLO-MIN105) flow cell
- Sequenced overnight (~12 hours)
- 30,422 reads generated
- 8086 high-QC reads aligned to database of 154 VEEV/EVEV genomes using NanoOK
- **5 reads mapped to two genomes**: ***Same two Everglades virus strains*** that were highly represented in MiSeq data.

Nanopore Read	Genome mapped to	Alignment Size	Alignment Percent Identity	Query Percent Identity
read5399	KR260737	2109	77.86	63.74
read193	KR260737	1218	80.54	47.83
read1199	KR260737	1104	73.64	43.64
read6407	KR260737	249	77.11	13.74
read3293	AF075251	328	79.57	59.18

Table 1: Out of 8,086 high-quality MinION reads analyzed, **five (5) MinION reads successfully mapped to EVG3-95 or Fe3-7c.**

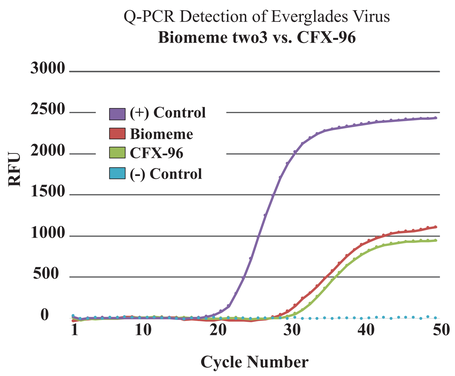


Figure 2: Comparison of Biomeme two3 performance with that of the Bio-Rad CFX-96, a "Gold Standard" q-PCR machine. Biomeme reported a Cq value of 33.92 for EVEV(+) sample well. CFX-96 reported a Cq value of 30.63.

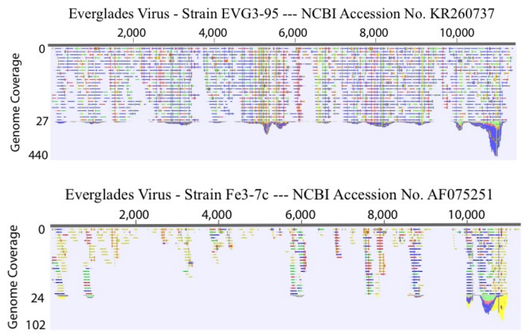


Figure 3: Genome coverage of Everglades Virus strains EVG3-95 (left) and Fe3-7c (right) from Illumina MiSeq sequencing of EVEV(+) mosquito-pool RNA. 3.21 million reads were generated. Of these reads, 12,680 reads successfully mapped to a curated list of 154 VEEV and EVEV genomes. **10,549 (>83%) of these reads mapped to strains EVG3-95 and Fe3-7c**.

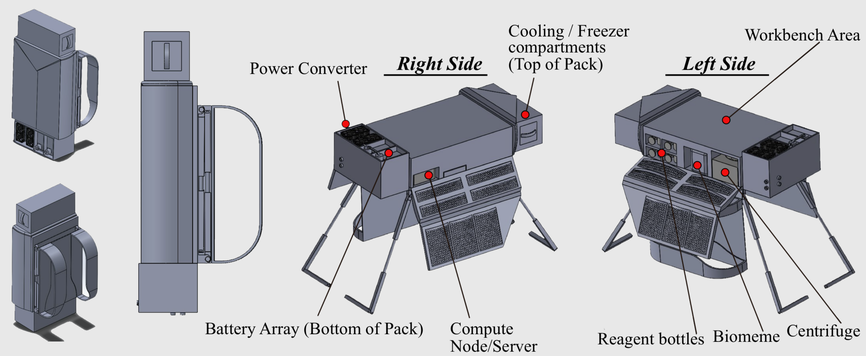
Results and Future Work

- Robust extraction of RNA from mosquito–pools for purposes of arbovirus surveillance using Biomeme's ultra portable Bulk Nucleic Acid Extraction kit (**mean RNA concentration from 25 mosquito pool: 1.02 ng/µl**)
- Effective detection of low-titer arbovirus (Everglades Virus) from single 25-mosquito sample pool using Biomeme two3 q-PCR thermocycler. Results comparable to current 'gold standard' q-PCR platforms.
- **Nanopore sequencing capable of detecting RNA virus from complex sample matrix** after eukaryote rRNA depletion and whole transcriptome amplification reaction.
- Designing and building modular systems for real-time, point-of-care molecular microbiology (see below)

Mercury Lab

- Fully-integrated, self-contained laboratory environment for fielding of hand-held genomics devices by a single operator
- Integrated 4ºC and -20ºC storage for samples, reagents, and consumable hardware (e.g., MinION flowcells).
- Integrated power for ~72 hours of nominal use in genomics-focused configuration
- Integrated computing with Intel NUC (32GB RAM, 2TB storage) for complex bioinformatics and cloud-based reach-back support

Mercury Lab – Backpack Molecular Microbiology Laboratory



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