

Development of a Genotyping Workflow for *Cyclospora cayetanensis* in Irrigation Waters Using Oxford Nanopore MinION Sequencing

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Cyclospora cayetanensis infections, characterized by watery diarrhea, vomiting, fatigue, and weight loss, have been increasing domestically in the United States in recent years. Transmission occurs via consumption of fresh produce contaminated with sporulated oocysts, which are then shed unsporulated into the environment. The presence of *C. cayetanensis* oocysts have been reported in irrigation waters, which are suspected to serve as a vehicle for transmission to fresh produce. However, there is a lack of a genotyping method to complement epidemiological investigations of outbreaks in a water matrix. The present study focused on developing a workflow to genotype *C. cayetanensis* in agricultural water samples of the Southeast United States using the Oxford Nanopore MinION. Taking a multi-locus sequence typing (MLST) approach, nine biomarkers (CDS-1, CDS-2, CDS-3, CDS-4, HC360i2, HC378, Mt Junction, MSR) developed by the Centers for Disease Control and Prevention (CDC) and the mit3PCR developed by the Food and Drug Administration (FDA) specific to *C. cayetanensis* were tested. Two rounds of Polymerase Chain Reaction (PCR) were conducted followed by gel electrophoresis and library preparation using the rapid sequencing DNA V14 kit (SQK-RBK114.96). With the Geneious sequencing software, a consensus sequence was obtained from both raw reads and assembled contigs following a map to reference step for each biomarker. Confirmation of *C. cayetanensis* in samples was performed with NCBI BLAST and utilization of a custom Kraken database with The University of Arizona's High Performance Computing (HPC) application. Comparison between consensus sequence from both reads and contigs were performed for accurate representation of each sample in preparation for concatenation and phylogenetic analysis. Successful *C. cayetanensis* sequencing in a water matrix with the MinION could reliably aid epidemiological traceback investigations of cyclosporiasis outbreaks as it would provide insights on environmental reservoirs and sources.