A method of rapid environmental sample preparation for real time PCR detection and quantification of HF183

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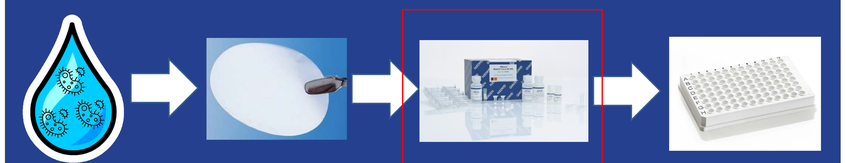
Introduction

- Measuring fecal indicator bacteria (FIB) is a major component of water quality monitoring
- Molecular based methods provide specific identification of species source of different FIBs
- Current standard for detecting human associated FIBs is the HF183 TaqMan quantitative polymerase chain reaction (qPCR) assay
  - DNA extraction is expensive, requires highly trained technicians, and time intensive
- **<u>Goal</u>: Design a method that is more cost effective, simpler, faster, and higher throughput for performing DNA extractions from environmental freshwater samples**

Current Method

Based on EPA Method 1696 (March 2019)



Collect Water Sample → Filter onto membrane to concentrate → Extract DNA using Qiagen DNeasy Kit → Run qPCR on sample with HF183 primers and probe

Bottleneck of method and expensive step

HF183 Primers and Probe

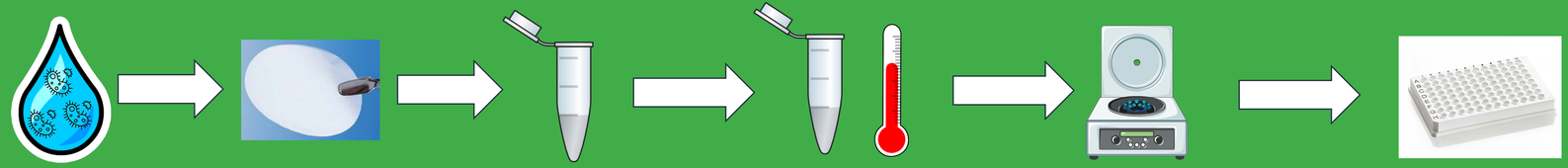
Forward: 5' – ATC ATG AGT TCA CAT GTC CG – 3'
Reverse: 5' – CGT AGG AGT TTG GAC CGT GT – 3'
Probe: 5' – [6FAM] - CTGAGAGGAAGGTCCCCCACATTGGA - (BHQ-1) – 3'

qPCR Assay Cycle Times and Temperatures

1. 10 minutes at 95°C for activation
2. 15 seconds at 95°C to denature
3. 1 minute at 60°C to anneal and extend then read plate

Repeat steps two and three 39 times for 40 total cycles

New Method



Collect Water Sample → Filter onto membrane to concentrate → Suspend filter in 500µL AE Buffer (10 mM Tris-Cl 0.5 mM EDTA; pH 9.0) → Heat solution at 95°C for 10 minutes then lightly vortex → Centrifuge at 12,000 x g for two minutes → Supernatant is template for qPCR

Results

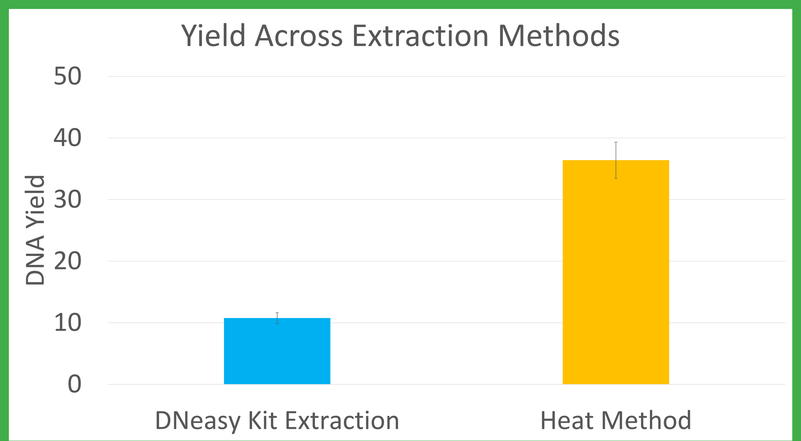


Figure 1. Overall yield of extraction using only heat as compared to using the Qiagen DNeasy Kit. Heat method shows significantly higher yield.

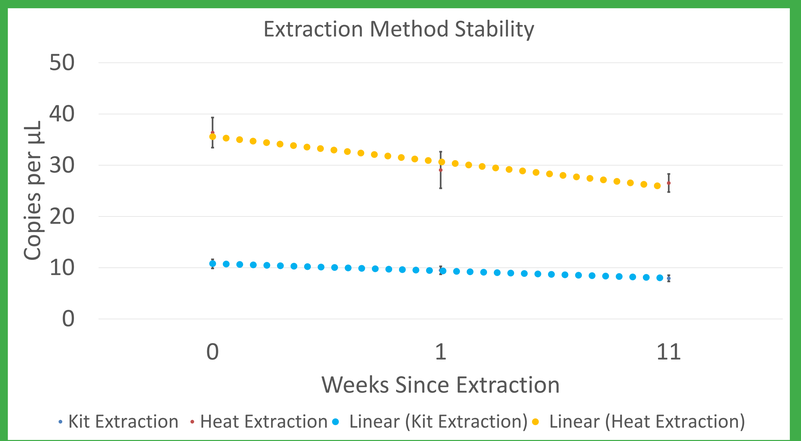


Figure 2. Overall stability of the two extraction methods over the course of 11 weeks. No statistically significant difference between week 0 and week 11.

Discussion

- Heat method is a viable alternative to a kit extraction that has comparable yield and maintains long term stability
- Heat method allows for significantly higher throughput and time savings than kit extraction
- Impurities from environment seem to not have an effect on qPCR reactions and don't cause DNA degredation

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Urban Ecosystems Stream Website

References and Additional Info