Promega

Product Application

Automated Purification of DNA from Microorganisms in Water Samples

Purify DNA from microorganisms present in water samples using the Maxwell® RSC Instrument and the Maxwell® RSC PureFood GMO and Authentication Kit.

Kit: Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

Analyses: Dye-based quantitation, qPCR and NGS

Sample Type(s): Water microorganisms captured on filters

Input: Water passed through a disc filter (volume to filter depends on biomass present in sample)

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM473 available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required:

- Polycarbonate disc filters with 0.2µm pore size (e.g. Whatman® Nuclepore™ Track-Etched Polycarbonate Membranes 47mm diameter, 0.2µm pore size, Millipore Sigma – Cat.# WHA111106)
- Filter apparatus (e.g. Millipore Classic Glass Filter Holder – Kit, 47mm, Glass frit membrane support, 300ml funnel, Millipore Sigma – Cat.# XX1014700)
- Vacuum pump
- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Heat block for 2ml tubes at 95°C and at 70°C

Protocol:

1. Filter desired amount of water by applying vacuum to collect microorganisms on filter in filter apparatus.
   Note: volume of water to filter depends on amount of biomass present in sample.
2. Transfer filters to 2ml microfuge tubes using clean, sterile forceps. It might be necessary to fold filters to fit them in the tubes.
3. Store filters at -80°C until ready to process or proceed to step 5 to process them fresh.
4. Remove filter from -80°C and bring to room temperature.
5. Add 1ml of CTAB Buffer to tube with filter and vortex for 30 seconds.
6. Incubate sample at 95°C for 5 minutes.
7. Remove sample from heat and allow to cool for 2 minutes at room temperature.
8. Vortex thoroughly for 1 minute. To facilitate processing, use a tube vortex adapter, such as a TurboMix vortex adapter.
9. Briefly centrifuge to bring volume to the bottom of the tube.
10. Add 40µl of Proteinase K and 20µl of RNase A to samples and vortex briefly to mix.
11. Incubate at 70°C for 10 minutes.
12. While heating, prepare the Maxwell® RSC cartridges.

a. Place cartridges in Maxwell® RSC cartridge rack and remove foil seals.
b. Add 100µl of Elution Buffer to supplied Elution Tubes and place tubes in cartridge rack.
c. Place plungers into well #8 of cartridge.
d. Add 300µl of Lysis Buffer into well #1 of cartridge.

13. After 70°C incubation, briefly centrifuge tubes to bring volume to the bottom of the tube.
14. Transfer 300µl of lysate to well #1 of the Maxwell® RSC cartridge.
15. Run Maxwell® RSC Instrument with the PureFood GMO Protocol, eluting in 100µl.
16. Store eluted DNA at 4°C (short-term) or -20°C (long-term).

Note: Depending on the biomass in the sample and the source of the water, it may be necessary to perform clean-up steps with PCR inhibitor removal columns, such as OneStep PCR Inhibitor Removal Kit (Zymo Research) prior to downstream amplification.

Clean-up for indicated samples (Fig. 3) was performed with Zymo Research OneStep PCR Inhibitor Removal Kit to determine effects on microbiome profiles by NGS.

Next-Generation Sequencing (NGS) Analysis of Microbiomes

Water DNA samples were analyzed with the LoopSeq™ 16S & 18S Low Biomass Microbiome SSC 24-plex Kit (Loop Genomics) to sequence full-length 16S and 18S rRNA genes. The library preparation was performed as indicated by the manufacturer, except for three modifications (1) sample concentration was measured by the QuantiFluor® ONE dsDNA System, (2) all clean-up steps in the workflow were performed with the ProNex® Size-Selective Purification System, and (3) library concentration was measured using the ProNex® NGS Library Quant Kit. Libraries were sequenced with 2x300 reads on an Illumina MiSeq instrument. Data were analyzed with the Loop Genomics bioinformatics workflow.

Results

Three independent purifications were performed from microorganisms collected on filters from four different water samples (Aquarium, 50ml; Feynman Pond, 50ml; Riva Pond, 10ml; Rain Barrel, 100ml). Yield of purified DNA per volume of water differed for each sample, reflecting the different amounts of biomass present (Fig. 1). DNA from filters prepared from the same water samples were also purified with Qiagen DNeasy® PowerWater® kit. Yields were similar to Maxwell® purifications (data not shown).

Purified DNA was amplifiable by qPCR with GoTaq® qPCR Master Mix (Cat.# A6001) using universal eubacterial primers at the DNA concentration required for NGS library preparation (Fig. 2). These DNA dilutions were also assessed for PCR inhibition by qPCR, and no appreciable inhibition was detected (data not shown).

NGS analyses of 16S and 18S rRNA genes revealed different microbiome profiles from each water type (Fig. 3). Eubacteria (92.2-95.6%) and eukarya were detected (4.4-7.8%), and organism abundance was specific to each water source. Microbiome profiles were consistent between water types, purification replicates and purification kits.

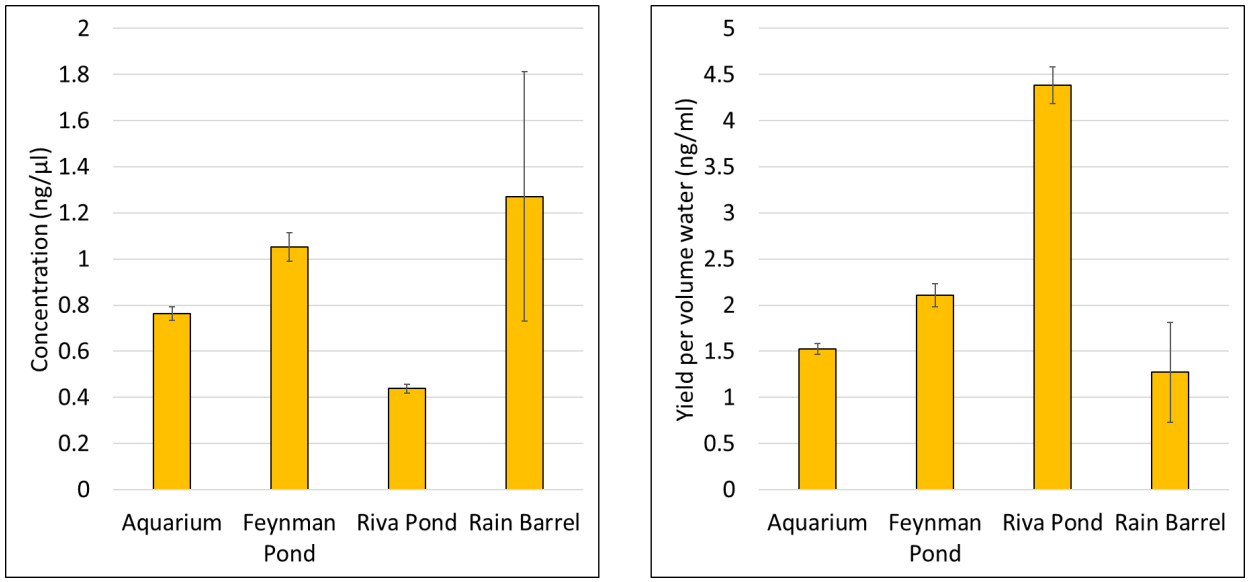

Figure 1. Concentration and yield of DNA from water samples using the Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600) on the Maxwell® RSC Instrument (Cat.# AS4500). Left, concentration measured by fluorescence with QuantiFluor® ONE dsDNA System (Cat.# E4871) on the Quantus™ Fluorometer (Cat.# E6150) relative to a Lambda DNA standard. Right, yield of DNA (ng) per volume of water (ml) filtered. Mean ± standard deviation (n=3) is shown for all measurements.

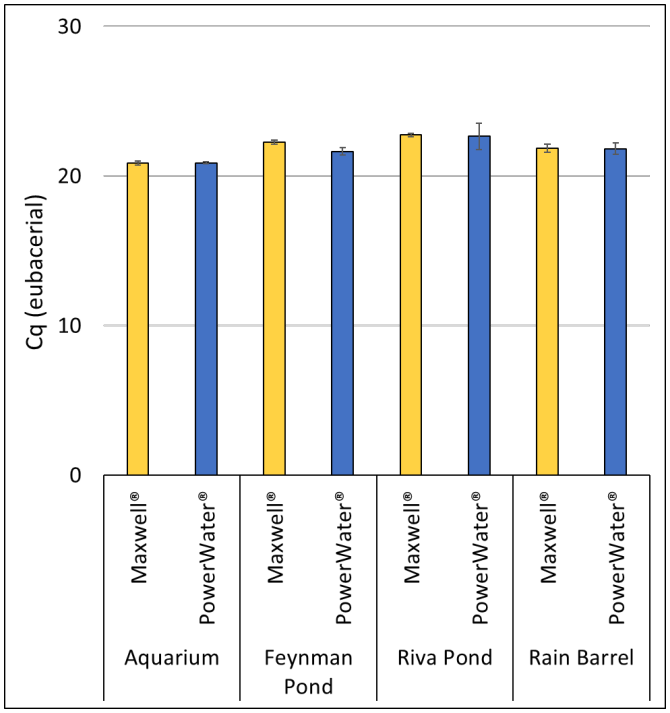

Figure 2. Amplification of eubacterial DNA by qPCR. DNA samples were diluted to 40pg/µl, and 2µl were amplified in duplicate reactions using universal eubacterial primers. The two Cq values were averaged for each purification replicate, and mean ± standard deviation is shown, n=3 (except for Feynman Pond PowerWater® samples, n=2). Gold, Maxwell® purifications; blue, PowerWater® purifications.

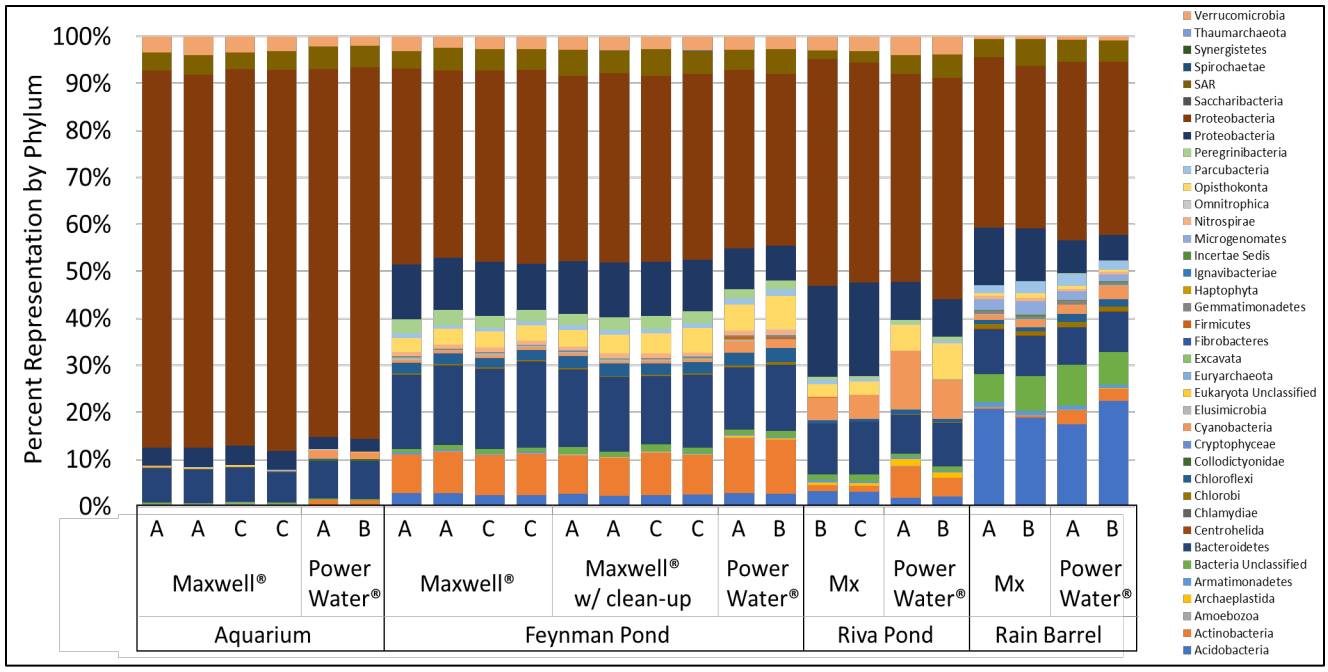

Figure 3. Analysis of water microbiomes by 16S and 18S rRNA gene sequencing. Phyla of organisms detected by analyzing LoopSeq™ 16S & 18S Low Biomass Microbiome SSC 24-plex Kit data are indicated by various colors (legend at the right side of the figure) and plotted relative to the water source, purification method (Maxwell®/Mx or PowerWater®) or replicate (A, B, C). Duplicate libraries were prepared from Maxwell® purifications from Aquarium and Feynman Pond samples to assess the effects of sampling on the microbiome profile. "W/ clean-up" indicates that samples were cleaned-up using Zymo Research OneStep PCR Inhibitor Removal Kit to determine if a clean-up step would alter the microbiome profile.