Promega

Product Application

Automated Purification of DNA from Wastewater and Biofilm

VEOLIA

Purify Bacterial DNA from wastewater and biofilm samples using the Maxwell® RSC Instrument and Maxwell® RSC PureFood GMO and Authentication Kit.

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

Analyses: UV absorbance, Dye-based quantitation and qPCR of bacterial 16S rRNA

Sample Type(s): Wastewater/ Biofilm

Input: 250mg/ 500µl

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:

- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Thermoblock
- Centrifuge
- Bead beater (e.g. FastPrep 24 from MP biomedical)
- Beads (e.g. lysing matrix E from MP biomedical)

Protocol:

1. Place 250mg (or 500µl) of sample into a bead-beating tube (Matrix E, MP biomedical).
2. Add 1ml of CTAB buffer. Vortex for 15 seconds.
3. Heat sample at 95°C for 5 minutes. Allow samples to cool down 2 minutes.
4. Bead-beat twice at 5.5m/s for 30 seconds.
5. Add 40µl of Proteinase K and 20µl of RNase A and incubate sample at 70°C for 10 minutes.
6. Centrifuge samples for 5 minutes at 12,000 x *g*.
7. Prepare cartridges
   a. Place cartridges in RSC cartridge rack and remove foil seals.
   b. Add 100µl of Elution Buffer to Elution Tubes and place tubes in cartridge rack.
   c. Place plungers into the well #8 of cartridge.
   d. Add 300µl Lysis Buffer into well #1 of cartridge.
8. Add 300µl of supernatant of well #1 of cartridge.
9. Run Maxwell® RSC with PureFood GMO and Authentication Protocol.
10. In case of amplification inhibition an optional clean-up can be done using ProNex® Size Selective Purification System (Cat.# NG2001) following TM508 with a 3:1 ProNex chemistry: sample.

Results:

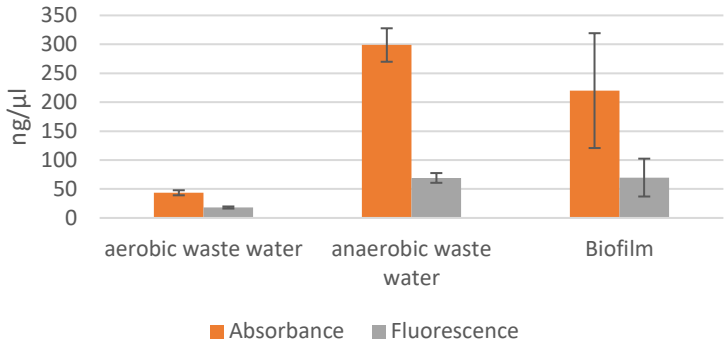

Figure 1: Concentration of DNA purified from 250mg of sample using the Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600) on the Maxwell® RSC Instrument (Cat.# AS4500). Samples were eluted in 100µl. Quantitation using a fluorescent based method (QuantiFluor® ONE dsDNA system, Cat.# E4870) or by absorbance using NanoDrop™ One spectrophotometer. Data are shown as mean ± StDev of n=3.

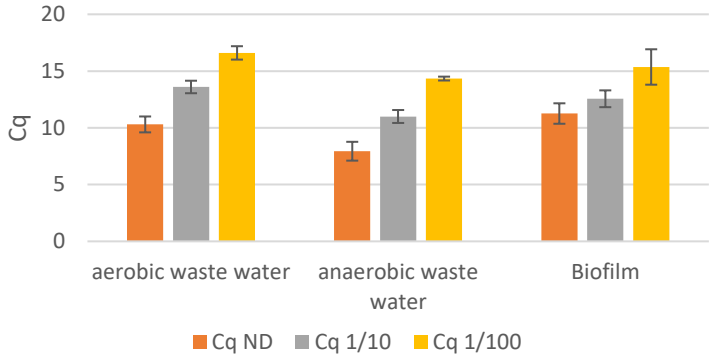

Figure 2: qPCR analysis of 16S rRNA gene of DNA purified from 250mg samples. Cq values for 2µl of undiluted (ND) 1:10 and 1:100 dilution of DNA amplified using GoTaq® qPCR Master Mix (Cat.# A6002) and 16S rRNA gene primers in a final volume of 20µl. N=3.

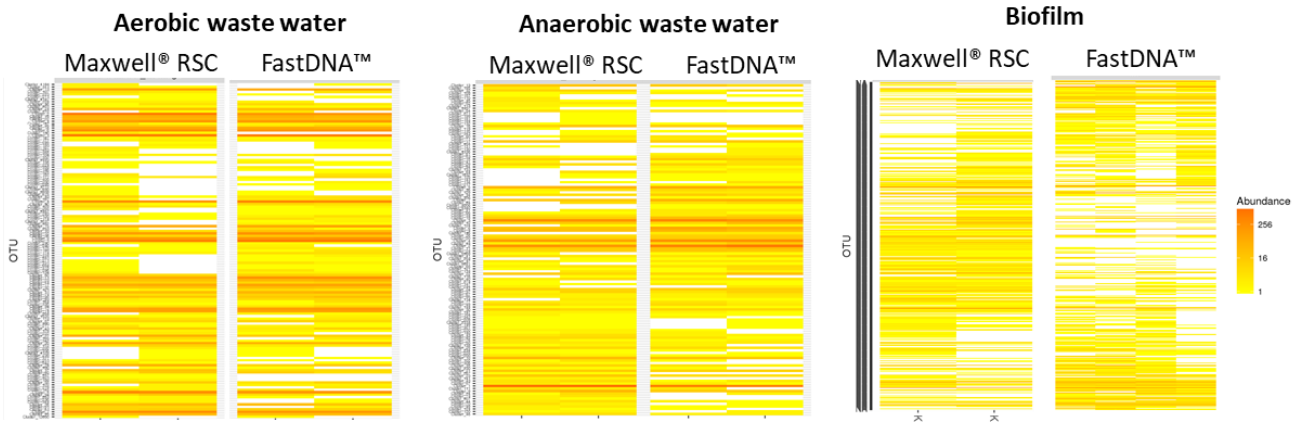

Figure 3: 16S NGS sequencing. Relative abundance of OTUs detected by 16S (V3-V4 area) Metagenomic sequencing on DNA extracted using Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600) and a FastDNA™ Spin Kit for soil. Sequencing data were processed using FROGS pipeline (FROGS: Find, Rapidly, OTUs with Galaxy Solution." Bioinformatics, Volume 34, Issue 8, 15 April 2018, Pages 1287–1294).
This protocol was tested by Veolia R&I.