Promega

Product Application

DNA extraction from waste water with ReliaPrep™ Blood gDNA MiniPrep System

Bacterial DNA was successfully extracted from waste water samples and biofilm samples using the ReliaPrep™ Blood gDNA MiniPrep System

Kit: ReliaPrep™ Blood gDNA MiniPrep System (Cat. #A5081)

Sample Type(s): Waste water/ 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.

Further information can be found in Technical Manual #TM330, available at: www.promega.com/protocols

or by e-mailing technical services at techserv@promega.com

Materials Required:

- ReliaPrep™ Blood gDNA MiniPrep System (Cat. #A5081)
- CTAB buffer (Cat. #MC1411)
- Optional: RNase A (Cat. #A7973)
- Beads (e.g: lysing matrix E from MP Biomedical)
- 100% isopropanol
- Thermoblock
- Centrifuge
- Bead Beater (e.g: FastPrep 24 from MP Biomedical)

Protocol:

1. Place 250mg (or 500µl) of sample into a bead-beating tube (Matrix E, MP biomedical).
2. Add 1 ml 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 (optionally add 20µl of RNase A) and incubate sample at 70°C for 10 minutes.
6. Centrifuge samples for 5 minutes at 12000xg.
7. Transfer 300µl of supernatant to a clean 1.5ml tube.
8. Add 300µl of CLD Buffer and 600µl of 100% isopropanol to supernatant. Invert to mix.
9. Load 600µl of sample to a ReliaPrep™ Binding Column placed into a collection tube, spin for 1 minute at max speed. Discard flow through.
10. Load the rest of sample to the ReliaPrep™ Binding column, spin for 1 minute at max speed. Place the Binding Column into a new collection tube.
11. Add 500µl of Column Wash Buffer (CWD) into the column, spin 2 minutes at max speed and discard flow through.
12. Repeat step 11 for a total of 3 washes.
13. Place Binding Column into a labeled elution tube. Add 100µl of Elution Buffer to Binding Column. Spin 1 minute at max speed.
14. In case of amplification inhibition an optional clean-up can be performed using ProNex® Size Selective Purification System (Cat. #NG2001) following TM508 with a 3:1 ProNex chemistry: sample.

Results:

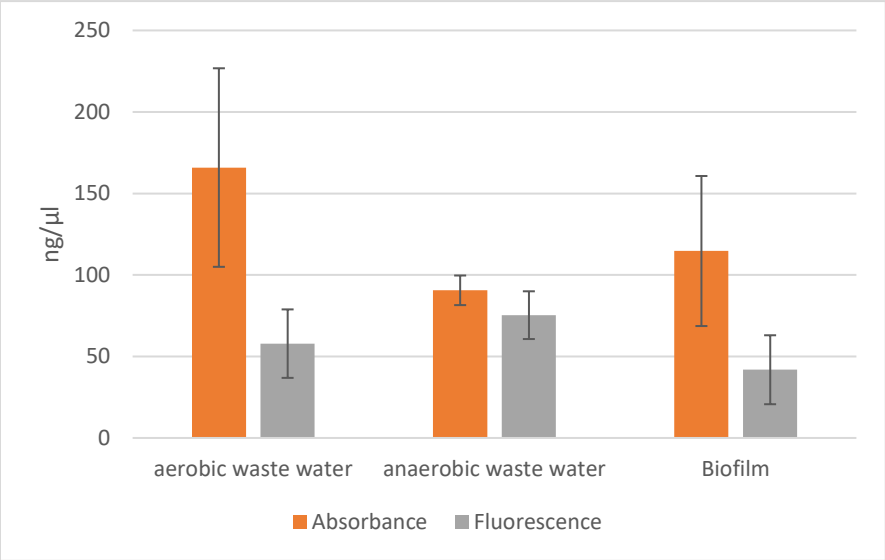

Figure 1: Concentration of DNA extracted from 250mg of sample using ReliaPrep™ Blood gDNA MiniPrep System.
Samples were eluted in 100µl. Quantitation using a fluorescent based method (QuantiFluor® ONE dsDNA system) or by absorbance using NanoDrop™ One spectrophotometer. Data are shown as mean ± StDev of n=3.

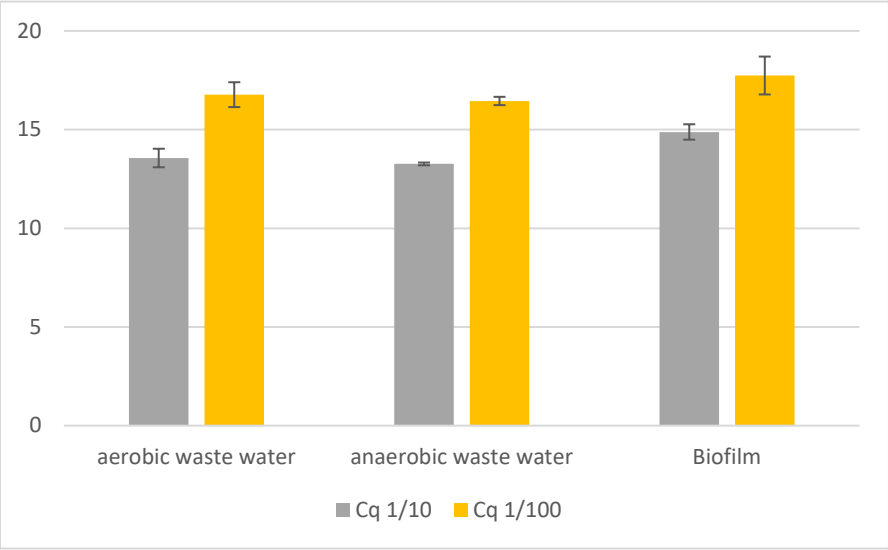

Figure 2: qPCR analysis of 16S rRNA gene of DNA extracted from 250mg samples. Cq values for 2µl of 1:10 and 1:100 dilution of DNA amplified using GoTaq® qPCR Master Mix (Cat. #A6002) and 16S rRNA gene primers in a final volume of 20µl. N=3