Quantitative PCR for Genetic Markers of Human Fecal Pollution.

Wastewater

CASE STUDY

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Overview

Keywords: Waterborne disease, environmental waters, microbial community, DNA extraction

Aim of the study: Development of a method to assess microbial community present in waste water samples

Application: Quantitative PCR

Sample name: Wastewater

Material: FastPrep-24™ instrument, FastDNA™ SPIN Kit for Soil containing Lysing Matrix E

Buffer: Sodium Phosphate Buffer and MT Buffer supplied with the FastDNA™ SPIN Kit for Soil

Protocol and Parameters

500 mL of primary effluent was collected and immediately stored on ice. 25 mL of each sample was filtered through a 0.2 µm-pore size supor-200 filters and each filter was placed in a sterile 1.5 mL microtube and stored at -80°C for DNA extraction.

1. Cut the frozen filters with a sterile cutter.
2. Add the cut filters to a Lysing Matrix E tube.
3. Add 978 µL of Sodium Phosphate Buffer and 122 µL of MT buffer, provided with the FastDNA™ SPIN Kit for Soil.
4. Homogenize in the FastPrep-24™ instrument for 120 seconds at a speed setting of 6.0
5. Centrifuge at 14,000 x g for 5-10 minutes to pellet debris.
6. Proceed with the FastDNA™ SPIN Kit for Soil extraction protocol.

Conclusion

The FastPrep-24™ and associated matrices have demonstrated successful lysis and DNA extraction from 20 samples of wastewater in only 120 seconds.

This method saves hours of work during sample preparation and ensures highly purified DNA for effective PCR amplification.

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