Purify Microbial DNA from Water Samples Using the WaterMaster™ DNA Purification Kit

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The WaterMaster™ DNA Purification Kit provides all of the reagents needed to purify high-molecular-weight microbial DNA from water samples, including DNA from most bacteria and many eukaryotes, such as protozoa. The purified DNA can be used for restriction digests, PCR, subcloning, sequencing, or microarray analysis. The simple, gentle procedure requires no toxic organic solvents or mechanical cell disruption. The final volume of the purified DNA solution is 60 µl, 50-fold less than other commercial kits, and does not need to be concentrated.

Environmental sample

To test the WaterMaster DNA Purification Kit on an environmental sample, spring-fed pond water (100 ml) was filtered, first through Miracloth (Calbiochem, EMD Biosciences) to remove large particles, and then through a 0.45-micron filter, the maximum pore size used to trap bacteria. The filter unit used in this study was a pre-sterilized Millipore 0.45 micron Microfil® V, 47 mm diameter filter available from Fisher Scientific. Because different applications require different filter sizes, the kit does not include filters.

DNA was purified from the collected bacteria using the WaterMaster Kit and assayed on an agarose gel. Figure 1 shows the high molecular weight DNA purified from the pond water sample. The DNA sample was further tested by PCR and shown to contain Bacillus sp., as indicated by amplification of the expected 600-bp product from the 16S ribosomal RNA gene (rDNA).1 Lane 1, 100-bp ladder; Lane 2, 5 ng DNA purified with the WaterMaster Kit.

Some bacteria are more difficult to lyse and many require an additional step for lysis, as outlined in the WaterMaster Kit protocol (available on EPICENTRE’s website at www.epicentre.com).

Limits of detection

To test the detection limit for bacteria in water samples, varying amounts of E. coli (10^5 to 10 cells) were added to distilled water (100 ml) and filtered through 0.45 micron filters. DNA was purified using the WaterMaster Kit and detected by PCR using the FailSafe™ PCR System with PreMix B and universal primers for eubacterial rDNA.3 Lane 1, 100-bp ladder; PCR products from DNA purified from varying numbers of E. coli cells: Lane 2, 10^5 cells; Lane 3, 10^4 cells; Lane 4, 10^3 cells; Lane 5, 10 cells; Lane 6, no template, negative control. The size of the expected amplicon is 350 bp.

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source, PCR may yield additional, slower migrating amplicons. These heteroduplex amplicons are created when rDNA from different species anneal together, and can be prevented by a method known as “PCR reconditioning.” In this method the amplification reaction is diluted 1:10 in fresh reaction mix before the last 3 PCR cycles.

Conclusion
The EPICENTRE WaterMaster DNA Purification Kit effectively purifies microbial DNA from environmental water sources using a simple procedure, with no toxic solvents or bead beating. DNA is recovered in a low, 60-µl volume and is ready for PCR or other techniques, as described in the poster presented at the 12th International Meeting on Microbial Genomes (2004) http://www.epicentre.com/posters/enviroDNAPosterweb.pdf.

References

Purify RNA from 10 to 10,000 Eukaryotic Cells. . . (continued from page 5)

produced only primer-dimers, as expected. ArrayPure RNA from as little as 10 HeLa cells produced cDNA that gave a specific real-time PCR product. RNA purified from 10 HeLa cells with the other supplier’s kit did not produce a significant amount of cDNA as indicated by the substantial primer-dimer peak in the 10-cell PCR reaction (1D).

Acknowledgments
We would like to thank Anupama Khanna and Ramesh Vaidyanathan for RNA amplification results and technical discussions.

Table 1. Standard curve data generated from quantitative RT-PCR amplification plots.

<table>
<thead>
<tr>
<th>RNA Purification Kit</th>
<th>Correlation Coefficient</th>
<th>Slope</th>
<th>PCR Efficiency (%)</th>
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<tbody>
<tr>
<td>ArrayPure™ Kit</td>
<td>0.998</td>
<td>-3.412</td>
<td>96.4</td>
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<tr>
<td>Other supplier’s kit</td>
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<td>-3.793</td>
<td>83.5</td>
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