Introduction

The construction of metagenomic libraries using a fosmid cloning system is rapidly becoming the method of choice for exploring environmental microbial communities that are unculturable or difficult to culture. Fosmid cloning systems typically use sheared DNA that results in an unbiased library with better sequence coverage compared to libraries prepared by partial restriction enzyme digests. Critical to the construction of metagenomic fosmid libraries are: i) the isolation of sufficient amounts of high molecular weight DNA; and ii) generation of ~40 kb fragments by mechanical shearing followed by size selection using pulse-field gel electrophoresis. This process, however, may take up to 3 days and results in significant sample loss.

The Metagenomic DNA Isolation Kit for Water provides the reagents to isolate random sheared, fosmid cloning-ready DNA fragments directly from microbes found in water samples. The majority of the isolated fragments using this method are in the range of 40 kb. The simple, 90-minute procedure requires no agarose plugs, no toxic solvents or mechanical cell disruption, and eliminates the need for shearing and size-fractionation of genomic DNA prior to constructing a fosmid library.

Methods and Results

DNA Isolation

To test the Metagenomic DNA Isolation Kit for Water, a 250-ml water sample was collected from Lake Mendota in Madison, WI and filtered, first through Miracloth (Calbiochem, EMD Biosciences) to remove large particles, and then through a 0.45-micron membrane filter to trap bacteria. Using the kit, the bacteria were washed off the filter and then lysed with lysis solution that contains ReadyLyse™ Lysozyme. The sheared DNA was purified by isopropanol precipitation and resuspended in 50 µl of TE buffer. Aliquots of the DNA were analyzed by agarose gel electrophoresis, confirming the size range of the sheared DNA was approximately 40 kb (Fig. 1).

Fosmid Library Construction

Using the CopyControl™ Fosmid Library Construction Kit (EPICENTRE), the sheared DNA was end-repaired to generate blunt, 5' phosphorylated ends and then ligated directly into the cloning-ready CopyControl pCC1FOS Vector. The ligated DNA was then packaged using an ultra-high efficiency MaxPlax™ Lambda Packaging Extract (EPICENTRE) and plated on phage T1-resistant E. coli cells to produce the library.

Packaging a single 10-µl ligation reaction generated 100,000 fosmid clones from the DNA isolated from the lake water sample. DNA from several randomly chosen clones was purified using the Direct Lysis Fosmid96 Kit (see p. 21) for high-throughput fosmid sequencing. As expected, Not I digestion of the fosmid DNA produced inserts that were approximately 40 kb in size (Fig. 2).

Assuming an average insert size of 40 kb, this 100,000-clone library represents 4 gigabases of DNA, which is sufficient for eight-fold coverage of one hundred 5-megabase genomes. A five-fold coverage indicates that the chance of finding a particular genomic sequence in a single genome is approximately 99%.

Sequence Analysis

DNA isolated from 15 randomly picked water metagenomic fosmid clones was end-sequenced using the pCC1Fosmid primer and BigDye chemistry (Applied Biosystems). The sequencing reactions were analyzed on a Genetic Analyzer (Applied Biosystems). BLAST
sequence homology searches were conducted against three databases: GenBank nr, Genome Survey Sequence (GSS), and environmental sample (Env-nt), after subtracting vector sequence. Two clones out of the 15 sequences analyzed showed significant hits in all three databases (a representative sample of the matches is shown in Fig. 3. for clone 2C), while the rest did not show significant matches in any database examined.

Conclusions
The Metagenomic DNA Isolation Kit for Water was developed for rapid isolation of 40-kb genomic DNA directly from microbes present in water samples. The kit maximizes DNA yields for fosmid library construction by making traditional shearing and size-fractionation steps obsolete. The kit facilitates construction of a high-coverage fosmid genomic library of the collective species present in any water sample.

Ordering Information
Metagenomic DNA Isolation Kit for Water
MGD08420 20 purifications

Fig. 2. Not I digestion of fosmid DNA isolated from randomly chosen clones verifies a 40-kb insert size. Lane M, kilobase ladder; lane 1, 40-kb control insert; lanes 2-19, Not I-digested fosmid DNA.

Fig. 3. Representative sample of BLAST search results of GenBank, GSS, and env-nt databases for clone 2C.