

Construction of an Environmental Genomic DNA Library from Soil using the EpiFOS™ Fosmid Library Production Kit

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Fosmid vectors^{1,2} provide an improved method for cloning and stably maintaining cosmid-sized (35 - 45 kb) libraries in *E. coli*. The pEpiFOS™-5 Fosmid Vector, provided linearized and dephosphorylated in the EpiFOS™ Fosmid Library Production Kit, is derived from the single copy F-factor of *E. coli* to insure that the clones produced are propagated as single copies in the cell to improve their stability compared to conventional cosmid libraries.

The EpiFOS Fosmid Library Production Kit utilizes a novel strategy (Figure 1) for cloning randomly sheared and end-repaired genomic DNA. Shearing of DNA into approximately 40-kb fragments leads to highly random generation of fragments in contrast to the conventional approach of generating DNA fragments by partial restriction endonuclease digestion. Like cosmids, fosmids are introduced into *E. coli* by high efficiency lambda packaging. The result is a complete and unbiased fosmid library.

Here we describe the production and preliminary analysis of an environmental genomic DNA fosmid library from soil using the EpiFOS Fosmid Library Production Kit.

DNA preparation

17.5 g of soil (taken from the grounds of EPICENTRE, Madison, WI) was suspended in buffer containing 1% SDS, Proteinase K, RiboShredder™ RNase Blend and RNase A and incubated at 70°C for 1 hour. The “slurry” was filtered and the DNA was precipitated from the filtrate. Following centrifugation, the DNA pellet was dried and resuspended in TE.

Fosmid cloning and library production

The crude soil DNA was further purified and size selected by agarose gel electrophoresis. The purified DNA was made blunt and 5'-phosphorylated, and then ligated into the provided linearized and dephosphorylated pEpiFOS-5 Fosmid vector. The EpiFOS Fosmid Library Production Kit provides all buffers and enzymes for the end repair of the DNA as well as for ligation into the pEpiFOS-5 vector. pEpiFOS clones were packaged using the provided high efficiency MaxPlax™ Lambda Packaging Extracts and plated on the supplied EPI100™ *E. coli* strain.

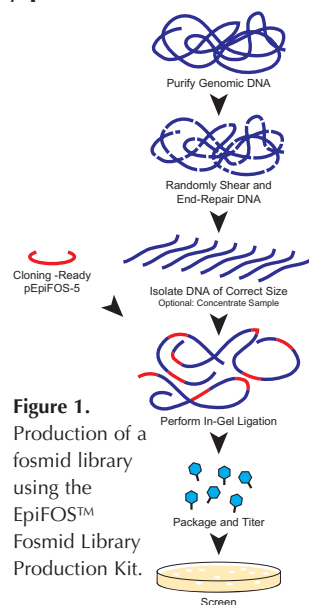


Figure 1. Production of a fosmid library using the EpiFOS™ Fosmid Library Production Kit.

Analysis of the Fosmid soil library

The EpiFOS Fosmid Library Production Kit produced a library of 6×10^5 colonies/ μg of soil DNA. Analysis of the fosmid clones was initially done by restriction endonuclease “fingerprinting” and fosmid end-sequencing using the pEpiFOS-5 Forward and Reverse Sequencing Primers.

Three fosmid clones were randomly chosen from the library and analyzed by *Not* I (GCGGCCGC) restriction

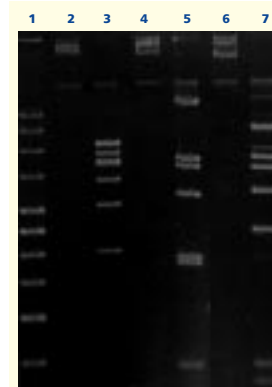


Figure 2. Gel analysis of 3 *Not* I-digested Fosmid clones.

Lane 1, size marker;
Lane 2-3, Clone F1 undigested and *Not* I-digested
Lane 4-5, Clone F2 undigested and *Not* I-digested
Lane 6-7, Clone F3 undigested and *Not* I-digested

digestion. These clones were found to contain multiple *Not* I sites (figure 2) - a surprising find considering the approximately 40kb size of the clones - providing our first evidence of high G+C content. The DNA sequences generated from end-sequencing of each of three other clones revealed 63% - 70% G+C content. BLAST homology search against GenBank revealed no significant homology between any of the 3 fosmid clones and any sequence in GenBank. Thus, these clones likely contain unique and previously uncharacterized sequences.

Analysis of these and other fosmid clones including one that demonstrates a phosphatase-like activity, is ongoing.

References

- Kim, UJ. *et al.* (1992) *Nucl. Acid Res.* **20**, 1083
- Birren, B. *et al.* (1999) *Construction of Bacterial Genomic Libraries in Genome Analysis: A Laboratory Manual v.3*, 24

EpiFOS™-Fosmid Library Production Kit

FOS0901 1 Kit

For producing up to 10 complete and unbiased fosmid libraries. Kit includes pEpiFOS™-5 Fosmid Vector*, End-repair Enzyme Mix, End-repair 10 X Buffer, dNTP Mix, Fast-Link™ DNA Ligase, Fast-Link™ 10X Ligation Buffer, ATP Solution, GELase™ Gel-digesting Preparation, GELase™ 50X Reaction Buffer, MaxPlax™ Lambda Packaging Extracts, Ligated Lambda Control DNA, Control DNA, EPI100™ Plating strain, Control Lambda Plating strain.

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pEpiFOS™-5 Forward Sequencing Primer

F5FP010 1 nmole 50 μM

pEpiFOS™-5 Reverse Sequencing Primer

F5RP011 1 nmole 50 μM

www.epicentre.com/catalog/fos5.htm