NEW PRODUCT!

Produce Unbiased Libraries of Single Copy Cosmid-Sized Clones Using the EpiFos™ Fosmid Library Production Kit

Fosmid vectors[1,2] provide an improved method for cloning and stably maintaining libraries of cosmid-sized (about 40 Kb) clones in *E. coli*. The pEpiFOS™-5 Fosmid Vector (Figure 1) provided in the EpiFOS™ Library Production Kit is derived from the single copy F-factor of *E. coli*. Thus, the cosmid-sized clones produced using pEpiFOS-5 are propagated as a single copy. Low copy number has been shown to improve clone stability compared to high copy cosmid clones. In addition, pEpiFOS-5 contains *cos* sites for *cos* site-mediated lambda phage packaging and high efficiency fosmid library production. The pEpiFOS-5 vector is provided linearized and dephosphorylated- ready for use in library production.

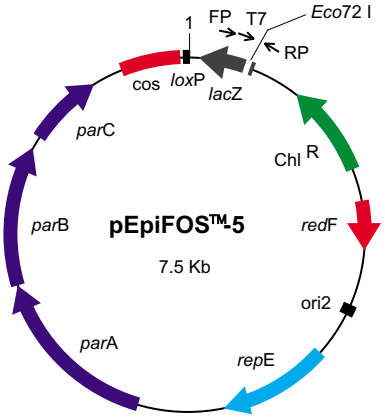

Figure 1. Map of pEpiFOS™-5 Fosmid Vector.

The EpiFOS Fosmid Library Production Kit provides all reagents needed to construct a complete and unbiased fosmid library in about 2 days using a novel cloning strategy (Figure 2). Genomic DNA is first sheared by passing it through a syringe needle (not supplied with the kit). Shearing the DNA into approximately 40 Kb fragments leads to the highly random generation of DNA fragments in contrast to more biased libraries that result from fragmenting the DNA by partial restriction endonuclease digestion. The sheared DNA is then end-repaired to generate blunt ends, using reagents supplied in the kit and size-selected using low melting point agarose gel. Finally, the size-selected DNA is ligated into the supplied linearized and dephosphorylated pEpiFOS-5 Fosmid Vector, packaged using ultra-high efficiency packaging extracts (>10^9 pfu/µg for phage lambda), also included in the kit, and plated on the supplied EPI100™ *E. coli* plating cells. The result is a complete and unbiased primary fosmid library.

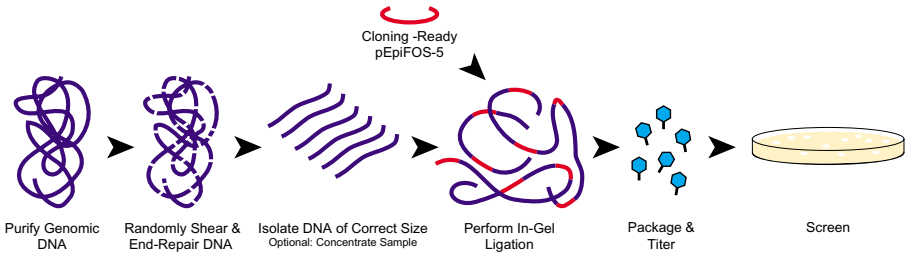

Figure 2. The process for preparing a complete and unbiased fosmid library using the EpiFOS™ Fosmid Library Production Kit. Cloning randomly sheared genomic DNA fragments results in a complete and unbiased fosmid library. The EpiFOS Fosmid Library Production Kit provides all necessary reagents (except for genomic DNA).

EpiFOS Fosmid Library Production Kit provides:

- Stable unbiased genomic libraries with single-copy clones averaging about 40 Kb in size.
- Reagents for 10 cloning and packaging reactions.
- High efficiency packaging extracts to maximize the number of clones produced.

References

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

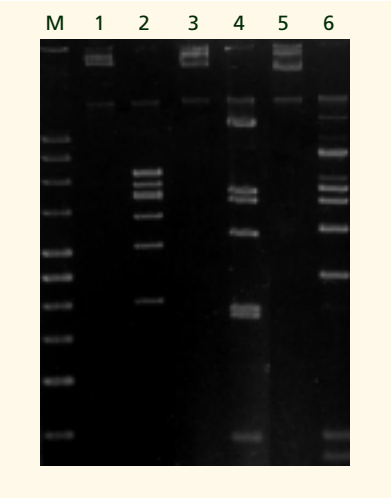

Figure 3. Gel analysis of three *Not* I-digested fosmid clones (even numbered lanes) from an environmental soil fosmid library produced using the EpiFOS Fosmid Library Production Kit.

EpiFOS™ Fosmid Library Production Kit
FOS0901 1 Kit
10 Reactions

Contents:
Kit includes pEpiFOS™-5* Fosmid Vector, End-repair Enzyme Mix, End-repair 10X 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.

pEpiFOS™-5 Forward Sequencing Primer
F5FP010 1 nmole 50 µM

pEpiFOS™-5 Reverse Sequencing Primer
F5RP011 1 nmole 50 µM

* pEpiFOS™-5 is exclusively licensed by EPICENTRE Technologies.