

The CopyControl™ Fosmid Library Production Kit Generates More Complete and Unbiased (Blunt-End) Libraries of Stable, 40-Kb Genomic Clones

Fosmid vectors, containing the single copy *E. coli* F-factor replicon, were developed as an improved method for constructing libraries of cosmid-sized (approximately 40 Kb) clones. The stability of inserts cloned into fosmid vectors has been shown to be substantially greater than in high copy vectors.¹ EPICENTRE's new CopyControl™ Fosmid Library Production Kit includes the pCC1FOS™ Fosmid vector that contains both the *E. coli* F-factor replicon and the *oriV* high-copy origin of replication, thus providing the user the clone stability afforded by single-copy fosmid cloning and the high yields of DNA that can be realized from cosmid clones.

The CopyControl Fosmid Library Production Process Facilitates Construction of Complete and Unbiased Libraries

The CopyControl Fosmid Kit uses a novel strategy (Figure 1) of cloning randomly-sheared, end-repaired and 5'-phosphorylated DNA fragments. Shearing the DNA to approximately 40 Kb generates highly random DNA fragments, as opposed to more biased libraries that result from partial restriction endonuclease digestion. A CopyControl Fosmid library of >10⁷ clones can be generated in about 2 days. Thus, the CopyControl Fosmid Library Production Kit is ideal for preparing genomic libraries containing 40 Kb inserts from all sources. Even a library with 10X coverage of a genome as large as human is much faster and easier to prepare, and is more unbiased in its coverage, using the CopyControl Fosmid Vector than using a BAC vector.

At Single Copy, CopyControl Fosmid Clones Are More Stable Than Cosmids

Selection and growth of CopyControl Fosmid clones at single copy greatly improves the stability of the cloned insert by reducing the likelihood of segment deletions or rearrangements that can occur with cosmids. Additionally, single copy cloning facilitates cloning of DNA segments encoding products that can be lethal or detrimental to the host cell at high copy number. This was reported recently by researchers at the University of Maryland² who were unable to find a specific gene in a 20X cosmid library - most likely due to the toxic nature of its encoded protein. They readily identified

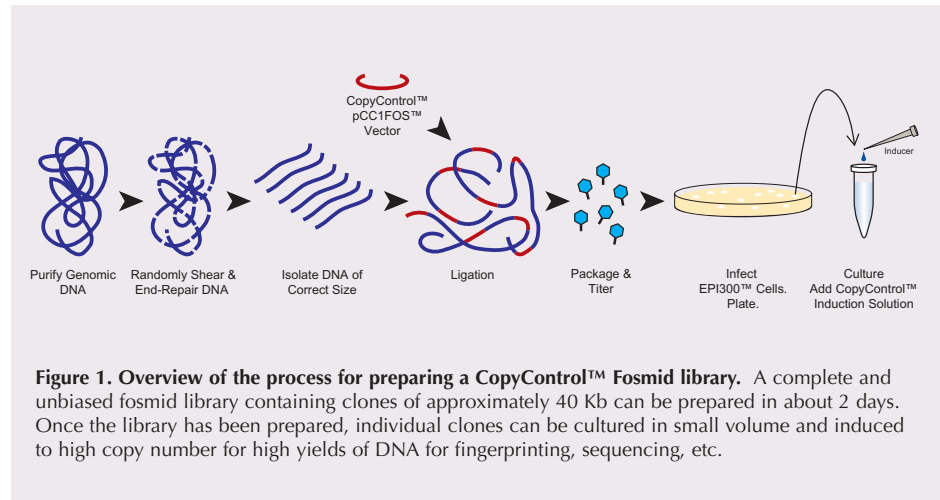


Figure 1. Overview of the process for preparing a CopyControl™ Fosmid library. A complete and unbiased fosmid library containing clones of approximately 40 Kb can be prepared in about 2 days. Once the library has been prepared, individual clones can be cultured in small volume and induced to high copy number for high yields of DNA for fingerprinting, sequencing, etc.

the gene in a fosmid library constructed using the CopyControl pCC1FOS Vector grown at single copy.

CopyControl Fosmid Clones Can Be Amplified to 10 – 50 Copies Per Cell for High Yields of DNA for Sequencing and Fingerprinting

Once the CopyControl Fosmid library has been produced, individual clones can be induced from single copy to 10 – 50 copies per cell (Figure 2) by the addition of the CopyControl Induction Solution. A small volume culture of an induced CopyControl Fosmid clone yields sufficient amounts of DNA for sequencing, fingerprinting, or other applications.

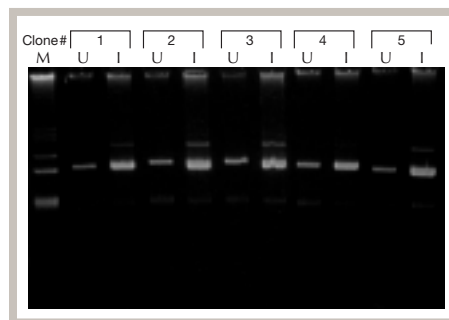


Figure 2. The copy number of CopyControl™ Fosmid clones can be induced 10 - 50 fold to greatly increase DNA yield. Five randomly chosen CopyControl Fosmid clones were grown in culture in duplicate. One sample of each was induced (I) to high copy number by addition of CopyControl™ Induction Solution. The other sample was an uninduced control (U). DNA was isolated from an equal number of cells of each and analyzed by agarose gel electrophoresis.

References

1. Kim, U.-J. *et al.* (1992) *Nucl. Acids Res.* **20**, 1083.
2. Dr. Nate Ekborg, University of Maryland, personal communication.

www.epicentre.com/ccfosmid.asp

CopyControl™ Fosmid Library Production Kit

CCFOS110 1 Kit
Kit contains sufficient reagents to produce up to 10 Fosmid Libraries.

Contents:

CopyControl™ pCC1FOS™ (Blunt) Vector, End-Repair Enzyme Mix and Buffer, Fast-Link™ DNA Ligase and Buffer, ATP, GELase™ Enzyme Preparation and Buffer, MaxPlax™ Lambda Packaging Extracts, EPI300™ *E. coli*, Control DNA, and CopyControl™ Induction Solution.

pCC1FOS™/pEpiFOS-5 Forward Sequencing Primer

F5FP010 50 µM 1 nmole

pCC1FOS™/pEpiFOS-5 Reverse Sequencing Primer

F5RP011 50 µM 1 nmole