

EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research

EPICENTRE Revolutionizes Cloning by Introducing CopyControl™ Cloning Systems for Single-Copy Cloning and “On-Demand” Induction of Clones to High Copy Number

EPICENTRE's new CopyControl™ Cloning Systems, with a choice of BAC, fosmid or plasmid CopyControl Vectors, allow researchers to make and maintain libraries or clones of genomic DNA, cDNA, or PCR products at a single copy and then, whenever desired, to induce the clones to high copy number (10-50+ copies per cell) (Figure 1). Thus, the CopyControl Systems combine the clone stability afforded by single copy cloning with the advantages of high yields of DNA obtained by high copy vectors.

The benefits of on-demand induction to obtain higher DNA yields from smaller amounts of bacterial culture will be obvious to genomics researchers using single-copy BAC or fosmid vectors. However, unexpectedly, single-copy vectors are also better for cloning low molecular weight DNA, such as cDNA or PCR products. For example, in one experiment, the number of cDNA clones was significantly higher using a single-copy vector compared to using a high-copy vector (personal communication). Overall, single-copy cloning results in more complete and more unbiased clone libraries.

CopyControl Cloning Systems are based on the revolutionary new technology invented in the well-known laboratory of Professor Waclaw Szybalski at the University of Wisconsin-Madison by the McArdle Laboratory team of Drs. Szybalski, Zdenka Hradecna, and Jadwiga Wild,^{1,2,3} and further developed during more than one year of intensive work by EPICENTRE's team of scientists.*

Until Now, Researchers Have Used High-Copy Vectors for Cloning Low Molecular Weight DNA and Single-Copy Vectors for Cloning High Molecular Weight Genomic DNA

Early work on molecular cloning concentrated on developing vectors with the

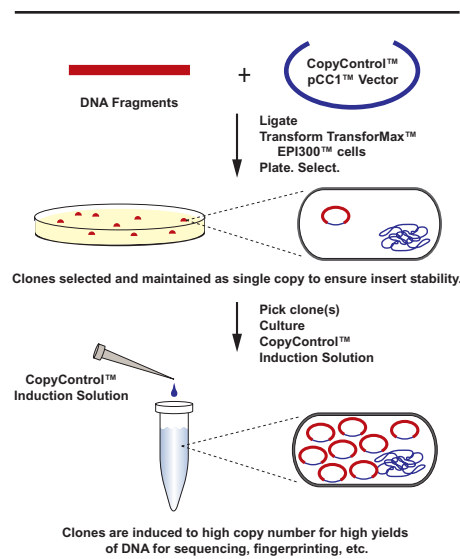


Figure 1. Overview of the CopyControl™ Cloning Systems. CopyControl BAC, Fosmid or PCR clones are initially grown as single copy clones in TransformMax™ EPI300™ *E. coli*. Individual clones are chosen, grown in small volume culture then induced to high copy by addition of the CopyControl™ Induction Solution.

highest possible copy number (e.g., pBR322, pUC18) without general concern about the not-infrequent cloning “artifacts” that likely resulted from instability of the cloned insert when clones were propagated at high copy number. The advent of genomic DNA cloning and sequencing projects has spurred the development and use of single copy Bacterial Artificial Chromosome (BAC)^{4,5} and fosmid⁶ vectors that better ensure the stability of the cloned genomic DNA and improve the probability of cloning expressed genes or DNA fragments that would be toxic or detrimental for growth of the *E. coli* host if cloned in a high-copy vector.

The disadvantage of cloning in single-copy cloning vectors is the very low

yield of cloned DNA that can be obtained for analysis. Also, since high-copy vectors yield more clone DNA relative to *E. coli* host genomic DNA than single-copy vectors, it is easier and faster to purify high-copy vector clone DNA from host DNA.

... continued on page 2

Now Order Online!

Visit us online at
www.epicentre.com

In This Issue

- 1 EPICENTRE Revolutionizes Cloning by Introducing CopyControl™ Cloning Systems
- New!** 3 The CopyControl™ Fosmid Library Production Kit Generates More Complete and Unbiased Libraries of Stable Genomic Clones
- New!** 4 Save At Least 2 Days Constructing a CopyControl™ BAC Library Then Amplify the Clones to 10-20 Copies Per Cell
- New!** 6 The CopyControl™ PCR Cloning Kit Enables Faster, More Reliable Cloning of Any PCR Product
- New!** 7 Retrofit Existing BAC and Fosmid Clones with CopyControl™ Capability
- New!** 7 BAC-Tracker™ Supercoiled DNA Ladder
- New!** 8 TransformMax™ EPI300™ *E. coli* and Electroporation Cuvettes
- 9 Obtain PCR-Ready Genomic DNA from a Variety of Sample Types
- New!** 10 *In Vitro* Synthesis of 2'-Fluoro-Modified RNA Transcripts That Are Completely Resistant to RNase A Digestion
- 12 Transposon Mutagenesis in *Trypanosoma brucei* with the EZ::TN™ Transposome™
- 14 *In Vitro* Insertion of a Transposon Containing an *E. coli* Origin of Replication Facilitates Propagation and Sequencing of Circular DNA Molecules
- 15 Consistent High-Fidelity PCR Amplification of DNA 20 Kb and Longer
- 16 DNA Ligations in 5 Minutes

CopyControl™ Cloning Systems Provide the Advantages of Both Single-Copy and High-Copy Vectors

EPICENTRE's new CopyControl Cloning Systems combine the advantages of both single-copy vectors and high-copy vectors without the disadvantages of either. With the CopyControl Cloning Systems, the user has complete control over the clone copy number. Clones are initially grown at single copy to ensure insert stability and cloning of potentially toxic expressed DNA segments and PCR products, and then induced to high copy number whenever desired, to maximize the yield and purity of DNA for sequencing, fingerprinting and other applications.

CopyControl Systems Use a Vector with Both Single-Copy and High-Copy Origins of Replication (*ori*) and an *E. coli* Host with an Inducible Protein Required for the High-Copy *ori*

CopyControl Systems are based on CopyControl pCC1™ Vectors (Figure 2) that have two origins of replication - the single copy *E. coli* F-factor replicon and a high-copy origin of replication called "*oriV*." Initiation of replication from *oriV* requires the "*trfA*" gene product. However, the *trfA* gene is absent in both the CopyControl vectors and common lab strains of *E. coli*. CopyControl Systems utilize a specially engineered *E. coli* host strain - called TransforMax™ EPI300™ - that contains a mutant *trfA* gene under tight control of an inducible promoter. In the absence of *trfA* gene induction agent, replication of pCC1 clones is controlled

by the F-factor replicon and the vector is present at one copy per cell. Addition of a simple sugar (the CopyControl Induction Solution) to the growth medium induces expression of *trfA* and subsequent amplification of the clone to high copy number (10 – 50+ copies per cell) to facilitate purification of microgram amounts of DNA.

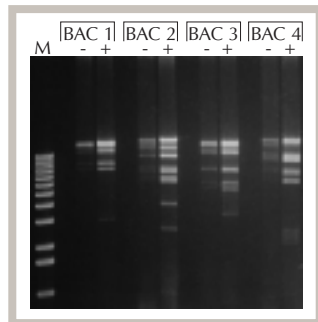


Figure 3. *Hind* III fingerprint of 1 µl of DNA isolated from an equal number of cells from induced (+) and uninduced (-) cultures of 4 CopyControl™ BAC clones. Lane M, DNA size ladder.

Existing BAC and Fosmid Clones Can Be Retrofitted with CopyControl™ Capability

BAC and fosmid clones generated in vectors that are exclusively single-copy can be easily retrofitted with CopyControl capability using the new EZ::TN™ <*oriV* /KAN-2> Insertion Kit. See page 7 to learn more about this transposon-based system for high yields of DNA and complete sequencing without primer walking or subcloning.

References

1. Hradecna, Z. *et al.* (1998) *Microbial and Comp. Genomics* **3**, 58.
2. Wild, J. *et al.* (2001) *Plasmid* **45**, 142.
3. Wild, J. *et al.* (2002) *Genomic Research* (submitted).
4. Shizuya, H. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* **89**, 8794.
5. Birren, B. *et al.* (1999) *Bacterial Artificial Chromosomes in Gene Analysis: A Laboratory Manual*, Cold Spring Harbor Press.
6. Kim, U. J. *et al.* (1992) *Nucl. Acids Res.* **20**, 1083.

*CopyControl™ products are covered by U.S. Patent No. 5,874,259 licensed to EPICENTRE and by other patents pending and assigned to EPICENTRE which cover specific vectors, including, without limitation, CopyControl™ pCC1™, pCC1BAC™, and pCC1FOS™, and specific cells, including, without limitation, TransforMax™ EPI300™. By purchasing CopyControl systems or vectors, the purchaser receives the right to use the product purchased from EPICENTRE or an authorized distributor for life science research.

CopyControl™ Cloning Kits and Related Products	
CopyControl™ Fosmid Library Production Kit	Page 3
CopyControl™ BAC Cloning Kits	Page 4
CopyControl™ PCR Cloning Kits	Page 6
EZ::TN™ < <i>oriV</i> /KAN-2> Insertion Kit	Page 7
BAC-Tracker™ Supercolied DNA Ladder	Page 7
TransforMax™ EPI300™ Electrocompetent and Chemically Competent <i>E. coli</i>	Page 8

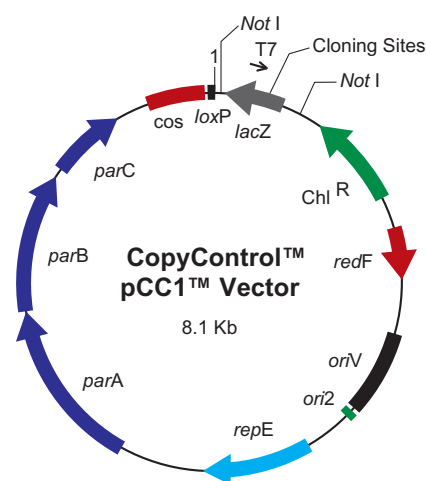


Figure 2. The CopyControl™ pCC1™ Cloning-Ready Vectors contain both a single copy and the *oriV* high copy origin of replication. The vector also contains a chloramphenicol selectable marker and T7 promoter. The CopyControl pCC1 Vector as supplied in the CopyControl Cloning Kits is linearized, completely dephosphorylated, and highly purified to ensure low background.

Benefits of the CopyControl Cloning Systems

- **Produce more complete genomic libraries and ensure successful cloning of all PCR products.**
Select and grow CopyControl clones at single copy to better ensure insert stability and cloning of encoded, expressed toxic proteins.
- **Get high yields of DNA for sequencing, fingerprinting or other applications.**
CopyControl clones, in small culture volumes, can be readily amplified from single copy to high copy number by addition of the CopyControl Induction Solution. CopyControl BAC clones are amplified to 10 – 20 copies per cell and CopyControl Fosmid and PCR clones to 10 – 50 copies per cell.
- **No need to prepare a cloning vector.**
The CopyControl Cloning Kits include a cloning-ready pCC1 vector which is linearized, dephosphorylated, highly purified and ready for ligation of the genomic DNA fragments or PCR product.
- **Rapidly screen CopyControl clones without minipreps or restriction digests.**
The size of CopyControl BAC clones can be estimated in 4 hours and CopyControl PCR clones in 1 hour without minipreps or restriction digests.
- **Retrofit existing BAC and fosmid clones with CopyControl capability.**
Existing BAC and fosmid clones can be retrofitted with the *oriV* high copy origin of replication using the transposon-based EZ::TN™ <*oriV* /KAN-2> Insertion Kit.