

Which Kit is Best for Your Genomic DNA Cloning Project— the CopyControl™ Fosmid Library Production Kit or the CopyControl™ BAC Cloning Kit?

EPICENTRE's new CopyControl™ Fosmid Library Production Kit and CopyControl™ BAC Cloning Kits are based on cloning technology developed in the laboratory of Dr. Waclaw Szybalski.¹ CopyControl cloning technology enables the user to grow CopyControl Fosmid or CopyControl BAC clones at single-copy number to ensure insert stability and successful cloning of sequences encoding expressed toxic protein. Then, whenever desired, the CopyControl clones can be induced from single copy to high copy for higher yields of higher purity DNA (Figures 1 and 2). Higher yields of higher purity DNA means more applications (e.g., fingerprinting, end-sequencing) from a single DNA prep, more DNA for sub-cloning and often longer DNA sequence reads. For more information on the CopyControl Cloning process, see the side bar on p. 11 or visit www.epicentre.com/cc_tutorial.asp.

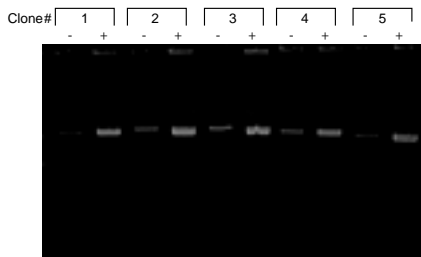


Figure 1. CopyControl™ Fosmid clones can be induced up to 50 copies per cell to greatly increase DNA yield. Five randomly chosen CopyControl Fosmid clones were grown in culture in duplicate. One sample of each was induced (+) to multiple-copy number by addition of CopyControl™ Induction Solution. The other sample was an uninduced control (-). DNA was isolated from an equal number of cells of each and analyzed by agarose gel electrophoresis.

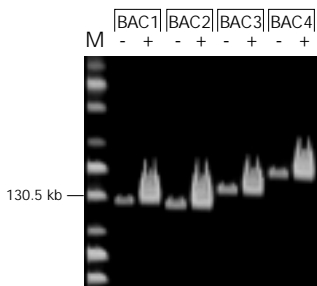


Figure 2. *Not I* digestion of DNA from an equal number of induced (+) and uninduced (-) cultures of 4 CopyControl™ BAC clones. The induced cultures yielded 10- to 20-fold more DNA, M = DNA marker.

Table 1. A comparison of the CopyControl™ Fosmid and the CopyControl™ BAC cloning processes.

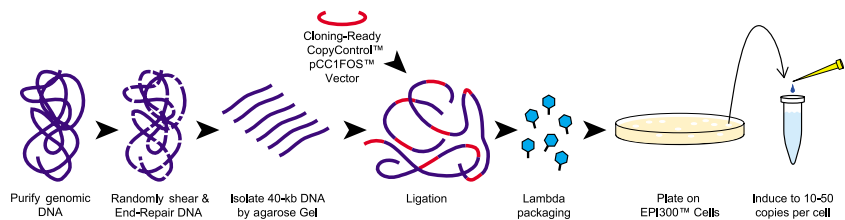
Library Construction Parameter	CopyControl™ Fosmid Library Production Kit (Figure 3A)	CopyControl™ BAC Cloning Kits (Figure 3B)
Vector	CopyControl™ pCC1FOS™ provided linearized at <i>Eco</i> 72 I (blunt) site, dephosphorylated, and purified.	CopyControl™ pCC1BAC™ vector provided linearized at either the <i>Bam</i> H I-, <i>Eco</i> R I-, or <i>Hind</i> III-site, dephosphorylated, and highly purified.
Preparation of genomic DNA	Random shearing followed by end-repair to 5'-phosphorylated, blunt-ended DNA. End-repaired DNA is size-selected by standard agarose gel electrophoresis.	Partial restriction endonuclease digestion. DNA fragments are size-selected by pulse field gel electrophoresis (PFGE).
Introduction into host	High efficiency lambda phage packaging followed by infection of TransforMax™ EPI300™ cells.	Electroporation of high efficiency TransforMax™ EPI300™ Electrocompetent <i>E. coli</i> .
Background	Virtually 100% white colonies.	Greater than 95% white colonies.
Insert size	All inserts are approximately 40 kb.	Typically an average size of 80 kb to 200 kb.
Cloning efficiency	Approximately 10-fold greater than BAC cloning.	Approximately 10-fold lower than fosmid cloning.
Induction of the CopyControl clones to high-copy number	Inducible from single-copy to 10-50 copies per cell.	Inducible from single-copy to 10-20 copies per cell.

Here we present a brief summary and comparison (Table 1 and Figure 3) of the processes and advantages of constructing genomic libraries using the CopyControl Fosmid Library Production Kit and the CopyControl BAC Cloning Kits in order to aid the user in selecting the best kit for their need.

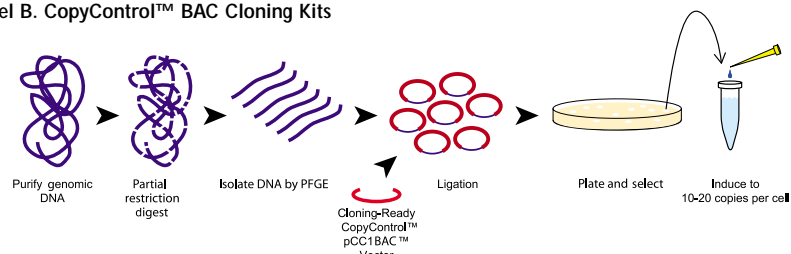
Additionally, CopyControl capability can be incorporated into existing single-copy BAC and fosmid clones using a simple transposon-based *in vitro* reaction. For more information, visit www.epicentre.com/transposomics.asp.

Figure 3. Overview of the process for preparing a genomic library using the CopyControl™ Fosmid Library Production Kit (Panel A) or the CopyControl™ BAC Cloning Kits (Panel B).

Panel A. CopyControl™ Fosmid Library Production Kit



Panel B. CopyControl™ BAC Cloning Kits



Discussion

The CopyControl Fosmid Library Production Kit and the CopyControl BAC Cloning Kits have been used successfully by many researchers to construct libraries from both large and small genomes. As a general rule, fosmid cloning can save the user time and labor constructing a genomic library while BAC cloning can save the user time and labor in analyzing the genomic clones.

For example, constructing a CopyControl Fosmid library does not require costly equipment (e.g., Pulse Field Gel Electrophoresis and electroporator) or labor (preparing and isolating large genomic fragments and assessing the quality of the library) that is needed for constructing a

BAC library. The CopyControl Fosmid Library Production Kit utilizes a process of random shearing and blunt-end cloning of the DNA which eliminates the need to optimize and then perform partial restriction endonuclease digestions, as required for BAC cloning. In addition, a fosmid library constructed from randomly sheared DNA will likely be a more complete and unbiased library than a BAC library that was constructed from partial restriction endonuclease digested DNA.

In contrast, BAC libraries typically produce clones with inserts 3- to 5-fold larger than a fosmid clone so that there are fewer clones required for complete genome coverage. The larger insert sizes of BAC clones can mean reduced time

and labor needed for fingerprinting and end-sequencing and ultimately, assembly of contigs—compared to the same library constructed in a fosmid vector.

Reference

1. Wild, J. *et al.* (2002) *Genome Research* **12**, 1434.

www.epicentre.com/ccbac.asp

CopyControl™ BAC Cloning Kit (*Bam*H I)

CCBAC1B 1 Kit

CopyControl™ BAC Cloning Kit (*Eco*R I)

CCBAC1E 1 Kit

CopyControl™ BAC Cloning Kit (*Hind* III)

CCBAC1H 1 Kit

Each kit contains sufficient reagents for constructing the equivalent of one 10X human genomic library.

Note: TransforMax™ EPI300™ Electrocompetent *E. coli* or Phage T1-Resistant TransforMax™ EPI300™-T1^R Electrocompetent *E. coli*, required for inducing CopyControl BAC clones to high-copy number, are available separately.

www.epicentre.com/epi300.asp

TransforMax™ EPI300™ Electrocompetent *E. coli*

EC300105 5 X 100 µl

EC300110 10 X 100 µl

Transformation efficiency > 1 x 10¹⁰ cfu/µg.

Includes CopyControl™ Induction Solution and pUC19 control DNA.

Phage T1-Resistant TransforMax™ EPI300™-T1^R Electrocompetent *E. coli*

EC02T15 5 X 100 µl

EC02T110 10 X 100 µl

Transformation efficiency > 1 x 10¹⁰ cfu/µg.

Includes CopyControl™ Induction Solution and pUC19 control DNA.

www.epicentre.com/ccfos.asp

CopyControl™ Fosmid Library Production Kit

CCFOS110 1 Kit

Kit contains sufficient reagents to produce up to 10 CopyControl Fosmid libraries.

Phage T1-Resistant EPI300™-T1^R *E. coli* cells, required for inducing CopyControl Fosmid clones to high-copy number, are supplied with the kit.

Incorporate CopyControl™ capability into existing single-copy BAC and fosmid clones.

www.epicentre.com/transposomics.asp

EZ::TN™ <*ori*V/KAN-2> Insertion Kit

EZ102VK 10 Reactions

How the CopyControl Cloning and Clone Induction Process Works

CopyControl™ cloning combines the advantages of both single-copy vectors and high-copy vectors without the disadvantages of either. The CopyControl Fosmid Library Production Kit and the CopyControl BAC Cloning Kits enable users to make and maintain fosmid or BAC libraries at single-copy number to ensure insert stability and cloning of potentially toxic expressed DNA segments, and then, whenever desired, to induce the clones to high-copy number for high yields of higher purity DNA for downstream processes such as fingerprinting and DNA sequencing (Figure 1).

The pCC1FOS™ Vector and the CopyControl pCC1BAC™ Vectors, provided in the respective kits contain two origins of replication—the single copy *E. coli* F-factor replicon and a high-copy origin of replication, *ori*V.

Initiation of replication from *ori*V requires the *trfA* gene product supplied by the TransforMax EPI300 *E. coli*—a host strain engineered by scientists at EPICENTRE to contain the *trfA* gene under tight control of an inducible promoter.

In the absence of the *trfA* gene induction, replication of pCC1FOS clones and CopyControl pCC1BAC is controlled by the F-factor replicon and the vector is present at one copy per cell. Addition of the CopyControl Induction Solution to CopyControl Fosmid and CopyControl BAC clones grown in individual cultures or in a 96-deep well format induces expression of the *trfA* gene resulting in initiation of replication from *ori*V and subsequent amplification of the clone to 10-50 copies per cell for the CopyControl Fosmid clones and 10-20 copies per cell for the CopyControl BAC clones.

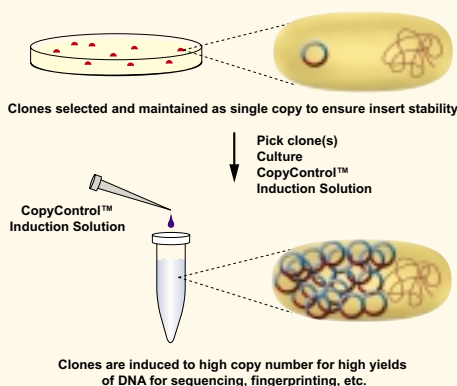


Figure 1. Overview of the CopyControl™ Cloning Systems.

CopyControl Fosmid or BAC clones are grown at single copy in TransforMax™ EPI300™ *E. coli*. Individual clones are chosen, grown in small volume culture, and then induced to high-copy number by addition of the CopyControl™ Induction Solution.