

Determining the Copy Number of Induced CopyControl™ Clones Using the FailSafe™ Real-Time PCR System

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Introduction

CopyControl™ Cloning Systems are based on an innovative technology originally developed in the laboratory of Dr. Waclaw Szybalski¹ and optimized at EPICENTRE.² Each CopyControl System includes a CopyControl™ pCC1™ Vector that has two origins of replication: a single-copy *E. coli* F-factor replicon and a high-copy origin of replication called “*oriV*”. The pCC1 vector confers chloramphenicol resistance. Initially, replication of CopyControl clones is controlled by the F-factor replicon and the vector is present at one copy per cell. Maintaining clones at single copy ensures insert stability and allows cloning of toxic gene products. Moreover, single-copy cloning results in more complete and less biased libraries (Figure 1).²

Initiation of replication from *oriV* requires the *trfA* gene product. CopyControl Systems use a specifically engineered *E. coli* host strain, TransforMax™ EPI300™, which contains a mutant *trfA* gene under tight control of an inducible promoter. Addition of the CopyControl™ Induction Solution to the growth medium induces expression of *trfA* and subsequent amplification of the clone to high-copy number (Figure 2). Higher copy number greatly improves the yield and purity of the DNA for sequencing, fingerprinting, and other applications.

CopyControl Systems are available in three cloning formats:

- CopyControl™ BAC Cloning Kits, for large inserts, typically 100 to 300 kb.
- CopyControl™ Fosmid Library Production Kits, for inserts of about 40 kb.
- CopyControl™ PCR Cloning Kits, for inserts less than 15 kb.

In this report, we use the FailSafe™ Real-Time PCR System to quantitate the pCC1 copy number in induced BAC, Fosmid and PCR CopyControl clones.

Methods

Each CopyControl clone was induced from single-copy to high-copy number using a procedure similar to the one described in the product literature. Briefly,

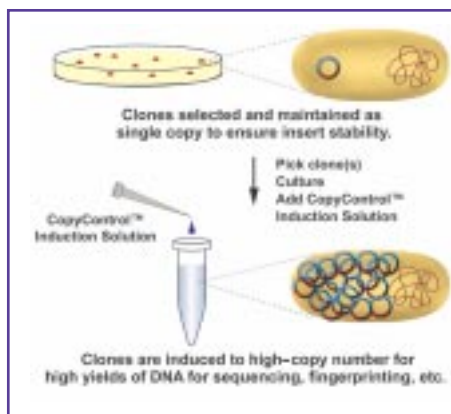


Figure 1. Overview of the CopyControl™ Cloning Systems.

after overnight growth at 37°C in LB medium containing chloramphenicol, duplicate samples were diluted into fresh media and grown for 30 to 60 minutes or to an O.D.₆₀₀ of 0.5. CopyControl Induction Solution was added to one culture, and both the uninduced and the induced cultures were shaken at 37°C for 3 hours.

Following the 3-hour induction period, EPICENTRE's Colony Fast-Screen™ Kit (PCR Screen) provided a rapid method for preparing PCR-ready template DNA. An

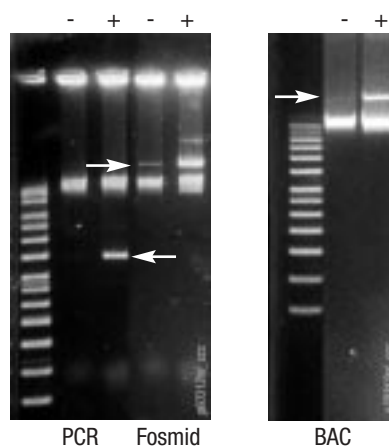


Figure 2. CopyControl™ clones can be induced from single-copy to high-copy number for higher yields of DNA. Samples contained an equal number of cells from uninduced (-) and induced (+) cultures of PCR, Fosmid, and BAC CopyControl clones. Each sample was processed with the Colony Fast-Screen™ Kit (Size Screen) and analyzed by agarose gel electrophoresis. Note: → indicates expected size of the clone

equal number of uninduced and induced cells (100 to 150 µl, based on the O.D.₆₀₀) were spun down, resuspended in 50 µl of the PCRLyse™ Solution, and heated at 99°C for 5 minutes. Lysates (1 µl for the BAC and fosmid clones; 1 µl of a 1:10 dilution for the PCR clone) were used directly in a real-time PCR assay.

EPICENTRE's FailSafe Real-Time PCR System was used to amplify a 317-bp region from the *oriV* sequence found in all pCC1 vectors. The 50-µl reactions were set-up at room temperature and included: 1 µl DNA template, 500 nmole each of the forward and reverse primers, 25 µl FailSafe™ PreMix E, and 2 Units of the FailSafe™ Enzyme Mix. PCR cycling conditions included an initial denaturation at 95°C (2 minutes), followed by 35 cycles of 95°C (10 seconds), 60°C (10 seconds), and 72°C (30 seconds). Amplification and data analysis were performed on Bio-Rad's iCycler iQ Real-Time PCR Detection System. A representative melt curve analysis graph is shown in Figure 3.

To quantify the copy number of each pCC1-derivative (PCR, Fosmid, BAC) in uninduced and induced samples, standard curves were generated for each type of CopyControl clone as follows. Ten-fold serial dilutions, ranging from 10⁶ to 10² copies of template per microliter, were made using purified DNA. Real-time PCR was then performed using the amplification conditions described above. Standard curves were generated by plotting the log of the dilution versus the threshold cycle (C_T) values. Results obtained on serial dilutions of the CopyControl Fosmid clone are shown in Figure 4.

Results and Discussion

CopyControl Cloning Systems provide efficient and time-saving methods for making BAC- and fosmid-sized libraries as well as cloning PCR fragments up to 15-kb. The pCC1 vector in each CopyControl System can be induced from single-copy to high-copy number simply by adding the CopyControl Induction Solution to the culture media. Quantitation by real-time PCR of a 13-kb clone constructed with the CopyControl PCR Cloning Kit indicated a 33.4-fold increase in copy number following induction

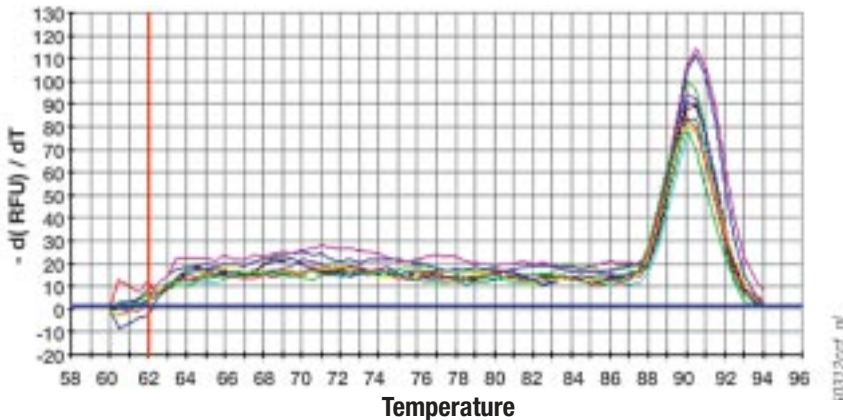


Figure 3. A melt curve analysis graph shows no nonspecific amplicon or primer/dimer formation using the FailSafe™ Real-Time PCR System and the CopyControl fosmid clone as template. Similar melt curves were obtained for all real-time PCR amplifications (data not shown).

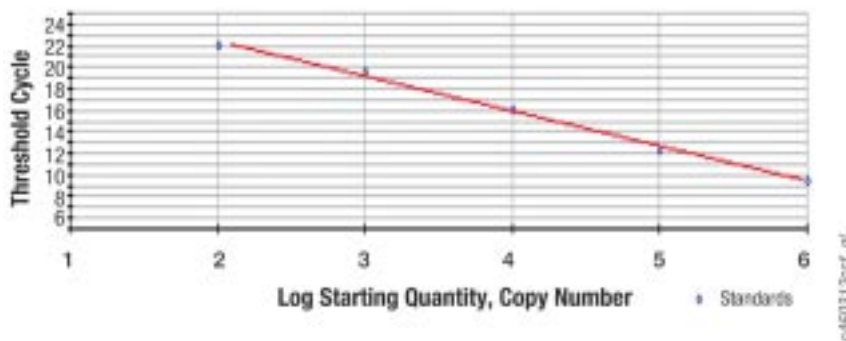


Figure 4. Standard curves with correlation coefficients greater than 0.99 and PCR efficiencies ranging from 98.8% to 104.7% were produced from the data collected for each CopyControl clone. Results obtained on serial dilutions of the CopyControl fosmid clone are shown here.

(Table 1). Induction of the larger 40-kb CopyControl Fosmid and 145-kb BAC clones resulted in 23.3-fold and 14.7-fold increases in copy number, respectively. The increase in copy number for other CopyControl clones will vary, depending on the insert size and sequence.

It is important to note that each CopyControl clone was induced for only three hours. Minimizing the amount of time each clone spends at high-copy number reduces the likelihood of DNA rearrange-

ment or the build-up of a toxic gene product. In contrast, when vectors are consistently held at high-copy number, the cloned DNA undergoes many more rounds of replication, which increases the probability that the clone will be mutated or lost.

References

1. Wild, J. et al. (2002) *Genome Research* **12**, 1434.
2. EPICENTRE Forum (2002) **9**(1), 1.

Clone (size)	CopyControl™ Induction Solution	C _T Value (average of triplicate reactions)	Copy Number (starting template quantity)	Fold Induction
PCR (13 kb)	-	18.28	(1.76 ± 0.1) × 10 ³	33.4
	+	13.19	(5.88 ± 0.9) × 10 ⁴	
Fosmid (40 kb)	-	15.92	(1.02 ± 0.03) × 10 ⁴	23.3
	+	11.53	(2.38 ± 0.2) × 10 ⁵	
BAC (145 kb)	-	16.65	(2.03 ± 0.2) × 10 ³	14.7
	+	12.79	(2.99 ± 0.1) × 10 ⁴	

Table 1. Results from real-time PCR assays used to determine the copy number of induced CopyControl™ clones.

www.epicentre.com/cbac.asp

CopyControl™ BAC Cloning Kit (BamH I)

CCBAC1B 1 Kit

CopyControl™ BAC Cloning Kit (EcoR 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.

CopyControl™ pCC1BAC™ (BamH I Cloning-Ready) Vector

CBAC311B 375 ng

CopyControl™ pCC1BAC™ (EcoR I Cloning-Ready) Vector

CBAC311E 375 ng

CopyControl™ pCC1BAC™ (Hind III Cloning-Ready) Vector

CBAC311H 375 ng

www.epicentre.com/epi300.asp

TransforMax™ EPI300™ Electrocompetent *E. coli*

EC300105 5 X 100 µl

EC300110 10 X 100 µl

Transformation efficiency > 1 × 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 × 10¹⁰ cfu/µg. Includes CopyControl™ Induction Solution and pUC19 control DNA.

www.epicentre.com/cfos.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.

www.epicentre.com/ccpcr.asp

CopyControl™ PCR Cloning Kit with TransforMax™ EPI300™ Electrocompetent *E. coli*

CCECPCR1 20 Reactions

CopyControl™ PCR Cloning Kit with TransforMax™ EPI300™ Chemically Competent *E. coli*

CCPCR1CC 20 Reactions