

An Improved Approach for Construction and Analysis of BAC Libraries Using the CopyControl™ pCC1BAC™ Vector Containing an Inducible High-Copy Origin

Dilara Begum, Mike Fianndt, and Jerry Jendrisak, EPICENTRE

Introduction

The low yield of DNA from single copy BACs is a bottleneck to high throughput processes such as BAC end sequencing, fingerprinting and shotgun library construction. EPICENTRE's new CopyControl™ BAC Cloning Kits, based on an important new cloning technology developed in the laboratory of Dr. Waclaw Szybalski,¹⁻³ allows the user to induce the BAC plasmid from single copy to 10–20 copies per cell, resulting in high yield of purer DNA, enough for several high-throughput processes from a single 1-ml DNA preparation.

The CopyControl BAC Cloning Kit utilizes the CopyControl™ pCC1BAC™ Vector and the TransforMax™ EPI300™ *E. coli* pCC1BAC (Figure 1), a derivative of pBeloBAC11 and EPICENTRE's pIndigoBAC-5, contains the *E. coli* F-factor single-copy origin of replication and *oriV*-a high-copy origin of replication. The TransforMax EPI300 cells have been engineered to contain a mutant *trfA* gene, whose gene product is required for initiation of replication of *oriV*, under tight control of an inducible promoter. BAC clones constructed in pCC1BAC and grown in TransforMax EPI300 cells are maintained as single copy when grown on LB-plates containing chloramphenicol. The CopyControl BAC clones can then be induced to multiple copies whenever desired by addition of CopyControl™ Induction Solution to a subculture of a clone.

Here we report an improved approach for construction of BAC libraries using the CopyControl BAC Cloning Kit and demonstrate the significant benefit of inducing the BAC clones from single copy to high copy number.

BAC library construction

A library of the *E. coli* genome was constructed using the CopyControl BAC Cloning Kit (*Hind* III). Complete protocols for CopyControl BAC Cloning Kits can be viewed on the EPICENTRE web site at www.epicentre.com/ccbac.asp. Briefly, high molecular weight (HMW) DNA plugs were prepared from *E. coli* genomic DNA and subjected to a pre-run followed by a partial digestion with *Hind* III

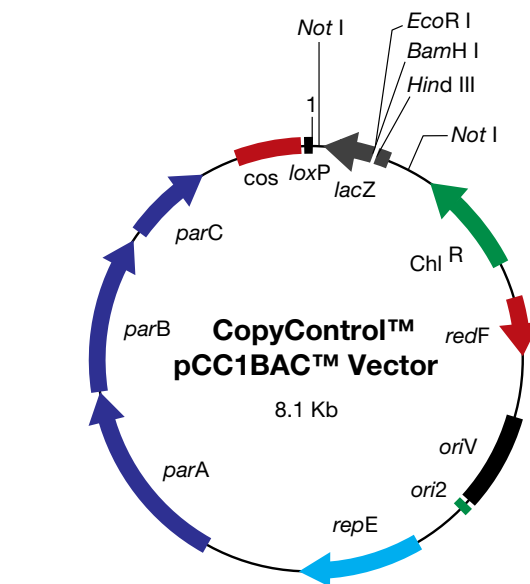


Figure 1. The CopyControl™ pCC1BAC™ Vector contains both the single-copy *E. coli* F-factor and the *oriV* high-copy origins of replication. pCC1BAC is supplied linearized (at its *Bam*H I, *Eco*R I, or *Hind* III site), dephosphorylated, and highly purified in the CopyControl™ BAC Cloning Kits.

restriction enzyme and finally size selection of the partially digested DNA by pulse field gel electrophoresis.^{4,5} Ligation reactions were performed using 100 ng of electro-eluted DNA from size selected 100-Kb to 150-Kb DNA fragments, 25 ng of the cloning-ready pCC1BAC (*Hind* III-cut) vector and 4 units of Fast-Link™ DNA Ligase at 16°C for 4 hours. The ligation reaction was desalted for 1 hour on ice and then 2 µl was electroporated into 50 µl of TransforMax EPI300 Electrocompetent *E. coli*. Following cell outgrowth, 100 µl of transformation reaction was plated on LB + chloramphenicol (12.5 µg/ml) + X-Gal + IPTG and the plate was incubated overnight at 37°C.

Sizing the BAC inserts without growing cultures and *Not* I digestions

The size of the inserts was determined by a rapid colony screening process without the need to grow cultures or perform restriction digests. Briefly, white colonies were randomly picked from the plate and processed, according to the product literature, using the EpiLyse™ Solution and EpiBlue™ Solution (included in the kits). An aliquot of each processed clone was individually loaded into a well of a 0.8% agarose gel and the BAC-Tracker™ Supercoiled DNA Ladder (included with the kit and available separately; see

page 12) was loaded as size markers. The gel was run for 3 hours at 4°C and stained with SYBR® Gold and the sizes of the BACs were estimated by comparing their mobility to that of the DNAs contained in the BAC-Tracker DNA Ladder. The size estimates obtained by this rapid colony screening process were confirmed by *Not* I digestion of each of the clones followed by CHEF gel electrophoresis.

Using the CopyControl BAC Cloning Kit (*Hind* III), we constructed an *E. coli* BAC library of 122,000 clones (3000X genome coverage) from a single 4-hour ligation reaction. The library contained >97% white colonies with an average insert size of 130 Kb as determined by both the 4-hour colony screening procedure (Figure 2A) and by *Not* I digest analysis (Figure 2B). The ability to screen a BAC library in 4 hours is a significant improvement because it eliminates the drop in transformation efficiency of the stored ligation reaction that occurs when BAC clones are sized by standard procedures that require overnight cultures and restriction digestions.

Induction of the CopyControl BAC clones to high-copy number

The most important feature of the CopyControl cloning technology is the

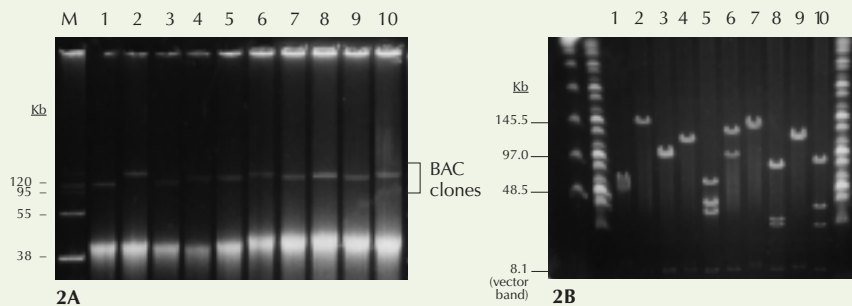


Figure 2. An average insert size of >120 Kb was determined in 4 hours. **2A:** Ten randomly picked CopyControl™ BAC clones were sized by the rapid colony screening procedure provided in the CopyControl™ BAC Cloning Kits and as described in the text. Total time including processing the clones, running and staining the gel was 4 hours. Lane M, BAC-Tracker™ Supercoiled DNA Ladder; Lanes 1-10, CopyControl BAC clones. **2B.** The same 10 clones were sized by standard methods which included overnight culture, DNA purification, *Not I* restriction digestion and CHEF gel electrophoresis. The two procedures gave comparable results thus validating the colony screening procedure provided in the kits.

ability to induce the CopyControl BAC clones from single-copy to high-copy number. Four CopyControl BAC clones, with insert sizes ranging from 128 Kb to 145 Kb were chosen to evaluate this process. An isolated colony of each of these clones was grown in 1 ml of culture medium and then induced to high-copy number by adding the CopyControl Induction Solution. These clones were designated as the “induced” clones. A duplicate sample of each clone was grown at single copy (designated “uninduced”) as control. DNA was isolated from an equal number of cells of both induced and uninduced cultures and digested with *Hind III* and the digests were analyzed by 1% agarose gel electrophoresis. The restriction analyses of the BAC DNAs from induced and uninduced cultures of the four CopyControl BAC clones is shown in Figure 3.

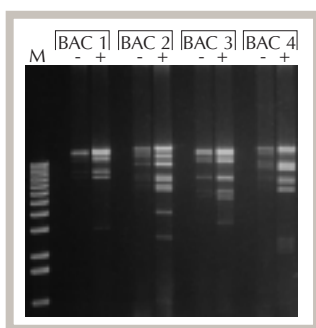


Figure 3. The copy number of CopyControl™ BAC clones induced to high copy number greatly improve analyses of the cloned inserts. One microliter of DNA, isolated from an equal number of cells from induced (+) and uninduced (-) cultures of four CopyControl BAC clones, was digested with *Hind III* and the digestion products analyzed by agarose gel electrophoresis.

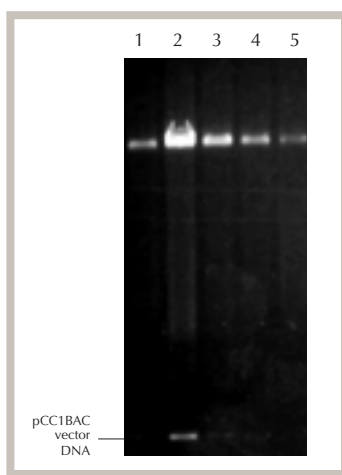


Figure 4. CopyControl™ BAC clones can be induced to 10 to 20 copies per cell. DNA was isolated from an equal number of cells of an induced and an uninduced culture of a 128-Kb CopyControl BAC clone. The DNA from the induced culture was diluted and then 1 µl of each sample was digested with *Not I* and digestion products resolved by PFGE followed by staining with SYBR®Gold. Visual comparison of the band intensity of DNA from the uninduced culture with the DNA dilutions from the induced culture indicate that induction resulted in 10 to 20 fold more BAC DNA from the same number of cells. Lane 1, DNA from uninduced 128-Kb clone; Lane 2, DNA from induced 128-Kb clone; Lanes 3-5, 1:5, 1:10 and 1:15 dilutions of DNA from the induced clone.

The clone copy number of the induced cultures was estimated by comparing the band intensity of the single-copy *Not I*-digested BAC DNA from the uninduced cultures with the band intensity of *Not I* digestion of 1:5, 1:10: and 1:15 serial dilutions of DNA from the induced cultures. As shown in Figure 4, the induced culture of the 128-Kb clone yielded ~15-fold more BAC DNA than the uninduced culture. Additionally, it was demonstrated that inserts are stably main-

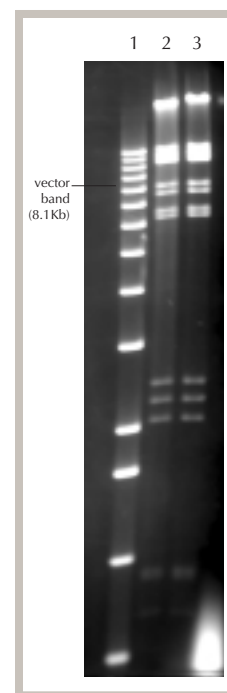
tained at single copy in the pCC1BAC Vector (Figure 5). A more complete investigation of the stability of CopyControl BAC clones at single copy and after induction to high-copy number will be presented in an upcoming issue of the *EPICENTRE Forum*.

Conclusion

The CopyControl BAC Cloning Kits provide an improved protocol for construction of BAC libraries that include a 4 hour ligation reaction and, significantly, the ability to screen the size of the clones in 4 hours.

The CopyControl pCC1BAC Vector, with its single-copy and *oriV* high-copy origins of replication, is the vector of choice for construction of large insert BAC libraries.

Figure 5. CopyControl™ BAC clones are stable at single copy. A 145-Kb CopyControl BAC clone was digested with *Hind III* before and after growth for 100 generations at single copy in TransformMax™ EPI300™ *E. coli*. The DNAs produced identical *Hind III* fingerprints demonstrating that the CopyControl BAC did not undergo deletions or rearrangements after 100 generations of growth. Lane 1, linear DNA size marker; Lane 2, *Hind III* fingerprint of the CopyControl BAC clone from generation 0; Lane 3, *Hind III* fingerprint of the CopyControl BAC clone after 100 generations.



Large inserts cloned into pCC1BAC Vectors and grown in TransformMax™ EPI300™ *E. coli* are stable when maintained at single copy and, when desired can be induced to 10 to 20 copies per cell for higher yields of purer DNA. Thus, the pCC1BAC Vector offers significant cost and time-saving advantages over all other low-copy BAC vectors for applications such as fingerprinting and BAC end-sequencing.

Construction of CopyControl™ BAC libraries from plant genomes

Drs. P. Piffanelli, C. Lanaud and J.C. Glaszmann BAC-TROP, CIRAD-AMIS, France have recently reported success in constructing BAC libraries from tropical

plant species using the CopyControl BAC Cloning Kits. Libraries constructed in the pCC1BAC Vector will be used as a resource for genome sequencing and map-based cloning of agronomically important genes. The ability of the pCC1BAC clones to induce to >15 fold makes the use of pCC1BAC Vector as an "ideal choice of vector" for high throughput sequencing and DNA fingerprinting.

Look for a complete article in an upcoming *Epicentre Forum*.

References

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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

Contents:

pCC1BAC™ (*Bam*H I) or pCC1BAC™ (*Eco*R I) or pCC1BAC™ (*Hind* III) Cloning-Ready Vector, Fast-Link™ DNA Ligase and Buffer, ATP, BAC-Tracker™ Supercoiled DNA Ladder, EpiBlue™ Solution, EpiLyse™ Solution, Control DNA Insert, and Control BAC Clone (145 Kb).

TransforMax™ EPI300™ Electrocompetent *E. coli*, required to induce CopyControl BAC clones to high copy number, are available separately.

TransforMax™ EPI300™ Electrocompetent *E. coli*

EC300105 5 X 100 µl

EC300110 10 X 100 µl

EC300150 50 X 100 µl

Contents:

Electrocompetent *E. coli* of >5 X 10⁹ cfu/µg, pUC19 Control DNA, and CopyControl™ Induction Solution.

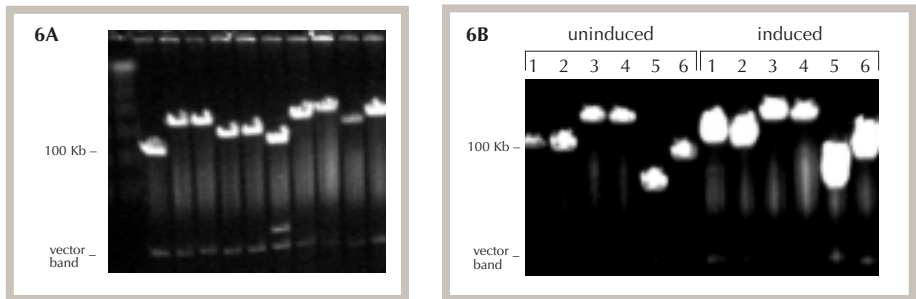


Figure 6. A cocoa plant genomic library was constructed at CIRAD-AMIS, France using the CopyControl™ BAC Cloning Kit (*Hind* III). **6A:** *Not* I-digest analysis of 10 CopyControl™ BAC clones. **6B:** Comparison of BAC DNA yield from an equal number of cells of uninduced and induced cultures of six clones.

Integrate CopyControl™ Capability into Existing BAC and Fosmid Clones

EPICENTRE's new EZ::TN™ <*ori*V /KAN-2> Insertion Kit enables researchers to integrate CopyControl™ capability into existing single-copy BAC and fosmid clones. The kit features the EZ::TN™ <*ori*V /KAN-2> Transposon, which contains the *ori*V high-copy origin of replication and a kanamycin selectable marker. A short, one-step *in vitro* reaction catalyzed by EZ::TN™ Transposase randomly inserts the transposon into existing BAC or fosmid clones. An aliquot of the transposition reaction is then used to transform TransforMax™ EPI300™ Electrocompetent *E. coli* (available separately) and insertion clones are selected by growth on kanamycin (Figure 1).

Obtain high yields of BAC and fosmid DNA for sequencing and fingerprinting

A single, reaction generates up to thousands of random transposon insertion clones. Like the CopyControl™

pCC1™ Vectors, BAC and fosmid clones containing the EZ::TN <*ori*V /KAN-2> Transposon can be maintained at single copy to ensure insert stability but can then be induced to 10 to 50 copies per cell whenever desired, to maximize the yield and purity of DNA for sequencing, fingerprinting and other applications.

Sequence bidirectionally from randomly distributed primer binding sites

Each insertion clone not only contains *ori*V and a kanamycin marker, but unique primer binding sites near the ends of the transposon. DNA flanking the transposon can be sequenced bidirectionally from these unique sites using the primers provided in the kit. Thus, insertion of the *ori*V-containing transposon accomplishes two functions; it converts a single-copy clone to one which has CopyControl capability and it generates a library of sequencing templates with random transposon insertions permitting complete sequencing of the clone with only two sequencing primers. The need for subcloning or primer walking strategies has been eliminated.

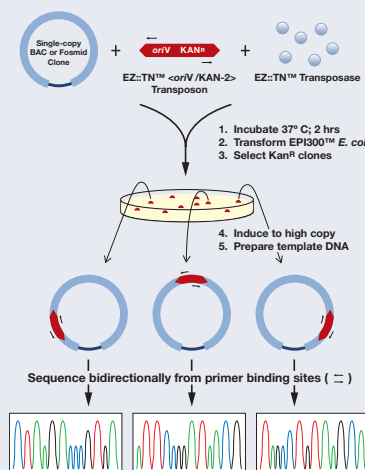


Figure 1. The process for generating EZ::TN™ <*ori*V /KAN-2> Transposon insertion clones for high yields of DNA and bidirectional sequencing.

www.epicentre.com/transposomics.asp

EZ::TN™ <*ori*V /KAN-2> Insertion Kit

EZI02VK 10 Reactions

Contents:

EZ::TN™ <*ori*V /KAN-2> Transposon, EZ::TN™ Transposase, EZ::TN™ 10X Reaction Buffer, EZ::TN™ 10X Stop Solution, Forward and Reverse Primers, Control Target DNA, and Sterile Water.

TransforMax™ EPI300™ Electrocompetent *E. coli*, required to induce EZ::TN™ <*ori*V /KAN-2> transposed clones to high copy number, are available separately.