

EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research

Construction of Four CopyControl™ BAC Libraries by BACTROP – a BAC-Based Platform to Study Tropical Plant Species

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BIOTROP is a unit of CIRAD (Centre de Cooperation Internationale en Recherche Agronomique pour le Development) the French scientific organization specializing in development-oriented agricultural research for the tropics and sub-tropics. The BIOTROP unit develops molecular technologies to study biodiversity of tropical species with the aim to create and identify well adapted genotypes, particularly in terms of their quality and their tolerance to the biotic and abiotic constraints of their environment.

At BIOTROP, we recently created BACTROP: a platform of BAC libraries for studying the genome structure and evolution of tropical plant species. BACTROP represents a valuable tool for map-based cloning of genes involved in quality traits (e.g., fruit maturation) and disease resistance and to initiate studies of linkage disequilibrium in tropical crop plants.

In 2001, we successfully constructed five BAC libraries using the pIndigoBAC-5 cloning vector from EPICENTRE. At the Plant and Animal Genome Conference (PAG X) in January, 2002 we learned of the advantages of EPICENTRE's new CopyControl™ BAC Cloning Kits. The ability to induce the CopyControl BAC clones from single-copy to higher-copy number to improve DNA yield for analysis was very appealing. Since January we have constructed an additional four BAC libraries using the CopyControl BAC Cloning Kits. Here we report our experiences constructing the CopyControl BAC libraries and inducing the clones to higher-copy number.

Constructing the CopyControl BAC libraries

Four CopyControl BAC libraries from banana, cocoa, coffee and coconut were

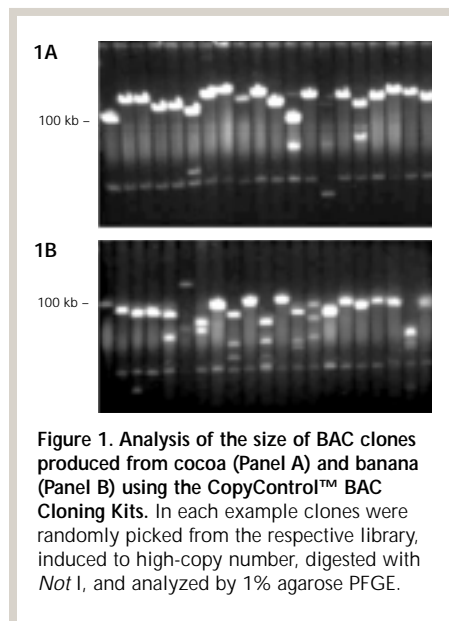


Figure 1. Analysis of the size of BAC clones produced from cocoa (Panel A) and banana (Panel B) using the CopyControl™ BAC Cloning Kits. In each example clones were randomly picked from the respective library, induced to high-copy number, digested with *Not* I, and analyzed by 1% agarose PFGE.

Species	Number of clones	Average insert size	Genome equivalents
Banana	36,864	135 kb	9
Coconut	92,160	135 kb	5
Coffee	55,296	135 kb	9
Cocoa	36,864	120 kb	11

Table 1. A summary of four CopyControl™ BAC libraries constructed by BACTROP using the CopyControl™ pCC1BAC™ (*Hind* III) Vector.

constructed using the CopyControl BAC Cloning Kit (*Hind* III). For each library, high molecular weight DNA was partially digested with *Hind* III. Ligation of the *Hind* III-cut DNA into the CopyControl™ pCC1BAC™ (*Hind* III) Vector was done for 3 hours at 30°C for the coconut library and overnight for the other three libraries. Each library contained less than 1% blue (non-recombinant)

colonies and very few empty white clones. The average insert size was determined by *Not* I digestion of randomly picked clones followed by 1% agarose PFGE (Figure 1). A summary of the four libraries is presented in Table 1.

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The low background and large insert sizes of the libraries demonstrated to us the high quality of the linearized and dephosphorylated pCC1BAC Vector preparation that is provided in the kits.

Induction of the CopyControl BAC clones to higher copy number

At BACTROP, like many laboratories, we have a need to rapidly analyze a large number of clones. We have developed a 96-deep well, high-throughput process for inducing the CopyControl BACs to high-copy number. We found the average induction level to be approximately 15-fold using this protocol. The large amount of DNA produced from each clone is sufficient for many applications including fingerprinting analysis for assembly of contigs, defining genomic

regions around genes of interest or Quantitative Trait Loci (QTLs).

The CopyControl system has also enabled high-throughput, direct sequencing of BAC-ends using template purified after induction to high-copy number.

Conclusion

The CopyControl technology developed at EPICENTRE has enabled the rapid development of the BACTROP platform and exploitation of BAC libraries representing the genomes of tropical species. The advantages of the CopyControl system will accelerate the analysis and sequencing of BAC clones of interest. We can then rapidly identify genomic regions containing quality traits or resistance genes to phytopathogens of tropical plants.

www.epicentre.com/ccbac.asp

CopyControl™ BAC Cloning Kit (<i>Bam</i>H I)	
CCBAC1B	1 Kit
CopyControl™ BAC Cloning Kit (<i>Eco</i>R I)	
CCBAC1E	1 Kit
CopyControl™ BAC Cloning Kit (<i>Hind</i> III)	
CCBAC1H	1 Kit

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

Contents:

Cloning-Ready pCC1BAC™ Vector (linearized at either its *Bam*H I, *Eco*R I or *Hind* III site and dephosphorylated), Fast-Link™ DNA Ligase, Fast-Link™ 10X Buffer, ATP, BAC-Tracker™ Supercoiled DNA Ladder, EpiBlue™ Solution, EpiLyse™ Solution, Control Genomic DNA Insert, and Control CopyControl™ BAC Clone.

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

www.epicentre.com/epi300.asp

TransforMax™ EPI300™ Electrocompetent <i>E. coli</i>	
EC300105	5 X 100 µl
EC300110	10 X 100 µl
EC300150	50 X 100 µl
TransforMax™ EPI300™ Electrocompetent <i>E. coli</i> are required to induce CopyControl™ BAC clones to high-copy number.	

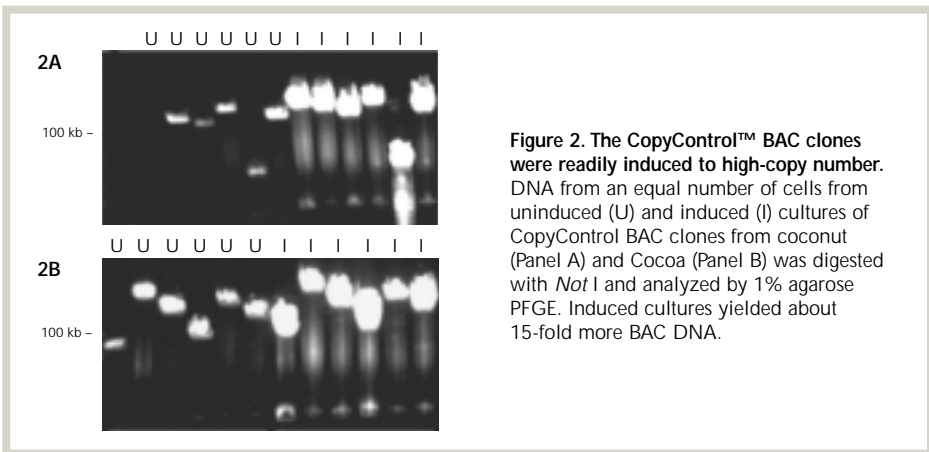


Figure 2. The CopyControl™ BAC clones were readily induced to high-copy number. DNA from an equal number of cells from uninduced (U) and induced (I) cultures of CopyControl BAC clones from coconut (Panel A) and Cocoa (Panel B) was digested with *Not*I and analyzed by 1% agarose PFGE. Induced cultures yielded about 15-fold more BAC DNA.

How the CopyControl™ BAC Cloning Kits Work

The CopyControl BAC Cloning Kits—based on technology developed in the laboratory of Dr. Waclaw Szybalski¹ at the University of Wisconsin-Madison—enable researchers to make and maintain BAC clones at single-copy number to ensure insert stability and then, whenever desired, to induce the clones to high-copy number for high yields of DNA for fingerprinting and DNA sequencing.

The pCC1BAC™ Vectors, provided in the kits, contain both 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 supplied by the TransforMax EPI300™ *E. coli* that contain the *trfA* gene under tight control of an inducible promoter.

In the absence of *trfA* gene induction, replication of CopyControl pCC1 clones 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 BAC clones grown in culture induces expression of the *trfA* gene resulting in initiation of replication from *oriV* and amplification of the clone to 10–20 copies per cell.

CopyControl capability can be easily introduced into existing single-copy BAC and fosmid clones (see the center insert). In addition, a CopyControl Fosmid Library Production Kit and CopyControl PCR Cloning Kits are available (see the center insert).

References

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

