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.

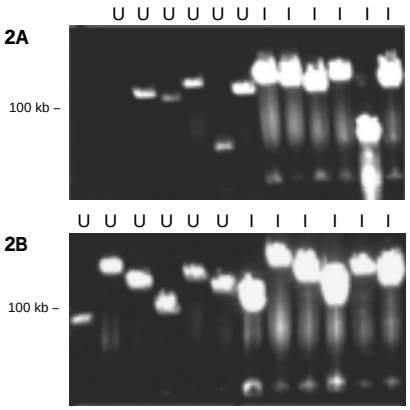


Figure 2. The CopyControl™ BAC clones were readily induced to high-copy number. DNA from an equal number of cells from uninduced (U) and induced (I) cultures of CopyControl BAC clones from coconut (Panel A) and Cocoa (Panel B) was digested with *Not* I and analyzed by 1% agarose PFGE. Induced cultures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

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.

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 *E. coli*

EC300105 5 X 100 µl
EC300110 10 X 100 µl
EC300150 50 X 100 µl

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

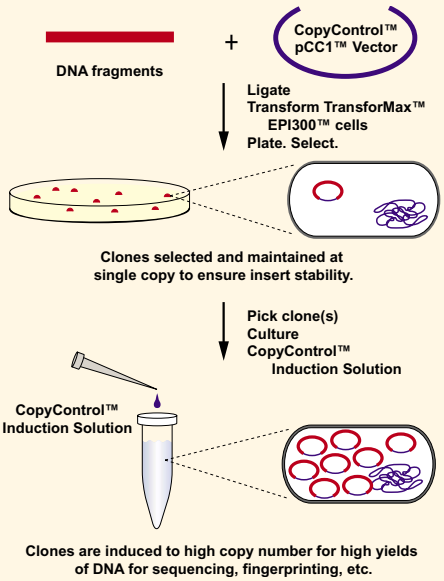
How the CopyControl™ BAC Cloning Kits Work

The CopyControl BAC Cloning Kits–based on technology developed in the laboratory of Dr. Waclaw Szybalski[1] 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.