

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

1. Hradecna, Z. et al. (1998) *Microbial and Comp. Genomics* **3**, 58.
2. Wild, J. et al. (2001) *Plasmid* **45**, 142.
3. Wild, J. et al. (2002) *Genomic Research* (submitted).
4. Daniel, G. et al. www.plantgenome.uga.edu/dgp/hub/pdf_files/bacman2.pdf
5. Osoegawa, K. et al. (1998) *Genomics* **52**, 1.
6. Shizuya, H., et al. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 8794.

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.

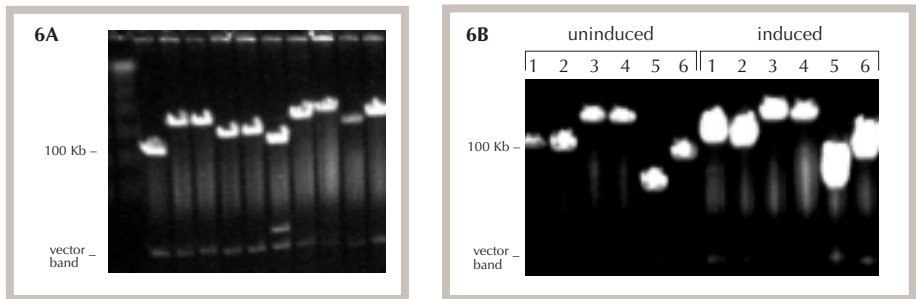


Figure 6. A cocoa plant genomic library was constructed at CIRAD-AMIS, France using the CopyControl™ BAC Cloning Kit (*Hind* III). **6A:** *Not* I-digest analysis of 10 CopyControl™ BAC clones. **6B:** Comparison of BAC DNA yield from an equal number of cells of uninduced and induced cultures of six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

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.

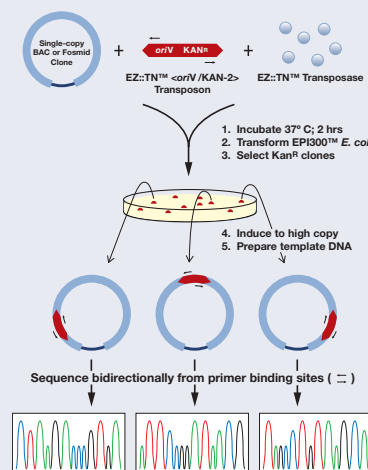


Figure 1. The process for generating EZ::TN™ <*ori*V /KAN-2> Transposon insertion clones for high yields of DNA and bidirectional sequencing.