

# Save At Least 2 Days Constructing a CopyControl™ BAC Library Then Amplify the Clones to 10 – 20 Copies Per Cell, for Higher DNA Yields and Purity

The CopyControl™ BAC Cloning Kits combine the advantages of the CopyControl cloning technology with an improved cloning protocol that significantly reduces the time and labor normally required to construct a BAC library. CopyControl BAC clones are initially grown in TransformMax™ EPI300™ Electrocompetent *E. coli* at single copy to ensure insert stability and cloning of potentially toxic expressed DNA segments. Once the library is produced, individual CopyControl BAC clones can be amplified, by addition of the CopyControl Induction Solution, to 10 – 20 copies per cell, to maximize the yield and purity of DNA for sequencing and fingerprinting.

Each of the CopyControl BAC Cloning Kits contain sufficient reagents to construct the equivalent of one 10X human library. The high transformation efficiency TransformMax™ EPI300™ Electrocompetent *E. coli* cells contain the inducible mutant *trfA* gene required for induction of CopyControl BAC clones to high copy number. These electrocompetent cells are available separately (see page 8).

## The CopyControl BAC Cloning Kits Reduce the Time Needed to Construct a BAC Library by At Least 2 Days

In constructing a BAC library, two of the most laborious and time-consuming steps are preparing the BAC cloning vector and

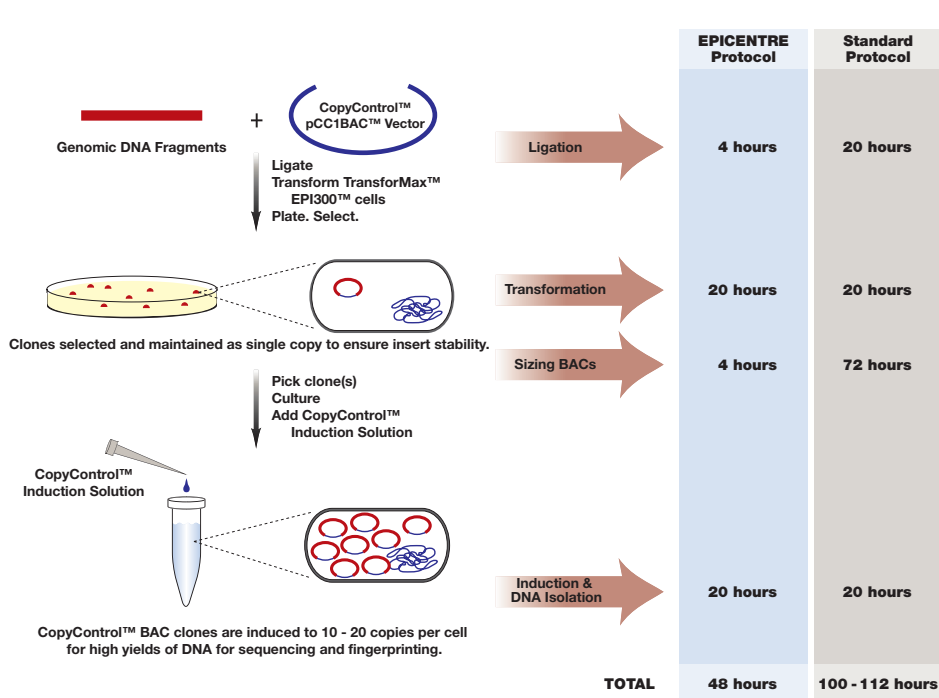


Figure 2. The CopyControl™ BAC Cloning Kits reduce the time to construct a BAC library by 2 days or more compared to standard methods.

size screening the BAC clones produced. The kits supply the cloning-ready CopyControl pCC1BAC™ Vector (Figure 1) - linearized at its *Bam*H I, *Eco*R I or *Hind* III site, dephosphorylated and then extensively purified – thus eliminating the need for and the quality concerns of vector preparation. The size of the BAC clones

produced can be estimated by agarose minigel electrophoresis in as little as 4 hours, without the need for minipreps or restriction endonuclease digestion (Figure 4B). Additionally, reaction conditions have been optimized to provide 4-hour DNA ligations. Other unique and time-saving components of the kits include the BAC-Tracker™ Supercoiled DNA Ladder, composed of supercoiled DNA gel markers of 38 Kb – 120 Kb (see page 7), a Control DNA Insert for testing ligation efficiency, and a 145 Kb CopyControl BAC clone for testing transformation efficiency and the clone amplification process.

## CopyControl BAC Clones Can Be Amplified to 10 – 20 Copies Per Cell for High DNA Yields for Sequencing and Fingerprinting

Addition of the CopyControl Induction Solution to individual cultures of CopyControl BAC clones results in amplification of the clones from single copy to 10 – 20 clone copies per cell. A 1 – 2 ml culture of an induced CopyControl BAC clone yields microgram amounts of BAC DNA for fingerprinting (Figure 3) and sequencing. An additional benefit of amplifying the BAC clones is the higher

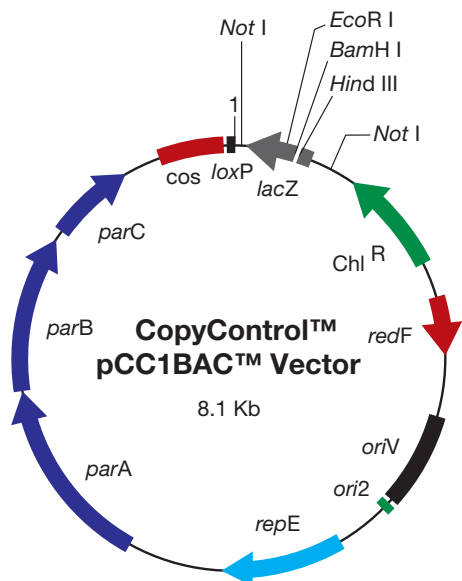
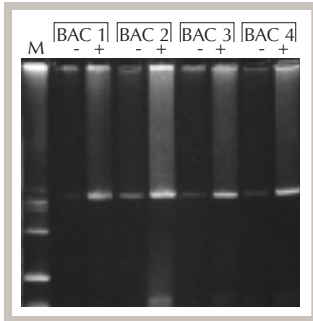


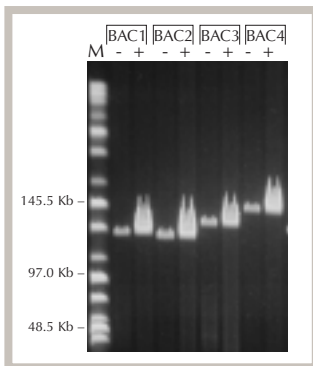
Figure 1. The CopyControl™ pCC1BAC™ vectors are supplied linearized at either the unique *Bam*H I, *Hind* III or *Eco*R I site, completely dephosphorylated and highly purified to ensure very low background. pCC1BAC is derived from pBeloBAC11 and EPICENTRE's pIndigoBAC-5.

proportion of BAC DNA relative to *E. coli* chromosomal DNA, resulting in higher BAC clone DNA purity.

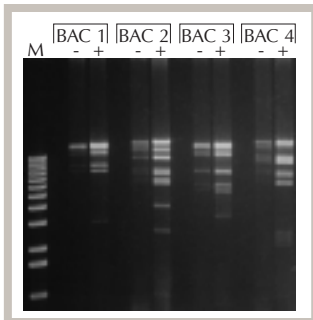
**Figure 3. The copy number of CopyControl™ BAC clones can be induced 10 - 20 fold to greatly increase DNA yield.** Four CopyControl BAC clones (BAC 1 = 130 Kb, BAC 2 = 125 Kb, BAC 3 = 140 Kb, BAC 4 = 145 Kb) were grown in culture in duplicate. One sample of each was induced (+) to high copy by addition of CopyControl™ Induction Solution. The other sample was an uninduced (-) control. DNA was isolated from an equal number of cells of each and analyzed by agarose gel (Panel A), *Not* I digestion (Panel B) and *Hind* III digestion (Panel C).



**3A. One microliter of DNA from induced (+) and uninduced (-) cultures were resolved on a 0.8% agarose gel for 3 hours at 4°C and stained with SYBR®Gold.** The induced cultures yielded 10 - 20 fold more CopyControl BAC clone DNA as determined by gel electrophoresis. M, supercoiled DNA ladder.



**3B. *Not* I digests of 2 µl of DNA from induced (+) and uninduced (-) cultures resolved by PFGE and stained with SYBR®Gold.** M, PFG marker.



**3C. *Hind* III digests of 1 µl of DNA from induced (+) and uninduced (-) cultures.** M, 1 Kb DNA ladder.

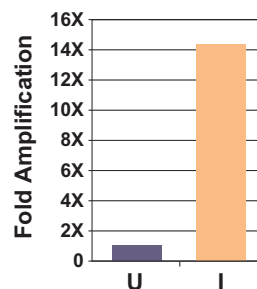
**4A. A *Hind* III genomic BAC library was constructed from *E. coli* using the CopyControl™ BAC Cloning Kit (*Hind* III).** A 100 µl aliquot from a 1 ml transformation of TransformMax™ EPI300™ Electrocompetent *E. coli* was plated. The kit produced > 95% white recombinant clones.

**4B. The size of the CopyControl™ clones can be estimated and the quality of the library can be assessed in 4 hours.** Nine randomly chosen CopyControl BAC clones were picked from the plate in Panel 3A and processed using the EpiLyse™ and EpiBlue™ screening solutions provided in the kit. The supercoiled DNA released from the cells was resolved in a 0.8% agarose gel at 4.5 V/cm for 3 hours at 4°C and stained with SYBR®Gold. M, supercoiled DNA ladder.

### Cloning-Ready pCC1BAC Vectors and TransformMax EPI300 Electrocompetent *E. coli* Are Extensively Tested for Quality

The cloning-ready CopyControl pCC1BAC Vector provided in each kit is tested to ensure low background and high cloning efficiency. Each batch of the vectors is tested to produce >95% white (recombinant) colonies after ligation of the Control Insert (provided with each kit) and transformation into TransformMax™ EPI300™ Electrocompetent *E. coli*. In addition, the integrity of the *Bam*H I, *Eco*R I or *Hind* III ends is confirmed by a vector self-ligation assay after 5'-phosphorylation of the cohesive ends.

The TransformMax EPI300 Electrocompetent *E. coli* (available separately; see page 8 for additional information) are tested to give >1 X 10<sup>7</sup> cfu/µg (>10<sup>21</sup> cfu/mole) when transformed with a 145 Kb CopyControl BAC clone.



**Figure 5. The yield of a BAC DNA from CopyControl™ BAC clones of 110 Kb - 145 Kb increased > 14 fold when grown in TransformMax™ EPI300™ cells and induced to high copy number by addition of the CopyControl™ Induction Solution.** I, induced cells. U, uninduced control cells.

[www.epicentre.com/ccbac.asp](http://www.epicentre.com/ccbac.asp)

- CopyControl™ BAC Cloning Kit (*Bam*H I)**  
CCBAC1B 1 Kit
- CopyControl™ BAC Cloning Kit (*Hind* III)**  
CCBAC1H 1 Kit
- CopyControl™ BAC Cloning Kit (*Eco*R I)**  
CCBAC1E 1 Kit

Each Kit contains sufficient reagents to construct the equivalent of one 10X human library.

**Contents:**

CopyControl™ pCC1BAC™ (*Bam*H I, *Eco*R I, or *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 CopyControl™ BAC Clone (145 Kb).

**TransformMax™ EPI300™ Electrocompetent *E. coli***

(formerly called TransformMax™ EC300™ Electrocompetent *E. coli*)

- EC300105 5 x 100 µl
- EC300110 10 x 100 µl
- EC300150 50 x 100 µl

**pCC1BAC™/pIndigoBAC-5 Forward Sequencing Primer**

- BFP0701 50 µM 1 nmole

**pCC1BAC™/pIndigoBAC-5 Reverse Sequencing Primer**

- BRP0801 50 µM 1 nmole