

Retrofit Existing BAC and Fosmid Clones with CopyControl™ Capability Using a Kit for Simple *In Vitro* Insertion of an *OriV*-containing EZ::TN™ Transposon

Many libraries have already been generated and are available in exclusively single-copy BAC or fosmid vectors. EPICENTRE's new EZ::TN™ <*oriV* /KAN-2> Insertion Kit enables researchers to integrate CopyControl™ capability into these clones. The kit features the EZ::TN™ <*oriV* /Kan-2> Transposon, which contains the *oriV* 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, see page 8) and insertion clones are selected by growth on kanamycin (Figure 1).

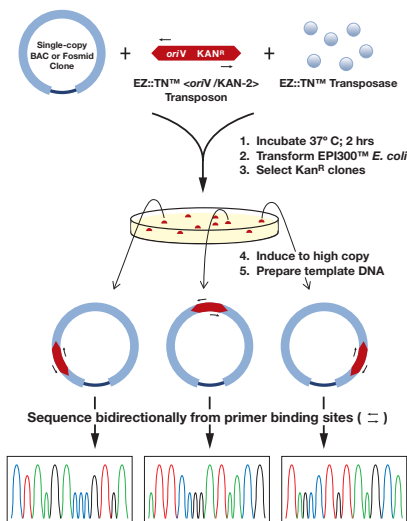


Figure 1. The process for generating EZ::TN™ <*oriV* /KAN-2> Transposon insertion clones for high yields of DNA and bidirectional sequencing.

Obtain High Yields of BAC and Fosmid DNA for Sequencing and Fingerprinting

A single, 10- μ l transposition reaction generates up to thousands of random transposon insertion clones. Each clone can be induced to 10 – 50 copies per cell by the addition of the CopyControl™ Induction Solution. Like the CopyControl™ pCC1™ Vectors, BAC and fosmid clones retrofitted with the EZ::TN <*oriV* /KAN-2> Transposon can be maintained at single copy to ensure insert stability but can then be induced to high copy number 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 *oriV* and a kanamycin marker, but unique primer binding sites near the ends of the transposon. DNA flanking the transposon can be sequenced bidirectionally from

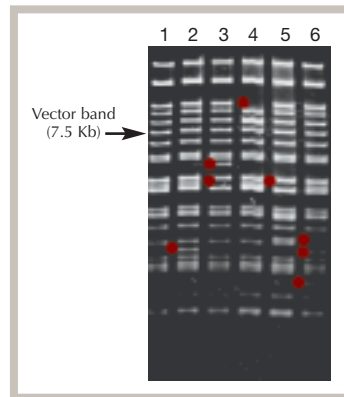


Figure 2. Unique *Hind* III fingerprints confirm that insertions of the EZ::TN™ <*oriV* /KAN-2> Transposon into a 165 Kb BAC are random.

Amplified DNA was isolated from individual insertion clones, digested with *Hind* III, and resolved on a 1% agarose gel. Lane 1, parental BAC clone; Lane 2-6, BAC insertion clones. ● indicate altered band positions resulting from a transposon insertion.

these unique sites using the primers provided in the kit. Thus, insertion of the *oriV*-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 (Figure 2), 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™ <*oriV* /KAN-2> Insertion Kit

EZI02VK 10 Reactions

Contents:

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

TransforMax™ EPI300™ Electrocompetent *E. coli*

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

EC300105 5 x 100 μ l
EC300110 10 x 100 μ l
EC300150 50 x 100 μ l

BAC-Tracker™ Supercoiled DNA Ladder

EPICENTRE's new BAC-Tracker™ Supercoiled DNA Ladder is suitable for estimating the size of large supercoiled DNAs, such as BAC (Bacterial Artificial Chromosome) clones, by agarose gel electrophoresis.

The BAC-Tracker Ladder contains four discreet supercoiled DNAs of 38 Kb, 55 Kb, 95 Kb, and 120 Kb.

The Ladder is provided in a ready-to-load solution. Simply load 10 μ l of the Ladder per gel lane. Run the gel and stain using ethidium bromide or SYBR® Gold.

Applications

- Size analysis of BAC clones by agarose minigel or Pulse Field Gel Electrophoresis (e.g., CHEF, FIGE).
- Size analysis of supercoiled plasmid or extra-chromosomal DNA from any source.

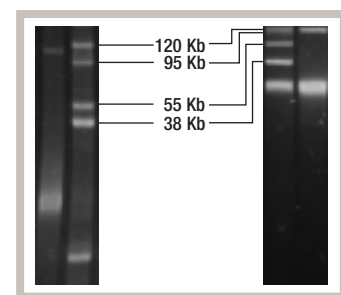


Figure 1. A purified 115 Kb BAC clone sized using the BAC-Tracker™ Supercoiled DNA Ladder by PFGE (Panel A) and by 1% agarose minigel (Panel B). Gels were stained using SYBR® Gold.

www.epicentre.com/bactrack.asp

BAC-Tracker™ Supercoiled DNA Ladder

BT010950 50 gel lanes (500 μ l)