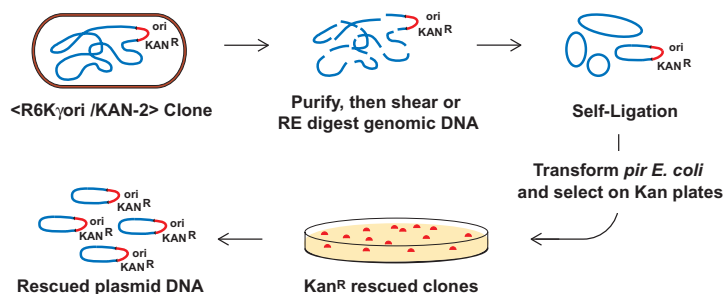


## Rescue Cloning of Bacterial Genomic DNA using the EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™

A «Transposome™» is a stable complex between an EZ::TN™ Transposon and EZ::TN™ Transposase that forms spontaneously in the absence of magnesium cations. The complex is so stable that it can be used to transform competent *E. coli* and other microorganisms. Once in the cell, the EZ::TN Transposase is activated by intracellular magnesium cations and randomly inserts a single EZ::TN Transposon into the chromosome or extrachromosomal DNA of each cell *in vivo*. EPICENTRE offers EZ::TN Transposomes with transposons that encode kanamycin-, tetracycline-, or trimethoprim-resistance genes. Insertion clones are selected by plating on medium containing the respective antibiotic. Also, insertions into genes can create functional gene knockouts, which can often be screened for or selected based on a change in phenotype.

In addition to encoding a kanamycin-resistance gene, the EZ::TN Transposon in the new EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™ contains a conditional origin of replication (R6Kγori). The presence of this origin of replication enables easy rescue cloning of the gene or region of DNA containing the transposon for each insertion clone (Figure 1).



**Figure 1. The process for rescue cloning of transposon insertion sites in genomic DNA using the EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™ and TransformMax™ EC100D™ *pir*<sup>+</sup> or TransformMax™ EC100D™ *pir*-116 Electrocompetent *E. coli*.**

Rescue of insertion clones is extremely powerful for functional and genetic analysis. With *E. coli*, greater than 10<sup>4</sup> transposon insertion clones are obtained following electrotransformation with only 1 µl of EZ::TN <R6Kγori /KAN-2>Tnp Transposome. A gel with examples of typical rescue clones is shown in Figure 2.



**Figure 2. Gel analysis of 11 rescue clones.**

Lane 1, size markers; Lanes 2-12, rescue clones of *E. coli* chromosomal DNA produced using EZ::TN <R6Kγori /KAN-2>Tnp Transposome.

Rescue cloning of the region of host genomic DNA containing the inserted transposon is a three step process:

1. Purify approximately 1 µg of genomic DNA from a single chosen insertion clone or from a pooled population of clones using the MasterPure™ DNA Purification Kit. Fragment the genomic DNA by digestion with restriction endonuclease(s) or by random shearing.
2. Self-ligate the genomic DNA fragments with a DNA Ligase (e.g. Fast-Link™ DNA Ligase). Genomic DNA fragments produced by random shearing or by digestion with multiple restriction endonucleases must first be end-repaired (made blunt-ended) and then 5'-phosphorylated using, for example, the End-It™ DNA End-Repair Kit (available separately).
3. Transform an aliquot of the ligation reaction into an *E. coli* cell line that expresses the Π protein (*pir* gene product), such as TransformMax™ EC100D™ *pir*<sup>+</sup> or TransformMax™ EC100D™ *pir*-116 Electrocompetent *E. coli* (see p. 15) and grow on plates containing kanamycin. Only those clones containing the EZ::TN <R6Kγori /KAN-2> Transposome will grow. Yields of >10<sup>4</sup> rescue clones per µg of genomic DNA are typically seen.

Two unlabeled sequencing primers that are homologous to the ends of the inserted transposon are provided for bidirectional sequencing of the rescue clones.

**Note:** To randomly insert an R6Kγori into a cloning vector or other DNA *in vitro*, use the new EZ::TN™ <R6Kγori /KAN-2> Insertion Kit described on p. 15.

### EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™

TSM08KR 10 Reactions

Includes two unlabeled sequencing primers.

### MasterPure™ Complete DNA & RNA Purification Kit

MC89010 10 Purifications

### End-It™ DNA End-Repair Kit

ER0720 20 Reactions

For end-repair of up to 100 µg of DNA.

[www.epicentre.com/catalog/r6ktnp.htm](http://www.epicentre.com/catalog/r6ktnp.htm)