

Rapidly Rescue Clone Transposed Genomic DNA

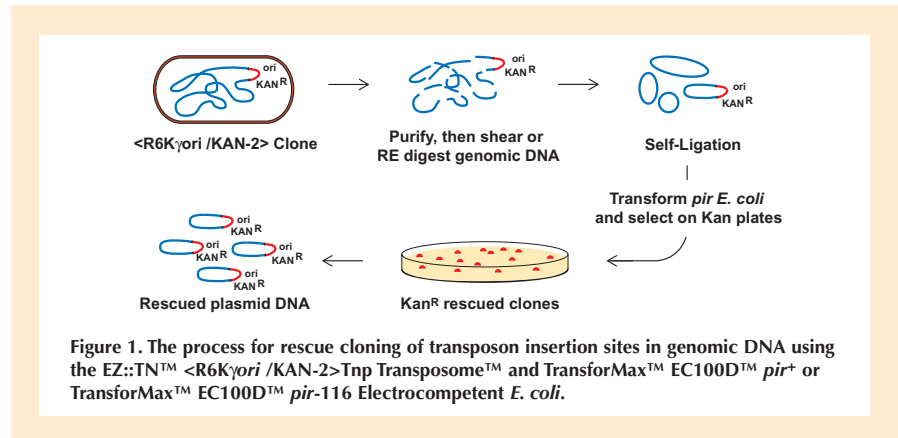
Among the advantages of transposon mutagenesis is that the transposon serves as a marker that can be used to clone and sequence the region of genomic DNA that has been disrupted. Nothing makes this cloning process easier than creating mutations *in vivo* with the EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™. In addition to encoding a kanamycin-resistance gene, the transposon contains an *E. coli* conditional origin of replication (R6Kγori). The presence of this origin of replication enables you to propagate or “rescue” the region of genomic DNA into which the transposon has been inserted in a three step process (Figure 1):

- 1** Purify genomic DNA from a single kanamycin resistant insertion clone or a pool of clones, and then fragment the DNA by digestion with restriction endonuclease(s) or by random shearing.
- 2** Self-ligate the genomic DNA fragments using Fast-Link™ DNA Ligase or another suitable ligase. For efficient ligation of randomly sheared DNA, the ends of the DNA can be repaired using the End-It™ DNA End-Repair Kit (see p. 11).
- 3** Transform an *E. coli* host expressing the π protein (*pir* gene product), such as TransforMax™ EC100D *pir*⁺ or TransforMax™ EC100D *pir*-116 Electrocompetent *E. coli* and select on kanamycin plates. Only those clones containing the EZ::TN <R6Kγori /KAN-2> Transposon will grow.

Rescue clones can then be sequenced bidirectionally using the provided primers that are homologous to the ends of the transposon.

Make your own R6Kγori containing transposon

EPICENTRE now offers the EZ::TN™ pMOD™-3<R6Kγori /MCS> Transposon Construction Vector (Figure 2) so that you can quickly and easily prepare a custom EZ::TN Transposon that can also be used for rescue cloning. To prepare your transposon, clone any DNA sequence of interest (e.g. selectable marker, control element, reporter gene) into the multiple cloning site and then prepare the transposon by PCR amplification using the Forward and Reverse PCR Primers provided with the vector, or by restriction enzyme digestion with *Pvu* II or *PshA* I.



The transposon can be incubated with EZ::TN Transposase in the absence of Mg²⁺ to form an EZ::TN Transposome for random insertion into the genomic DNA of living cells (see page 14). DNA flanking the transposon insertion site can then be rescued by modifying the three step process outlined above to include a selection process other than kanamycin resistance. Your custom EZ::TN Transposon can also be used for insertion into any target DNA *in vitro*. *In vitro* transposition of R6Kγori containing transposons can be used, for example, to rescue plasmids which ordinarily do not replicate in *E. coli* because they lack a recognizable origin of replication and/or a selectable marker.

Your custom EZ::TN Transposon will also include unique primer binding sites at either end for bidirectional sequencing of the insertion site using the pMOD™ <MCS> Forward and Reverse Sequencing Primers (available separately). No need to design your own primers.

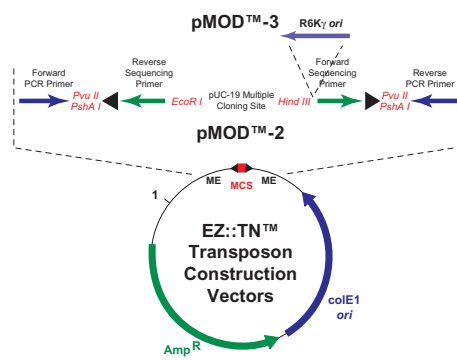


Figure 2. A custom EZ::TN™ Transposon containing any sequence of interest can be prepared using a Transposon Construction Vector. Transposons made with the pMOD™-3 vector can also be used for rescue cloning.

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

TSM08KR-F83 10 Reactions
Kit contains pre-formed Transposome™ and two unlabeled sequencing primers.

EZ::TN™ pMOD™-2<MCS> Transposon Construction Vector

MOD0602-F83 20 μg
Includes: pMOD™-2<MCS> Vector and the Forward and Reverse PCR Primers.

EZ::TN™ pMOD™-3<R6Kγori /MCS> Transposon Construction Vector

NEW!
MOD1503-F83 20 μg
Includes: pMOD™-3<R6Kγori /MCS> Vector and the Forward and Reverse PCR Primers.

EZ::TN™ Transposase

TNP92110-F83 10 Units

TransforMax™ EC100D™ *pir*⁺ Electrocompetent *E. coli*

ECP09500-F83 5 X 100 μl
(10 Electroporations)
Maintains clones at 15 copies per cell. Includes control vector containing an R6Kγori.

TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli*

EC6P095H-F83 5 X 100 μl
(10 Electroporations)
Maintains clones at 250 copies per cell. Includes control vector containing an R6Kγori.