

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

The new EZ::TN™ <R6Kγori /KAN-2> Insertion Kit can be used to randomly insert the *E. coli* R6Kγ conditional origin of replication (R6Kγori) into target DNA *in vitro*. The EZ::TN <R6Kγori /KAN-2> Transposon contains the R6Kγori and a kanamycin selection marker. A single 2-hour *in vitro* reaction randomly inserts the <R6Kγori /KAN-2> Transposon into the target DNA. Use an aliquot of the reaction to transform *E. coli* hosts expressing the *pir* gene product (Π protein) such as EPICENTRE's TransforMax™ EC100D™ *pir*⁺ or TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli* and select on kanamycin plates. Only those clones harboring DNA containing the EZ::TN <R6Kγori /KAN-2> Transposon will grow.

Use the EZ::TN <R6Kγori /KAN-2> Insertion Kit to:

- Introduce the R6Kγori into any cloning vector.
- Propagate vectors from non-*E. coli* species in *E. coli*.
- Propagate genomic DNA fragments from any species as independently replicating DNA in *E. coli*.

Two unlabeled Sequencing Primers, homologous to the ends of the inserted transposon, are provided in the kit for bidirectional sequencing of the transposon insertion clones.

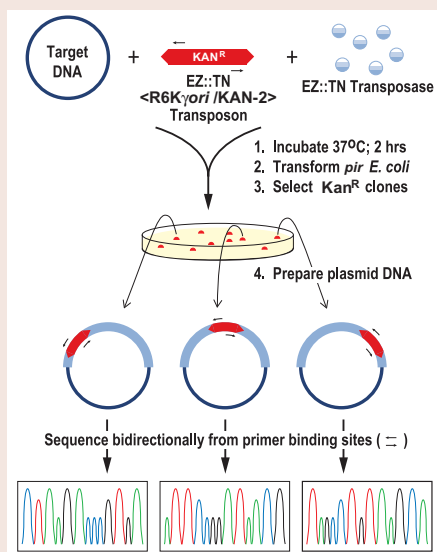


Figure 1. The process for generating and sequencing R6γori - containing clones.

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

EZ1011RK

10 Reactions

Contains: EZ::TN™ <R6Kγori /KAN-2> Transposon, EZ::TN™ Transposase, EZ::TN™ 10X Reaction Buffer, EZ::TN™ 10X Stop Solution, KAN-2 FP-1 Forward Sequencing Primer, R6KAN-2 Reverse Sequencing Primer, Control Target DNA, Sterile Water

Electrocompetent *E. coli* for Rescue Cloning

TransforMax™ EC100D™ *pir*⁺ Electrocompetent *E. coli* and TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli* each express the Π protein (*pir* gene product) for replication of vectors containing the R6Kγ conditional origin of replication (R6Kγori). The cells are derived from EPICENTRE's TransforMax™ EC100™ Electrocompetent *E. coli* by P1 phage transduction with a strain containing the *pir*⁺ or *pir*-116 gene linked to a dihydrofolate reductase (DHFR) marker. Both cell strains can be used for propagation of vectors and rescue clones transposed by the EZ::TN <R6Kγori /KAN-2> Transposon.

Transformation Efficiency

Greater than 1 X 10⁹ cfu/μg supercoiled DNA

Genotypes

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

Maintains plasmids at approximately 15 copies per cell¹. F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80d*lacZ*ΔM15 Δ*lacX74* *recA1* *endA1* *araD139* Δ(*ara, leu*)7697 *galU* *galK* λ⁻ *rpsL* *nupG* *pir*⁺(DHFR).

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

Maintains plasmids at approximately 250 copies per cell¹. F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80d*lacZ*ΔM15 Δ*lacX74* *recA1* *endA1* *araD139* Δ(*ara, leu*)7697 *galU* *galK* λ⁻ *rpsL* *nupG* *pir*-116(DHFR).

Applications and Important Phenotypes

- Expresses the Π protein for propagation of vectors containing the R6Kγori.
- Blue/white screening of vectors expressing the LacZ' α-complementing peptide.
- Restriction minus for efficient cloning of methylated (e.g. mammalian genomic) DNA.
- Recombination minus (*recA1*) to ensure the stability of large cloned inserts.

Reference

1. Metcalf, W.M. *et al.* (1994) *Gene* **138**,1

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

ECP09500

5 X 100 μl

(10 Electroporations)

Includes control vector containing an R6Kγori.

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

EC6P095H

5 X 100 μl

(10 Electroporations)

Includes control vector containing an R6Kγori.

www.epicentre.com/catalog/ecpir.htm