

New

Direct Selection of Genes Encoding Non-Cytoplasmic Proteins

The EZ::TN™ β -Lactamase Fusion Kit was developed for the direct selection of genes encoding membrane and secreted proteins. The kit features the EZ::TN™ $\langle blaM/R6K\gamma ori \rangle$ Transposon, which contains a β -lactamase gene (*blaM*) that lacks both promoter and secretory signal sequences.

Screen a clone or library of clones with a simple, one-step *in vitro* reaction that randomly inserts a single EZ::TN™ $\langle blaM/R6K\gamma ori \rangle$ Transposon into the target DNA. Then, transform *E. coli* cells with an aliquot of the reaction and plate on media containing ampicillin (Figure 1, A). Only insertion clones with transcriptional fusions to genes encoding extracytoplasmic proteins will grow. These fusions generate hybrid proteins that can transport the *blaM* moiety through the inner membrane and confer resistance to ampicillin (Figure 1, B).

Once Amp^R clones are selected, use the primer binding sites at the ends of the EZ::TN™ $\langle blaM/R6K\gamma ori \rangle$ Transposon to map or bidirectionally sequence the

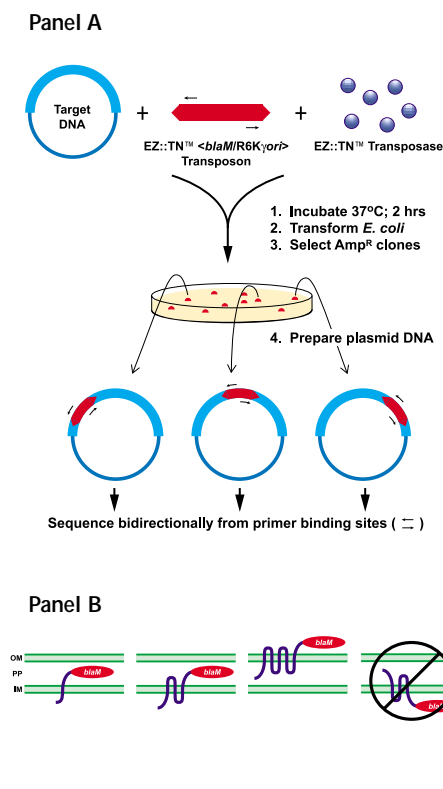


Figure 1. The process for selecting cloned genes encoding membrane and secreted proteins with the EZ::TN™ β -Lactamase Fusion Kit (Panel A). Only fusions that transport the *blaM* moiety through the inner membrane will grow on ampicillin (Panel B).

insertion site with primers provided in the kit. When large genomic clones (e.g., fosmids, cosmids, or BACs) are screened, DNA flanking the insertion site can be subcloned or “rescued” as an Amp^R plasmid that replicates from the R6K γ origin of replication also contained on this EZ::TN Transposon (see center insert).

www.epicentre.com/transposomics.asp

EZ::TN™ β -Lactamase Fusion Kit

EZ131BL 10 Reactions

Contents:

EZ::TN™ Transposase, EZ::TN™ $\langle blaM/R6\gamma ori \rangle$ Transposon, Reaction Buffer, Stop Solution, Forward and Reverse Primers, Control Target DNA, and Sterile Water.

Efficient Mapping of Functional Domains or Epitopes of Proteins

The EZ::TN™ In-Frame Linker Insertion Kit was designed to rapidly and easily produce random 19-amino acid (19 codon) in-frame insertions into genes of expressed proteins to facilitate mapping of functional domains or epitopes.

The kit features the EZ::TN™ $\langle Not I/KAN-3 \rangle$ Transposon, which contains a kanamycin resistance marker flanked by *Not I* restriction sites. A simple *in vitro* reaction catalyzed by EZ::TN™ Transposase randomly inserts this transposon into target DNA. Following transformation of *E. coli*, a library of $>10^6$ independent Kan^R insertion clones is obtained. Insertion clones can be identified for further analysis by altered activity, restriction mapping, or sequencing from the ends of the transposon.

Once clones are chosen, the kanamycin-resistance gene is excised from the EZ::TN™ $\langle Not I/KAN-3 \rangle$ Transposon by *Not I* digestion. Each *Not I*-digested clone is then ligated and re-transformed into

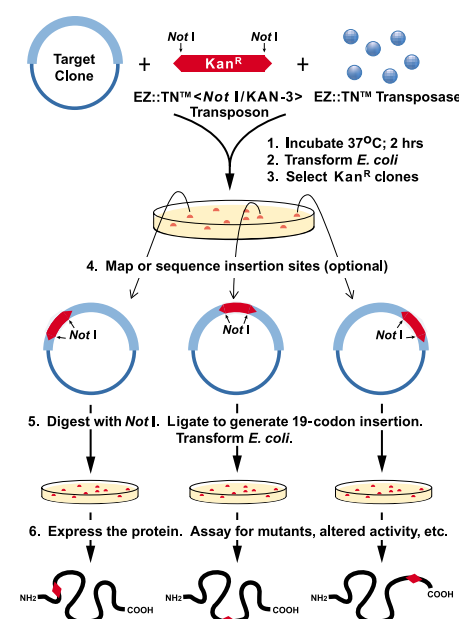


Figure 1. The EZ::TN™ $\langle Not I/KAN-3 \rangle$ Transposon contains a kanamycin resistance gene flanked by *Not I* restriction sites. A 19-codon insertion that can be read in all three reading frames is generated following *Not I* digestion and ligation.

E. coli (Figure 1). Since the ends of the transposon have been modified to eliminate translational stops, the resulting clones each contain a random 19-codon insertion that can be read in all three reading frames. Thus, the protein is unchanged except for the random insertion of 19 amino acids.

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EZ::TN™ In-Frame Linker Insertion Kit

EZ104KN 10 Reactions

Contents:

EZ::TN™ Transposase, EZ::TN™ $\langle Not I/KAN-3 \rangle$ Transposon, Reaction Buffer, Stop Solution, Forward and Reverse Primers, Control Target DNA, and Sterile Water.