

# Create Random Gene Knockouts in Living Cells Using the EZ::TN™ Transposome™

A transposon is a DNA sequence that “hops” or transposes into a DNA molecule in a reaction catalyzed by a transposase enzyme. These genetic elements have been used in applications such as distributing sequencing primer binding sites, creating gene knockouts, and introducing a variety of markers for genetic analysis. EZ::TN™ Transposomes™ provide an efficient and reliable method of randomly inserting transposons into the genomes of many different genera.

## A simple one-step transposition system

An EZ::TN Transposome is the stable complex formed by incubating an EZ::TN™ Transposon with EZ::TN™ Transposase in the absence of Mg<sup>2+</sup>. EZ::TN Transposomes are so stable that they can be electroporated into many living cells that can be transformed by electroporation. Once in the cell, the EZ::TN Transposome is activated by Mg<sup>2+</sup> and the EZ::TN Transposon is randomly inserted into the host's genomic DNA (Figure 1).<sup>1</sup>

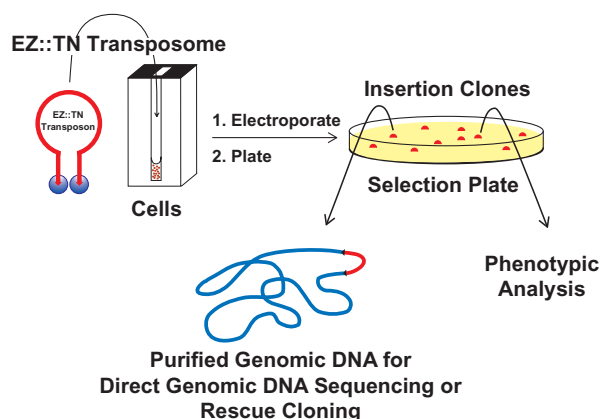
No other transposition system is so simple or versatile. The only requirement is that DNA can be introduced into the host by electroporation. There is no need for cell conjugation, suicide vectors or specific host factors.

## A broad host range system

EZ::TN Transposomes have already been used successfully by scientists working with a variety of different species including *Escherichia*, *Corynebacterium*, *Mycobacterium*, *Pseudomonas*, *Proteus*, *Salmonella*, *Xylella*, *Saccharomyces* and more.<sup>1-4</sup> Various methods (e.g. Southern blot and sequence analysis) have verified that insertion mutants from these studies are both random and stable. Combined with ease of use, these properties make the EZ::TN Transposome ideal for generating libraries of mutants in species that have poorly described genetic systems or lack adequate molecular tools.

**Table 1. Average number of Kan<sup>R</sup> transposon insertion clones produced from electroporation of 1 µl of EZ::TN™ <KAN>Tnp Transposome™.**

<i>E. coli</i>	>10 <sup>5</sup>
<i>Salmonella typhimurium</i>	>10 <sup>4</sup>
<i>Pseudomonas sp</i>	>10 <sup>2</sup>
<i>Proteus vulgaris</i>	>10 <sup>3</sup>
<i>Mycobacterium smegmatis</i>	>10 <sup>2</sup>



**Figure 1.** An EZ::TN™ Transposome™ can be electroporated into living cells where it randomly inserts the transposon component into the host's genomic DNA. The EZ::TN Transposon insertion site can be analyzed by a variety of methods.

The number of transposition clones obtained is highly dependent on the transformation efficiency of the host cell (Table 1). The higher the transformation efficiency of the cell, the more clones will be produced. Electroporation of *E. coli* with a transformation efficiency of >10<sup>9</sup> cfu/µg typically results in >10<sup>5</sup> independent insertion clones when 1 µl of EZ::TN Transposome is used.

Pre-formed EZ::TN Transposomes are available containing either a kanamycin selectable marker (<KAN-2> or <R6Kγori /KAN-2>) derived from Tn903 or a dihydrofolate reductase gene (<DHFR-1>) that can be selected on plates containing trimethoprim.

## Create your own Transposome

You can create your own EZ::TN Transposome using one of the EZ::TN™ pMOD™<MCS> Transposon Construction Vectors (see page 15) and EZ::TN Transposase. To prepare your transposon, clone the DNA of interest into the multiple cloning site and then release the transposon by PCR or by digestion with *Pvu* II or *Psh* A I. Nanogram amounts of transposon DNA are then incubated with EZ::TN Transposase in the presence of glycerol to form an EZ::TN Transposome for random insertion into genomic DNA. A custom EZ::TN Transposon might include antibiotic resistant determinants for insertional mutagenesis, a reporter gene to facilitate studies of gene regulation and protein localization, or rare restriction enzyme sites for genome mapping.

## Sequencing your gene knockout is easy

EZ::TN Transposons contain unique primer binding sites at either end for bidirectional sequencing. Hence, once a gene knockout has been selected the affected gene can be sequenced directly using bacterial genomic DNA as template and primers homologous to the ends of the inserted transposon. The transposon insertion can also be “rescued” and the flanking DNA sequenced when mutations are made with an EZ::TN Transposon containing an *E. coli* conditional origin of replication (R6Kγori) (see page 15).

## References

- Goryshin, I.Y. et al. (2000) *Nature Biotechnol.* **18**, 97.
- Derbyshire, K. M. et al. (2000) *EPICENTRE Forum* **7**(2), 1.
- Guilhabert, M. R. et al. (2000) *EPICENTRE Forum* **8**(2), 1.
- Abstr. Annu. Meet. Am. Soc. Microbiol. (2001) Losada, L. C. et al., B107, p. 64, Marra, D. et al., B99, p.62.

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

TSM99K2-F83 10 Reactions

### EZ::TN™<DHFR-1>Tnp Transposome™ Kit

TSM99D1-F83 10 Reactions

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

TSM08KR-F83 10 Reactions

Each Transposome™ kit contains the specific EZ::TN Transposome complex and two unlabeled sequencing primers.