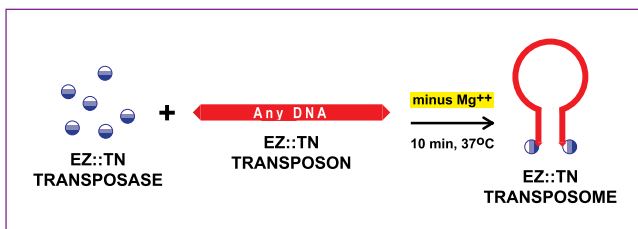


EZ::TN™ Transposome™ Frequently Asked Questions

In the previous article Dr. Keith Derbyshire *et al.* demonstrate the use of an EZ::TN Transposome to generate auxotrophic mutations in mycobacteria. EZ::TN Transposomes, which can be used to create gene knockouts in living cells and to facilitate direct sequencing of bacterial genomic DNA without cloning, have generated a great deal of interest among scientists working with a variety of organisms. Here, we provide answers to some of the most frequently asked questions received by our Technical Consultants.

What is an EZ::TN Transposome?

An EZ::TN Transposome is the stable complex formed between an EZ::TN Transposon—containing any DNA sequence of interest—and the hyperactive EZ::TN Transposase in the absence of Mg²⁺. An EZ::TN Transposome is so stable that it can be electroporated directly into cells where it is activated by the intracellular Mg²⁺. Once activated, the transposome randomly inserts its transposon component into the host's genomic DNA.



How efficient is an EZ::TN Transposome?

The most critical parameter affecting transposition efficiency is the transformation efficiency of the cell. The higher the transformation efficiency of the cell, the more clones will be produced. Electroporation of competent cells using an EZ::TN Transposome is less efficient than transformation with a small plasmid. Therefore, use cells with the highest transformation efficiency possible.

Additionally, the selectable marker in the transposon must be expressed in the cell to a high enough level to confer resistance to the insertion clones. Therefore, it may be necessary to plate electroporated cells on media containing different amounts of the antibiotic or other selection agent to detect the insertion clones.

Table 1. Number Kan^R transposon insertion clones generated by electroporation of 1 µl of EZ::TN <KAN>Tnp Transposome.

| <i>E. coli</i> | <i>Salmonella typhimurium</i> | <i>Proteus vulgaris</i> | <i>Pseudomonas sp.</i> | <i>Mycobacteria smegmatis</i> |
|-----------------------|-------------------------------|-------------------------|------------------------|-------------------------------|
| 1-5 x 10 ⁵ | 1-5 x 10 ⁴ | 1-5 x 10 ³ | 1-5 x 10 ² | 1-5 x 10 ² |

How random is an EZ::TN Transposon insertion?

The Tn5 transposition system, upon which the EZ::TN system is based, is a highly random transposon system. However, keep in mind that transposon insertions into essential host genes are likely to be lethal and thus will not be represented in the insertion clone library produced.

Can I make a custom EZ::TN Transposon containing a DNA sequence of my own design?

Yes. Custom EZ::TN Transposons containing virtually any DNA sequence of interest (e.g., species-specific selectable markers, control elements, cDNA) can be constructed using EPICENTRE's EZ::TN™ pMOD<MCS> Transposon Construction Vector. For a special offer on this vector see page 4.

Can EZ::TN Transposomes be used to transform gram-positive microorganisms, yeast or mammalian cells?

In theory, yes. However the transformation efficiency of the cell and availability of a selectable marker expressed in the host will be important parameters to success. Again, the higher the transformation efficiency of the cell, the more clones that will be generated. EZ::TN Transposons containing species-specific selection markers and control elements can be constructed using the EZ::TN pMOD<MCS> Transposon Construction Vector.

“I consider the EZ::TN Transposome one of the most important new tools for genetic manipulation of non-E. coli bacteria that has come along in the last 5 years.”

— Dr. Barry Hall,
Univ. of Rochester
Biology Dept.

To learn what others are saying about EZ::TN Transposomes, visit www.biowire.com and search for EZ::TN Transposome.