

# Ask Frank

by Fred and Hank



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## Questions about *In Vivo* Transposomics

**Q.** What is an EZ-Tn5™ Transposome™?

**A.** A Transposome is the stable synaptic complex formed between a transposase, and a transposon. In the case of EPICENTRE's patented EZ-Tn5 System, an EZ-Tn5 Transposome is generated by incubating an EZ-Tn5 Transposon with EZ-Tn5 Transposase in the absence of Mg<sup>++</sup>.

**Q.** Does the EZ-Tn5 Transposome system use a suicide plasmid to deliver the transposon to the chromosomal DNA of living cells?

**A.** No, it does not. The beauty of the EZ-Tn5™ Transposome™ system is that no suicide plasmids are required. The purpose of suicide plasmids in other *in vivo* transposition systems (such as mini-Tn5 and Himar mariner), is to provide the transposase gene, which is required for these systems to function. By eliminating the need for a suicide plasmid, and by using the engineered EZ-Tn5 Transposase (a hyperactive form of Tn5 transposase available from EPICENTRE Biotechnologies), researchers can realize much higher transposon mutagenesis frequencies in a wide variety of microorganisms.

**Q.** Can I make my own EZ-Tn5 Transposome with my gene of choice?

**A.** Yes, but first you need to create an EZ-Tn5 Transposon (any piece of DNA that is between 19 bp inverted repeat Mosaic Ends [ME] sequences). EPICENTRE offers the family of pMOD™ vectors with these Mosaic Ends to make this cloning easier. The pMOD vectors also offer a variety of origins of replication useful for different downstream applications.

Once the DNA marker is cloned into the pMOD vector, the transposon DNA can be generated either by the Polymerase Chain Reaction (PCR) or by a restriction enzyme digest. To build your Transposome, simply mix the appropriate amounts of your purified EZ-Tn5 Transposon DNA, EZ-Tn5 Transposase and Glycerol, and incubate for 30 minutes at room temperature. The length of the DNA portion of the transposome will have an affect on transformation efficiency. The longest Transposome we are aware of that has been used in bacteria is about 12.0 kb.

**Q.** Has my bacteria been mutagenized by EZ-Tn5 Transposomes? Do the Transposomes function well in gram positive bacteria?

**A.** The number of bacterial types that have been used by our customers continues to grow; we have citations for 57 different bacteria (both gram positive and gram negative), one for *Saccharomyces cerevisiae* and one for *Trypanosoma brucei*. These citations can be found at our regularly updated website (<http://www.EpiBio.com/transcite.asp>). We encourage our customers to share their successes with EZ-Tn5 Transposomes so that they benefit all of our customers.

In general, gram positive organisms are more difficult to transform using electroporation. In some cases the selectable markers used (other than EPICENTRE's standard Kanamycin and dihydrofolate reductase genes) require more 'gram positive-friendly' promoters. We recommend the pMOD Transposon Construction Vectors for researchers looking to generate their own transposons.

**Q.** What factors do I need to consider in order to assure a successful Transposome mutagenesis experiment?

**A.** There are three major factors that affect the outcome of EZ-Tn5 transposon mutagenesis experiments. The first is the transformation efficiency of the bacterium by electroporation. We recommend that the transformation efficiency by electroporation be at least  $1 \times 10^6$  cfu/μg using a plasmid that will replicate in that particular bacterial strain. Chemically mediated transformation has never yielded successful results. The second factor is the selectable marker used in the transposon. It must be robust and able to function in that strain of bacteria; this may require the construction of a custom transposon containing a 'good' promoter and a selectable marker that is known to function in the strain. Finally, the presence of endogenous restriction enzymes in the cells can cause greatly reduced transformation efficiency. Restriction enzymes will degrade transposon DNA readily if there is a recognition site for that endonuclease in the cell. To help reduce this problem, we offer the TypeOne™ Restriction Inhibitor (Catalog number TY0261H) for use in Transposome mutagenesis experiments where the possibility of restriction enzyme interference is present. TypeOne will not work against Type II restriction endonucleases.

For more information on *in vivo* transposomics and other EPICENTRE products, visit us at:

ASM Meeting

May 21-25 - Toronto  
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