

The EZ-Tn5™ Transposome™ Complex Simplifies Microbial Engineering

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Introduction

Man has exploited the metabolic diversity of the microbial world in many different disciplines. A list includes bioremediation, industrial synthesis of pharmaceutical and agrochemical intermediates, and the search for alternative energy sources. A critical step in this exploitation of microbial diversity is often the introduction of a gene or set of genes from one organism into a heterologous host. However, as the definition of heterologous host moves well beyond that of familiar lab standards, like *Escherichia coli* and *Bacillus subtilis*, effective tools for genetic manipulations have not.

Plasmids, for example, require a functional origin of replication, constant selective pressure, and are notoriously unstable—especially at high-copy numbers. The high levels of gene expression often associated with plasmids can also be problematic, resulting in inclusion body formation, an increase in amino acid misincorporation, and incomplete or inaccurate posttranslational modification. Each of these constraints makes it even more challenging to achieve the correct balance of enzyme activities and metabolites *in vivo*.

Homologous recombination as a means of strain construction also has limitations. If the host lacks efficient natural recombination capabilities this must be overcome by the addition of recombination functions from other organisms. Moreover, when using linear DNA constructs, native nuclease activities must be deleted or repressed. Homologous recombination also requires that a portion of the DNA to be inserted shares significant homology with the host.



FIG 1. An EZ-Tn5™ Transposome™ is the stable complex formed by incubating an EZ-Tn5™ Transposon with EZ-Tn5™ Transposase in the absence of Mg²⁺. ‘ME’ stands for ‘Mosaic Ends’ referring to modified transposon end sequences.

EPICENTRE Biotechnologies’ patented EZ-Tn5™ Transposition System

EZ-Tn5 Transposome™ complexes, in contrast to the methods described above, are ideal for introducing DNA into microorganisms that have poorly described genetic systems or lack adequate molecular tools. An EZ-Tn5 Transposome is the stable synaptic complex formed between an EZ-Tn5™ Transposon and EZ-Tn5™ Transposase in the absence of Mg²⁺ (FIG 1). EZ-Tn5 Transposomes are so stable that they can be electroporated into many living cells. Once in the cell, the Transposome is activated by Mg²⁺ within the hosts’ cellular environment, enabling the Transposome to efficiently and randomly insert its transposon into the genomic DNA of the host cell. The transposase is subsequently degraded in the cell, resulting in no further transposition events. Although not required, the transposons typically contain a selectable marker such as an antibiotic resistance gene. Cells containing the transposon are then selected by screening for the desired antibiotic resistance phenotype (FIG 2).

An EZ-Tn5 Transposon can be any DNA sequence that is between two 19-bp Mosaic End sequences specifically and uniquely recognized by EZ-Tn5 Transposase. Custom Transposomes are easily generated using one of EPICENTRE

Biotechnologies’ EZ-Tn5 pMOD™ Transposon Construction Vectors and EZ-Tn5 Transposase. Unlike other *in vivo* transposition systems, the only requirement for using the EZ-Tn5 Transposome is that DNA is electroporated into the host. There is no need for cell conjugation, suicide vectors, or specific host factors. Scientists have successfully used Transposomes with a variety of different organisms including gram-negative and gram-positive bacteria, yeast, and even a protozoan (visit: www.EpiBio.com/transcite.asp for more than 30 EZ-Tn5 Transposome citations). Various methods—including Southern blot and sequence analysis—have verified that the insertions in these studies are both random and stable.

Conclusion

EPICENTRE’s EZ-Tn5™ Transposomes—stable synaptic complexes between transposase and a transposon—greatly simplify microbial engineering. Following electroporation into a host cell, Transposomes generate highly random transposon insertions that are completely stable because there is no transposase gene lingering in the cell. Transposomes are ideal for use in species that have a poorly described genetic system, and are readily customized with any DNA sequence of interest.

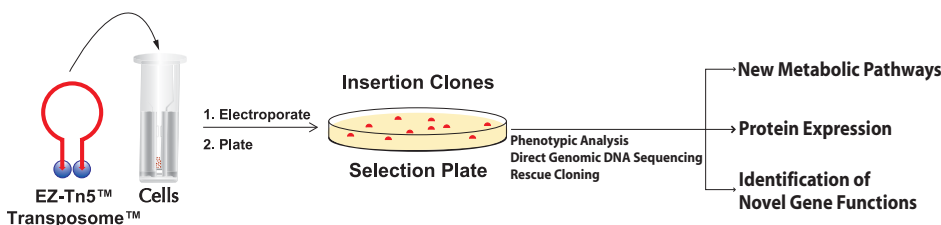


FIG 2. An EZ-Tn5™ Transposome™ Complex can be electroporated into living cells where it randomly inserts the transposon component into the host’s genomic DNA.

www.EpiBio.com/transposomics.asp

EZ-Tn5™ Transposase	
TNP92110	10 Units
Contents: EZ-Tn5™ Transposase, EZ-Tn5™ 10X Reaction Buffer, EZ-Tn5™ 10X Stop Solution and Sterile Water. EZ-Tn5™ Transposase is also available in bulk. Please inquire.	
EZ-Tn5™ pMOD™-2<MCS> Transposon Construction Vector	
MOD0602	20 µg
EZ-Tn5™ pMOD™-3<R6Kγori/MCS> Transposon Construction Vector	
MOD1503	20 µg
EZ-Tn5™ pMOD™-4<MCS> Transposon Construction Vector	
MOD4804	20 µg
EZ-Tn5™ pMOD™-5<R6Kγori/MCS> Transposon Construction Vector	
MOD4805	20 µg