

Prepare Custom EZ::TN™ Transposons by PCR Using Primers with Transposase-Specific Mosaic End (ME) Sequences

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An EZ::TN™ Transposon can be made from any DNA sequence by placing it between two 19-basepair inverted repeat Mosaic End (ME) sequences that are uniquely and specifically recognized by EZ::TN Transposase (Figure 1). EPICENTRE provides a number of pre-made EZ::TN Transposons for a variety of applications (e.g., see www.epicentre.com/transposomics.htm). One way to make custom EZ::TN Transposons is to clone DNA of interest into the multiple cloning site of one of a series of EZ::TN pMOD™<MCS> Transposon Construction Vectors available from EPICENTRE (see page 15). In this report we present an alternative method, for generating an EZ::TN Transposon called the “ME-Tailed PCR Method”.

The ME-Tailed PCR Method for making custom EZ::TN Transposons is shown in Figure 2. The DNA of interest, which can be anything - a resistance marker, a gene, a control element, etc. - is amplified by PCR using primers that, in addition to having 3'-sequences homologous to the template, also have non-homologous tails with 19-base ME sequences at their 5'-ends. PCR amplification of the template using these ME-Tailed primers produces an EZ::TN Transposon that can be used directly, without further purification, for *in vitro* insertion into any DNA target.

Materials and Methods

PCR templates

Three different DNA templates – a plasmid, genomic DNA, and a PCR product - were amplified using the ME-Tailed PCR Method. The three templates were an

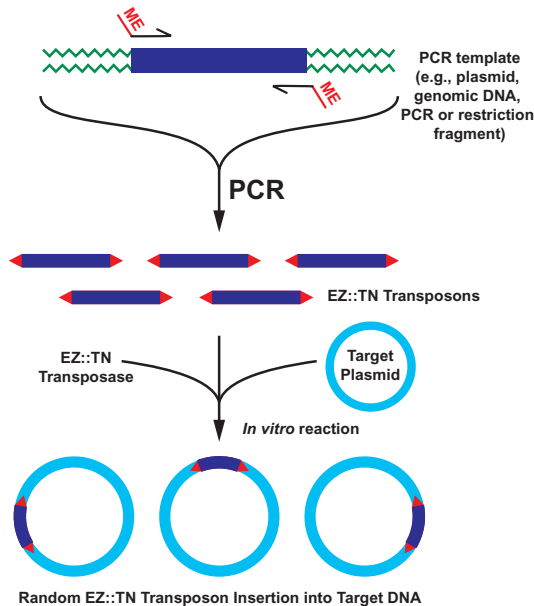


Figure 2. An EZ::TN™ Transposon can be rapidly produced from any DNA template by PCR using primers that contain the 19-base ME sequence at their 5'-ends. The EZ::TN Transposon produced can be used, without purification, for efficient and random insertion into a target DNA, *in vitro*. The ◀▶ designate the ME sequence at the ends of the EZ::TN Transposon.

1108-bp kanamycin resistance marker (Kan^R) in pJK1; an 887-bp Dihydrofolate Reductase (DHFR) gene in *E. coli* strain BW19851 genomic DNA; and the same 887-bp DHFR gene as a PCR product.

ME-tailed PCR primers

ME-Tailed PCR primers were designed to contain the 19-base EZ::TN Transposon ME sequence at their 5'-ends in addition to at least a 19-base sequence, homologous to the template at the 3'-ends. For example, the PCR primers used to produce a Kan^R EZ::TN Transposon from pJK1 were:

5'-CTGTCTCTTATACACATCTCT-
CAAAATCTCGATGTTACATTGC-3'

5'-CTGTCTCTTATACACATCTGGTTGAT-
GAGAGCTTTGTTGTAGGT-3'

The sequence shown in black is homologous to the template while sequence shown in red is the 19-base ME sequence.

PCR conditions

PCR reactions were performed in 50 µl containing 50 pmoles of each primer, 1 ng of template DNA, 2.5 U of FailSafe™ PCR Enzyme Mix, and either FailSafe PCR 2X PreMix C for amplifying the Kan^R gene or FailSafe PCR 2X PreMix D for amplifying the DHFR gene. Cycling conditions used for the Kan^R EZ::TN Transposon production were: 94°C for 2 minutes followed by 30 cycles of 1 minute at 94°C, 1 minute at 63°C, 1.5 minutes at 72°C.

The yield of each EZ::TN Transposon PCR product was determined by fluorometry. The EZ::TN Transposons produced by the ME-Tailed PCR Method were designated EZ::TN <KAN> and EZ::TN <DHFR>. The “< >” denote the 19-base ME sequence.

In vitro insertion of EZ::TN Transposons into target DNA

The EZ::TN <KAN> and EZ::TN <DHFR> Transposons produced by PCR were used

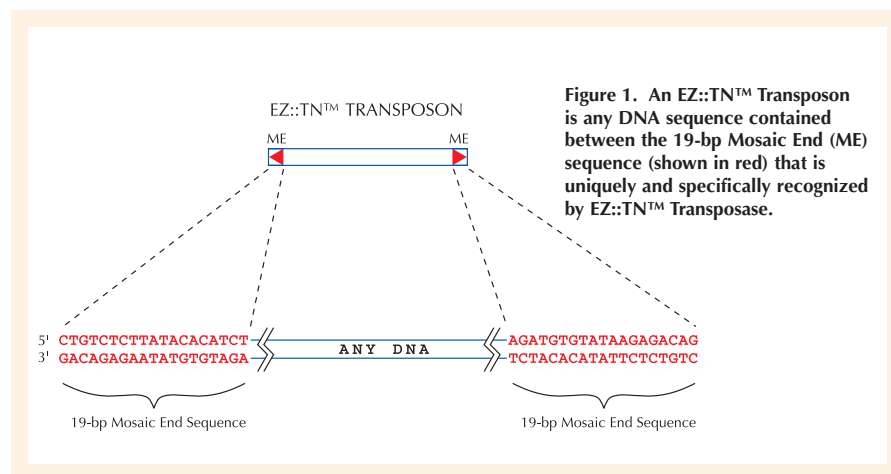


Figure 1. An EZ::TN™ Transposon is any DNA sequence contained between the 19-bp Mosaic End (ME) sequence (shown in red) that is uniquely and specifically recognized by EZ::TN™ Transposase.

for *in vitro* insertion into target DNA without further purification or modification (e.g. end-polishing). The target DNA contained a 3.4-Kb insert in pUC19 (designated as pUC19/3.4). Standard EZ::TN Transposon *in vitro* insertion reactions were:

1 μ l	10X Reaction Buffer (0.50 M Tris-acetate, pH 7.5; 1.5 M potassium acetate; 100 mM magnesium acetate; 40 mM spermidine)
2 μ l	0.2 μ g of pUC19/3.4 target DNA (100 ng/ μ l)
X μ l	Molar equivalent of EZ::TN <KAN> or EZ::TN <DHFR> Transposon
X μ l	Sterile water to a final volume of 9 μ l
1 μ l	EZ::TN Transposase (10 U/ μ l)
10 μ l	Total Volume

Insertion reactions were incubated for 2 hours at 37°C, and then terminated by the addition of 1 μ l of 1% SDS and heating for 10 minutes at 70°C.

A 1 μ l aliquot of each insertion reaction was used for electroporation of TransforMax™ EC100™ *E. coli* cells. Transposon insertion clones were selected by overnight growth on plates containing 100 μ g/ml of ampicillin to select for the vector and either 50 μ g/ml of kanamycin to select for EZ::TN <KAN> Transposon insertion clones or 10 μ g/ml of trimethoprim to select for EZ::TN <DHFR> Transposon insertion clones.

Results

Yield and integrity of EZ::TN Transposons made using the ME-Tailed PCR Method

The FailSafe PCR system was used for PCR in order to assure reliable, high fidelity synthesis of functional EZ::TN Transposons. As determined by agarose gel electrophoresis, full-length transposons were produced for both EZ::TN <KAN> and EZ::TN <DHFR> Transposons (Figure 3). Approximately 7.5 μ g of EZ::TN Transposon was obtained from each 50 μ l PCR reaction – enough for >100 *in vitro* insertion reactions.

In vitro transposition efficiency of the EZ::TN <KAN> and EZ::TN <DHFR> Transposons

Transposition efficiencies, defined as the ratio of the number of Amp^R/Kan^R or Amp^R/Trimethoprim^R EZ::TN Transposon

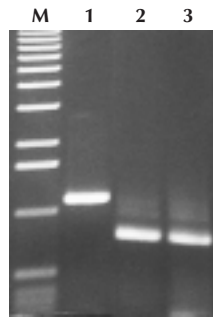


Figure 3. The ME-Tailed PCR Method produced full-length 1108 bp EZ::TN <KAN> Transposon from a plasmid DNA template (Lane 1) and 887 bp EZ::TN <DHFR> Transposon from *E. coli* genomic DNA template (Lane 2) and from a PCR product template (Lane 3). Lane M, DNA size markers.

insertion clones to Amp^R target clones in seven independent *in vitro* insertion reactions, averaged 0.13% for the EZ::TN <KAN> Transposon and 0.68% and 0.30%, respectively, for the genomic- and PCR template-derived EZ::TN <DHFR> Transposons. Although the transposition efficiencies of transposons prepared using the ME-Tailed PCR Method were about 10-fold lower than those of EPICENTRE's commercially available EZ::TN <KAN-2> and EZ::TN <DHFR-1> Transposons, transposons prepared using the ME-Tailed PCR Method still generate sufficient numbers of *in vitro* insertions for many applications. For example, the three transposons prepared using the ME-Tailed PCR Method described here generated >10⁵ *in vitro* insertion clones per 0.2 μ g of

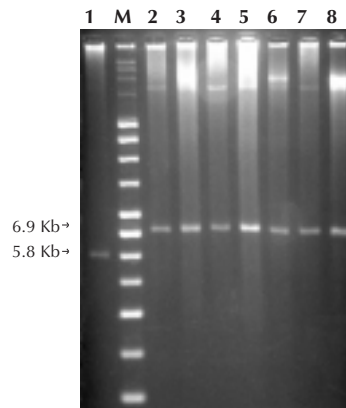


Figure 4. An EZ::TN Transposon produced by the ME-Tailed PCR Method is efficiently inserted into a target DNA, *in vitro*. Seven randomly chosen Amp^R/Kan^R clones were individually processed using the Colony Fast-Screen™ Kit (see p. 7) and the size of the clones analyzed by agarose gel electrophoresis in less than 1 hour. Lane M, Supercoiled DNA size marker; Lane 1, 5.8 Kb pUC19/3.4 DNA; Lanes 2-8, randomly chosen EZ::TN <KAN> Transposon insertion clones. The size of these clones (6.9 Kb) reflect the insertion of a single 1.1 Kb EZ::TN <KAN> Transposon into pUC19/3.4.

pUC19/3.4 as a target. These results compare to >10⁶ insertion clones per insertion reaction obtained using the comparable commercially available EZ::TN Transposons from EPICENTRE.

Analysis of the insertion clones

Putative EZ::TN <KAN> Transposon and EZ::TN <DHFR> Transposon insertion clones were analyzed based on size in less than an hour using EPICENTRE's Colony Fast-Screen™ Kit (see p. 7). All clones analyzed had the expected size for insertion of a single 1108-bp EZ::TN <KAN> Transposon or 887-bp EZ::TN <DHFR> Transposon, respectively. Representative gel data are shown in Figure 4.

Conclusions

Custom EZ::TN Transposons can be prepared by PCR amplification of plasmid-, genomic- or PCR-derived templates using PCR primers with a non-template-homologous 5'-tail, consisting of a 19-base ME sequence that is recognized by the transposase. Transposons generated using this ME-Tailed PCR Method can be used directly, without purification, for random *in vitro* insertion into any target DNA in a simple 2-hour reaction. The *in vitro* transposition efficiencies of unpurified transposons generated using the ME-Tailed PCR Method are about 10-fold less than the corresponding purified commercially available EZ::TN Transposons. These unpurified transposons are more than active enough (e.g., transposition efficiency of about 10⁵ insertion clones per 0.2 μ g of pUC19/3.4 target) for use in many *in vitro* insertion applications.

At this time, we recommend using EZ::TN Transposons made using the EZ::TN pMOD™<MCS> series of Transposon Construction Vectors (see p. 15) for making EZ::TN Transposomes - stable complexes of an EZ::TN Transposon and EZ::TN Transposase that can be electroporated directly into living cells.

EZ::TN™ Transposase

TNP92110-F83 10 Units

Colony Fast-Screen™ Kit

FS08250-F83 1 Kit

Reagents sufficient for screening 250 colonies.

*FailSafe™ PCR PreMix Selection Kit

FS99060-F83 48 Reactions

* See center insert for details.

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