

## EZ::TN™ pMOD™-2<MCS> Transposon Construction Vector Frequently Asked Questions

The EZ::TN™ pMOD™-2<MCS> Transposon Construction Vector enables researchers to easily construct custom EZ::TN Transposons containing virtually any DNA sequence of interest (e.g. species-specific selectable markers, genetic control elements, cDNA, genes) for use in *in vitro* insertion reactions or for preparation of EZ::TN Transposomes™. Here, we provide answers to the questions regarding preparation of EZ::TN Transposons using pMOD-2<MCS> Vector.

**Q1** What is the general protocol for preparing a custom EZ::TN Transposon using pMOD-2<MCS>?

**A1** Preparation of an EZ::TN Transposon using pMOD-2<MCS> is a three step process:

1. First, clone your DNA of interest into the multiple cloning site of pMOD-2<MCS>.
2. Then, isolate the custom EZ::TN Transposon either by digestion with *Pvu* II or *Psh* AI (*Box* I) or by PCR amplification of the transposon region.
3. Finally, purify (see Q6) the newly constructed EZ::TN Transposon.

**Q2** How can I make an EZ::TN Transposome from the custom EZ::TN Transposon?

**A2** It's easy!. Just incubate the EZ::TN Transposon with EZ::TN Transposase in the absence of Mg<sup>2+</sup>.

**Q3** What is the largest EZ::TN Transposon that I can make?

**A3** To date, the largest custom EZ::TN Transposon reported by our customers is about 7 Kb. In theory, larger EZ::TN Transposons can be prepared. However, in general, the larger the transposon, the less efficiently it is inserted into the target DNA either *in vitro* or *in vivo* as an EZ::TN Transposome. One theory as to why large transposons are inserted into target DNA less efficiently is that there is a higher probability of a large transposon undergoing *intramolecular* transposition into itself rather *intermolecular* insertion into the target DNA.

**Q4** What is the smallest EZ::TN Transposon that I can make?

**A4** To date, the smallest EZ::TN Transposon that has been used successfully is about 290 bp.

**Q5** Does the pMOD-2<MCS> vector contain genetic control elements?

**A5** No. The DNA of interest (e.g. a selectable marker, gene or cDNA) must contain all necessary genetic control elements when cloned into pMOD-2<MCS> if it is to be expressed in a cell. Also, when an EZ::TN Transposon is used to make an EZ::TN Transposome for *in vivo* insertion into the cell's genome, it will be replicated as a single copy. Therefore, the level of expression of the gene or selectable marker is likely

to be diminished compared to expression in a high copy plasmid. If the transposon contains an antibiotic selection marker, it may be necessary to plate transposoned cells on two or more plates containing different levels of antibiotic in order to detect transpositions into different cellular targets.

**Q6** Does the newly prepared EZ::TN Transposon need to be purified prior to use?

**A6** Whether the custom EZ::TN Transposon is released from pMOD-2<MCS> by *Pvu* II or *Psh* AI (*Box* I) digestion or by PCR amplification, it should be purified away from the vector and any trace of uncut pMOD-2 <MCS>. Agarose gel electrophoresis is one of the most effective methods of purification. Avoid or limit exposure of the EZ::TN Transposon to maintain its activity and integrity.

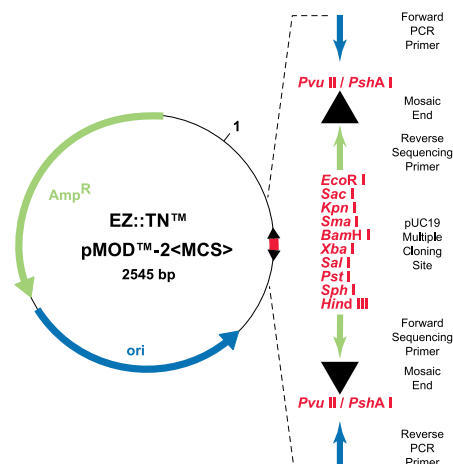


Figure 1. EZ::TN™ pMOD™-2<MCS> Transposon Construction Vector.

<b>EZ::TN™ pMOD-2™&lt;MCS&gt; Transposon Construction Vector</b>	
MOD0602	20 µg
Includes: pMOD-2™<MCS> Vector and the Forward and Reverse PCR Primers	
<b>EZ::TN™ Transposase</b>	
TNP92110	10 Units
<b>pMOD™&lt;MCS&gt; Forward Sequencing Primer</b>	
MODFSP201	1 nmole
<b>pMOD™&lt;MCS&gt; Reverse Sequencing Primer</b>	
MODRSP202	1 nmole

[www.epicentre.com/catalog/pmod2.htm](http://www.epicentre.com/catalog/pmod2.htm)