

# TypeOne™ Inhibitor Improves Transformation Efficiencies by Blocking Type I Restriction and Modification Systems *In Vivo*

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## Introduction

DNA transformation can be difficult to achieve in many bacterial strains due to the presence of one or more restriction and modification (R-M) systems which cleave unmodified DNA. Here we demonstrate that EPICENTRE's new TypeOne™ Restriction Inhibitor\* significantly increases transformation efficiencies of unmodified DNA in bacterial strains with type I R-M systems. Developed as a unique preparation of a phage protein called "ocr",<sup>1</sup> TypeOne Inhibitor can be electroporated into cells along with transforming DNA. *In vivo* this protein acts as a molecular decoy that blocks the DNA binding site of type I R-M enzymes and inhibits cleavage of unmodified DNA.

## Methods

Fifty microliters of electrocompetent cells, prepared according to standard methods, were electroporated with the amount of unmodified DNA indicated in Table 1, with or without 5 µg (1 µl) of TypeOne Inhibitor. Transformed cells were plated on media containing ampicillin (pUC19), chloramphenicol (48 Kb fosmid), or kanamycin (EZ::TN™ Transposon).

## Results

Type I R-M systems are widespread in Eubacteria and Archaeobacteria.<sup>2</sup> For example, *Salmonella typhimurium* strain LT2 has a type I R-M enzyme called StyL TIII, that cleaves pUC19 DNA at three sites. When unmodified pUC19 plasmid DNA was electroporated into *S. typhimurium* strain LT2 in the presence of TypeOne Restriction Inhibitor, 100-fold more ampicillin-resistant colonies were obtained than when the inhibitor was not included in the transformation mixture (Table 1). Addition of TypeOne Inhibitor made transformation efficiencies much more comparable to those of the non-restricting *S. typhimurium* strain LB5000.

TypeOne Inhibitor blocks type I R-M enzymes that recognize different DNA target sequences and therefore can be used to increase transformation efficiencies in a variety of host cells.<sup>1</sup> *E. coli* strain MG1655, for example, contains the EcoKI type I R-M enzyme that recognizes a different target sequence than the R-M enzyme found in *S. typhimurium* strain

Strain (Type I R-M system)	TypeOne™ Inhibitor	Type of DNA or Transposome™	Recombinants per µg DNA
<i>S. typhimurium</i> LT2 (StyL TIII)	-	pUC19 (100 pg)	3.0 X 10 <sup>6</sup>
<i>S. typhimurium</i> LT2 (StyL TIII)	+	pUC19 (100 pg)	3.0 X 10 <sup>8</sup>
<i>S. typhimurium</i> LB5000 (none)	-	pUC19 (100 pg)	2.0 X 10 <sup>10</sup>
<i>E. coli</i> MG1655 (EcoK1)	-	48 Kb fosmid (50 ng)	3.0 X 10 <sup>3</sup>
<i>E. coli</i> MG1655 (EcoK1)	+	48 Kb fosmid (50 ng)	1.4 X 10 <sup>6</sup>
<i>S. typhimurium</i> LT2 (StyL TIII)	-	EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™ (1 µl)	1.3 X 10 <sup>4</sup>
<i>S. typhimurium</i> LT2 (StyL TIII)	+	EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™ (1 µl)	1.0 X 10 <sup>6</sup>
<i>A. tumefaciens</i> (none)	-	EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™ (1 µl)	2.2 X 10 <sup>5</sup>
<i>A. tumefaciens</i> (none)	+	EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™ (1 µl)	1.3 X 10 <sup>5</sup>

Table 1. Effect of TypeOne™ Restriction Inhibitor on transformation efficiencies.

LT2. As shown in Table 1, the addition of TypeOne Inhibitor to electroporations of *E. coli* strain MG1655 with an uncharacterized 48 Kb fosmid clone resulted in a nearly 500-fold increase in transformation efficiency.

EZ::TN™ Transposomes™ - the stable complex between EZ::TN™ Transposase and an EZ::TN™ Transposon - have been used in a variety of microorganisms to create gene knockouts and facilitate sequencing of genomic DNA (see the center insert for more information on this product).<sup>3</sup> The EZ::TN™ <R6Kγori /KAN-2>Tnp Transposome™ contains six recognition sites for the type I *S. typhimurium* StyL TIII nuclease. When this Transposome was electroporated into *S. typhimurium* LT2 together with TypeOne Inhibitor, the number of insertion clones was increased by 75-fold (Table 1). The addition of TypeOne Inhibitor did not cause a significant change (neither an increase nor a decrease) in insertion efficiencies in other bacterial species when there was either no restriction activity in the cell (e.g., *Agrobacterium tumefaciens*, Table 1) or no recognition sites for the host type I R-M enzyme in the transposon (data not shown).

## Conclusion

Electroporation of TypeOne Restriction Inhibitor provides a new and powerful method for increasing DNA transformation efficiencies in bacterial strains with type I R-M systems. Use of TypeOne Inhibitor does not require prior knowledge of the restriction sites on the transforming DNA or the restriction specificity of the type I R-M system.

## References

1. Walkinshaw, M.D. et al. (2002) *Molec. Cell* **9**, 187.
2. Murray, N.E. et al. (2000) *Microbial. Molec. Biol. Rev.* **64**, 412.
3. Hoffman, L.M. et al. (2000) *Genetica* **108**, 19.

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TypeOne™ Restriction Inhibitor\*  
TY0261H 100 µg

\*Patent pending.