

# EZ::TN<sup>TM</sup> Transposon Insertions Into Target DNA *In Vitro* are Highly Random

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## Introduction

EZ::TN<sup>TM</sup> Transposon Tools, based on a hyperactive *in vitro* Tn5 transposition system<sup>1</sup>, can be used to speed up and simplify a myriad of genomics and proteomics procedures. For many applications, including complete sequencing of a DNA target that is too large to sequence with a single set of sequencing reactions, it is important that insertion of the EZ::TN Transposon into target DNA is highly random. In order to further evaluate randomness of *in vitro* insertion obtained using EZ::TN Insertion Kits, the transposon insertion sites were sequenced for 55 colonies picked at random from among >10<sup>6</sup> insertion clones obtained following transformation of *E. coli* cells with an aliquot of the insertion reaction.

## Materials and Methods

The DNA target used for the insertion reaction, referred to as “pIndigoBAC-5/269,” was a 7775-bp DNA comprising a 269-bp fragment cloned in the pIndigoBAC-5 vector.

*In vitro* insertions into the pIndigoBAC-5/269 target were made by incubating the target with an equal molar amount of an EZ::TN <TET-1> Transposon, encoding tetracycline resistance, and EZ::TN Transposase for 2 hours at 37°C as described in the EZ::TN <TET-1> Insertion Kit literature. Following transformation of TransforMax<sup>TM</sup> EC100<sup>TM</sup> Electrocompetent *E. coli* cells with an aliquot of the *in vitro* insertion reaction, insertion clones were selected by growth on tetracycline plates. Greater than >10<sup>6</sup> tetracycline-resistant pIndigoBAC-5/269 insertion clones were obtained. Fifty-five of these insertion clones, each containing a single EZ::TN <TET-1> Transposon, were randomly chosen from the plate, and used to prepare DNA for use as templates in DNA cycle sequencing reactions. The DNAs were sequenced using the SequiTherm EXCEL<sup>TM</sup> II DNA

Sequencing Kit LC and IRD800-labeled forward and reverse transposon-specific primers. See Figure 1 on page 4 for an overview of the EZ::TN Transposon insertion, selection and sequencing process.

## Results

The map position of each of the 55 EZ::TN Transposon insertions into pIndigoBAC-5/269 is shown in Figure 1A. An analysis of the localized G+C content of pIndigoBAC-5/269, based on a 50-bp window, is shown in Figure 1B. Further data and a detailed analysis of the results will be published separately at a later date. However, from the insertion data presented in Figure 1, the following conclusions can be surmised:

1. There was no transposon insertion bias either for or against high G+C or high A+T regions in the target DNA - see for example, insertion clones 679, 683, 752, 705, 727, 740, 688, 718, and 724 in Figure 1B.
2. The complete sequence of the target was easily obtained. The longest regions of pIndigoBAC-5/269 without an insertion were 510 bp between clones 683 and 752 and 488 bp between clones 745 and 678. Both of these spans were easily sequenced from clones 683/752 and 745/678, respectively.

## Conclusion

Although the sample size of 55 insertion clones was small relative to the >10<sup>6</sup> transposon insertion clones generated in the *in vitro* EZ::TN Transposon reaction, the data strongly supported the conclusion that EZ::TN Transposon insertions into target DNA are highly random.

## Reference

1. Goryshin, I.Y. and Reznikoff, W.S. (1998) *J. Biol. Chem.* **273**: 7367-7374.

**Figure 1. Insertion data for 55 EZ::TN<sup>TM</sup> <TET-1> Transposon insertions into pIndigo BAC-5/269: (1A) Map position of insertions; (1B) G+C content of target sequence determined using a 50-bp window.**

