

EZ-Tn5™ <T7/KAN-2> Promoter Insertion Kit

Cat. No. EZI03T7

Connect with Epicentre on our blog (epicentral.blogspot.com),
Facebook (facebook.com/EpicentreBio), and Twitter ([@EpicentreBio](https://twitter.com/EpicentreBio)).

1. Introduction

Transposons are mobile DNA sequences found in the genomes of prokaryotes and eukaryotes. Transposon tagging has long been recognized as a powerful research tool for randomly distributing primer binding sites, creating gene “knockouts”, and introducing a physical tag or a genetic tag into large target DNAs. One frequently used transposition system is the Tn5 system isolated from gram-negative bacteria.

Though a naturally occurring transposition system, the Tn5 system can be readily adapted for routine use in research laboratories for the following reasons:

- 1) Tn5 transposase is a small, single subunit enzyme that has been cloned and purified to high specific activity.
- 2) Tn5 transposase carries out transposition without the need for host cell factors.
- 3) Tn5 transposon insertions into target DNA are highly random.
- 4) Tn5 transposition proceeds by a simple “cut and paste” process. Although the chemistry is unique, the result is similar to using a restriction endonuclease, with random sequence specificity, accompanied by a DNA ligase activity.
- 5) Tn5 transposase will transpose any DNA sequence contained between its short 19 basepair Mosaic End (ME) Tn5 transposase recognition sequences.

In 1998 Goryshin and Reznikoff¹ demonstrated that a fully functional Tn5 transposition system could be reconstituted *in vitro*. Additionally, the transposition efficiency of this system has been increased more than 1,000-fold compared to wild-type Tn5 by introducing mutations in the transposase gene and in the 19-bp Tn5 ME transposase recognition sequence.

Epicentre’s EZ-Tn5 Transposon Tools (kits and reagents) are based on the hyperactive Tn5 transposition system developed by Goryshin and Reznikoff.

How the EZ-Tn5 <T7/KAN-2> Promoter Insertion Kit Works.

The EZ-Tn5 <T7/KAN-2> Promoter Insertion Kit can be used to randomly insert a phage T7 RNA polymerase transcriptional promoter, primer binding sites and a kanamycin resistance selection marker into target DNA *in vitro*. A single 2-hour *in vitro* reaction randomly inserts the <T7/KAN-2> Transposon into the target DNA. Use an aliquot of the reaction to transform *E. coli* such as Epicentre’s TransformMax™ EC100™ Electrocompetent *E. coli* strain and select on kanamycin plates. Only those clones harboring DNA containing the <T7/KAN-2> Transposon will grow.

The EZ-Tn5 <T7/KAN-2> Promoter Insertion Kit can be used for:

- Introducing a phage T7 RNA polymerase transcriptional promoter into any DNA.
- Faster sequencing of large DNA molecules, as compared to primer walking, random subcloning, or generating nested deletions with exonuclease III and mung bean nuclease.
- Making insertion mutants or gene “knockouts” *in vitro*.
- Introducing a kanamycin resistance selection marker into any DNA

Fig. 1 describes the steps involved when using the EZ-Tn5 <T7/KAN-2> Promoter Insertion Kit. The process can be summarized as follows:

Preparation

- Prepare 0.2 µg of recombinant DNA for the EZ-Tn5 <T7/KAN-2> promoter insertion reaction.

Day 1

- Perform the 2-hour *in vitro* EZ-Tn5 <T7/KAN-2> insertion reaction.
- Transform competent *recA*⁻ *E. coli* with 1 ml of the reaction mix.
- Select for kanamycin-resistant transposon insertion clones on kanamycin plates overnight.

Day 2

- Prepare DNA from kanamycin-resistant colonies.
- (Optional) Map the EZ-Tn5 <T7/KAN-2> Transposon insertion sites.
- Linearize the DNA and perform *in vitro* transcription using T7 RNA polymerase - or - transform (DE3) pLysS *E. coli* for *in vivo* transcription.
- (Optional) DNA sequence chosen clones bidirectionally using the unlabeled forward and reverse transposon-specific primers supplied in the kit.

2. Kit Contents

| Desc. | Concentration | Quantity |
|---|---------------|----------|
| Reagents included in the kit are sufficient for 10 <i>in vitro</i> transposon promoter insertion reactions. | | |
| EZ-Tn5™ Transposase: 10 U in 50% glycerol containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, and 0.1% Triton®X-100. | @ 1 U/µl | 10 µl |
| EZ-Tn5™ <T7/KAN-2> Transposon: 1 pmol @ 0.1 pmol/µl (0.08 µg/µl) in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA). | | 10 µl |
| EZ-Tn5™ 10X Reaction Buffer 0.50 M Tris-acetate (pH 7.5), 1.5 M potassium acetate, 100 mM magnesium acetate, and 40 mM spermidine. | | 100 µl |
| EZ-Tn5™ 10X Stop Solution: 1% SDS solution. | | 100 µl |
| T7/KAN-2 FP-1 Forward Primer: 1 nmol in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA). | @ 50 µM | 20 µl |
| T7/KAN-2 RP-1 Reverse Primer: 1 nmol in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA). | @ 50 µM | 20 µl |
| pUC19/3.4 Control Target DNA: 1 µg A 3.4-kb <i>Hpa</i> II fragment of bacteriophage DNA cloned into the <i>Acc</i> I site of pUC19, in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA). | @ 0.1 µg/µl | 10 µl |
| Sterile Water: | | 1 µl |

3. Related Products

The following products are also available:

- TransformMax™ EC100™ Electrocompetent *E. coli*
 - Transformation efficiency >10⁹ cfu/μg DNA.
- AmpliScribe™ T7 High Yield Transcription Kit
- AmpliCap™ T7 High Yield Message Maker Kit
- Colony Fast-Screen™ Kit
- MasterPure™ DNA Purification Kits
- EZ-Tn5™ Tnp Transposome™ Kits
- EZ-Tn5™ Transposase
- EZ-Tn5™ Transposons
- EZ-Tn5™ Insertion Kits

4. Materials

Storage

Store EZ-Tn5 Insertion Kits only at –20°C in a freezer without a defrost cycle. The enzyme solution will not freeze. Other component solutions will freeze. After thawing, be sure to mix the contents of each tube thoroughly before using. After use, return all of the kit components to –20°C for storage.

Performance Specifications and Quality Control

The EZ-Tn5 <T7/KAN-2> Promoter Insertion Kit is function-tested by performance of the kit's *in vitro* control reaction followed by electroporation into a *recA*[–] *E. coli* host strain having a transformation efficiency of >10⁹ cfu/μg DNA. Transposition frequency, defined as the ratio of the number of Kan^R clones divided by the number of transformants resistant to the antibiotic marker of the target vector, (Kan^R colonies/Amp^R colonies; for the control DNA) must be >0.5% (commonly at 10%) and transposition efficiency must be >10⁶ Kan^R colonies/μg target DNA. Random transposition clones are tested in *in vitro* transcription reactions using the AmpliScribe™ T7 High Yield Transcription Kit. Primers are function-tested in a DNA cycle sequencing reaction using the SequiTherm EXCEL™ II DNA Sequencing Kit and in a PCR reaction using a plasmid containing an EZ-Tn5 <T7/KAN-2> Transposon as template. All components of the EZ-Tn5 <T7/KAN-2> Promoter Insertion Kit are free of detectable DNase and RNase activities as judged by agarose gel electrophoresis following over-digestion assays, with the exception of the inherent endonucleolytic function of the EZ-Tn5 Transposase.

5. Transposon Insertion Reaction

Target DNA Preparation

The target DNA must not contain a kanamycin resistance gene. The transposon insertion reaction is not significantly inhibited by high levels of RNA contamination in target DNA preparations. However, if the target DNA is heavily contaminated with chromosomal DNA, which is a direct competitor for target transposition, the number of clones will be greatly reduced.

Plasmid and cosmid clones can be purified by standard miniprep procedures and used as target DNA in the insertion reaction without further clean-up. Low copy-number vectors, for example BAC or fosmid clones, are often contaminated with a higher molar proportion of *E. coli* chromosomal DNA thus reducing the transposon insertion frequency. Therefore, BAC and fosmid DNA should be purified, to remove the chromosomal DNA prior to the insertion reaction.

In Vitro Transposon Insertion Reaction

Reaction conditions have been optimized to maximize the efficiency of the EZ-Tn5 Transposon insertion while minimizing multiple insertion events. **Be sure to calculate the moles of target DNA used in the reaction and add an equimolar amount of the EZ-Tn5 <T7/KAN-2> Transposon.** If necessary, dilute the EZ-Tn5 <T7/KAN-2> Transposon with TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

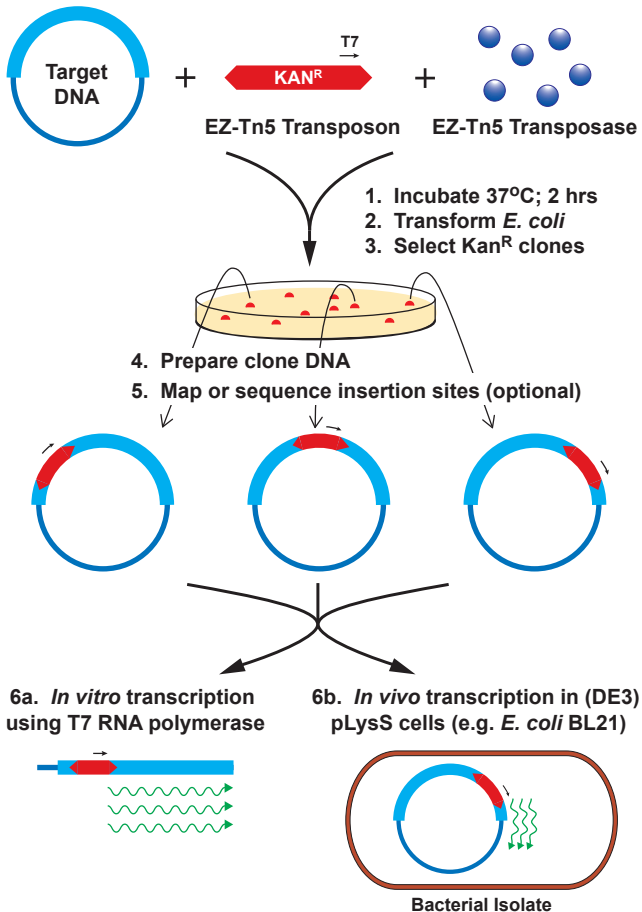


Figure 1. EZ-Tn5 <T7/KAN-2> Transposon Insertion Protocol.

1. Prepare the transposon insertion reaction mixture by adding in the following order:

| | | |
|-------|----|---|
| 1 | µl | EZ-Tn5 10X Reaction Buffer |
| 0.2 | µg | target DNA* |
| x | µl | molar equivalent EZ-Tn5 <T7/KAN-2> Transposon |
| x | µl | sterile water to a reaction volume of 9 µl |
| 1 | µl | EZ-Tn5 Transposase |
| <hr/> | | |
| 10 | µl | Total reaction volume |
2. Incubate the reaction mixture for 2 hours at 37°C.
3. Stop the reaction by adding 1 µl EZ-Tn5 10X Stop Solution. Mix and heat for 10 minutes at 70°C. Proceed to section 4, **Transformation and Recovery**, or store the reaction mixture at -20°C.

*Calculation of µmol target DNA:

$$\mu\text{mol target DNA} = \mu\text{g target DNA} / [(\# \text{ base pairs in target DNA}) \times 660]$$

For example: 0.2 µg of control pUC19/3.4 DNA which is 6,100 bp
= 0.2 µg / [6,100 bp x 660] = 0.05 x 10⁻⁶ µmol = 0.05 pmol

6. Selection of Transposon Insertion Clones

Transformation and Recovery

The number of EZ-Tn5 Transposon insertion clones obtained per reaction depends on, among other factors, the transformation efficiency of the competent cells used. The greater the transformation efficiency of the competent cells, the greater the number of insertion clones obtained. We recommend using electrocompetent or chemically competent *recA*⁻ *E. coli* with a transformation efficiency of >10⁸ cfu/µg of DNA. A *recA*⁻ strain of *E. coli* is recommended to eliminate the possibility of generating multimeric forms of the vector. Finally, the host strain used **must not** have a kanamycin resistance marker when used with the EZ-Tn5 <T7/KAN-2> Transposon. Epicentre's TransforMax EC-100 Electrocompetent *E. coli* (available separately) have a transformation efficiency of >1 x 10⁹ cfu/µg and are ideal for this application.

- 1) Using 1 µl of the insertion reaction mixture, transform *recA*⁻ *E. coli*. If electrocompetent cells are used, perform electroporation according to the equipment manufacturer's recommended conditions. Use of >1 ml of the transposon insertion reaction for transformation may result in arcing. The unused portion of the transposon insertion reaction can be stored at -20°C for future use.
- 2) Recover the electroporated cells by adding SOC medium to the electroporation cuvette to 1 ml final volume immediately after electroporation. Pipette the medium/cells gently to mix. Transfer to a tube and incubate on a 37°C shaker for 30-60 minutes to facilitate cell outgrowth.

Plating and Selecting Transformants

EZ-Tn5 <T7/KAN-2> Transposon insertion clones are selected on kanamycin-containing plates, however, the transposon will also confer resistance to neomycin and G418 in *E. coli*.

- 1) If transformation was done using cells with an efficiency of $>5 \times 10^8$ cfu/ μ g DNA, it may be necessary to dilute the cells 1:10 or 1:100 prior to plating. Plate portions (e.g., 100 μ l) of cells onto LB plates containing 50 μ g/ml kanamycin. Store the unused portion of the recovered cells at +4°C for up to 2 days in the event additional plates need to be prepared.
- 2) (Optional) To determine the transposon insertion efficiency, plate identical dilutions and dilution aliquots of the transformation reaction on a second plate containing an antibiotic specific for selecting target DNA (e.g., 100 μ g/ml ampicillin for the control DNA). The transposition frequency is given by the ratio of Kan^R/Amp^R clones for the control DNA.
- 3) Grow plates overnight at 37°C. Assuming a transposon insertion efficiency of 1% and use of high purity target DNA (i.e., little or no chromosomal DNA contamination), one should see 100-500 Kan^R clones per plate. If too few (or too many) colonies appear, replate the transformed cells at a lower (or higher) dilution.

Typical results obtained with the EZ-Tn5 <T7/KAN-2> Promoter Insertion Kit are:

- Transposon insertion frequency = 0.5-20%
- Transposition clones per μ g target DNA = 1×10^5 - 1×10^8
- Transposition clones per 10 μ l transposon insertion reaction = 1×10^4 - 1×10^7

The actual number of EZ-Tn5 <T7/KAN-2> promoter insertion clones obtained will vary depending on factors such as target DNA size and the transformation efficiency of the competent cells used to recover the transposon insertion clones.

7. Transcription and DNA Sequencing of Transposon Insertion Clones

Transposon Insertion Mapping

EZ-Tn5 Transposon insertion clones can be sequenced bidirectionally using the unlabeled forward and reverse transposon-specific primers provided in the kit. The insertion site of each clone can also be mapped prior to *in vitro* or *in vivo* transcription, depending on the cost-effectiveness and experience of the user.

EZ-Tn5 <T7/KAN-2> Transposon insertion sites can be mapped by size analysis of PCR products using colony minilysate DNA as a template. To map the insertion sites, use the T7/KAN-2 FP-1 or T7/KAN-2 RP-1 primers provided with the kit and a vector-specific flanking primers (not provided).

Alternatively, insertion sites can be mapped by restriction endonuclease digest(s). Use the nucleotide sequence and restriction information of the EZ-Tn5 <T7/KAN-2> Transposon provided in the Appendix for reference.

Transcription of <T7/KAN-2> Transposition Clones

The EZ-Tn5 <T7/KAN-2> Transposon contains a single phage T7 transcriptional promoter. Since transposon insertion is random, the location and orientation of the T7 promoter should be determined for each clone prior to *in vitro* transcription. Epicentre's AmpliScribe T7 High Yield Transcription Kit is ideal for *in vitro* transcription reactions. Templates should be linearized prior to *in vitro* transcription. For *in vivo* expression,

transform desired clones into cells containing an inducible T7 RNA Polymerase gene (genetic phenotype [DE3] pLysS; such as *E. coli* BL21). The first nucleotide incorporated into the transcript is a “G” at nucleotide 1,209 of the EZ-Tn5 <T7/KAN-2> Transposon sequence. Thus the first 40 nucleotides (nt) of the transcript will be transposon sequence. In addition, EZ-Tn5 Transposase-catalyzed transposon insertion results in the generation of a 9-bp target site sequence duplication immediately flanking the inserted transposon (see Fig. 2). This is important to consider when analyzing the transcription products.

Note: *There is a potential “Shine-Dalgarno” sequence at nts. 31-40 of the RNA product (transposon nts. 1,239-1,248).*

Primer Considerations

The T7/KAN-2 FP-1 Forward and T7/KAN-2 RP-1 Reverse Primers supplied with the kit have been constructed to minimize homology to commonly used cloning vectors.

However, the sequence of each primer should be compared to that of the user’s specific cloning vector to ensure minimal sequence homology to the vector. The sequence and theoretical melting temperatures for each primer are presented in the Appendix.

Note: *Occasionally a clone will yield the sequence of the cloning vector. This occurs when the EZ-Tn5 Transposon randomly inserts into a non-essential region of the vector rather than into the DNA insert. The frequency of this occurrence is dependent on the size of the DNA insert relative to the size of non-essential regions of vector. The larger the DNA insert, the less frequently an insertion will occur into the vector.*

Target Site Duplication

EZ-Tn5 Transposase-catalyzed transposon insertion results in the generation of a 9-bp target site sequence duplication where one copy immediately flanks each side of the inserted transposon. This is important to consider when assembling the nucleotide sequence of a recombinant clone insert. The process of transposon insertion site duplication is depicted in Fig. 2.

Distinguishing Transposon Sequence from Insert Sequence

Since the primers provided in the EZ-Tn5 <T7/KAN-2> Promoter Insertion Kit anneal to a region near the ends of the transposon, the first sequence data obtained from each sequencing reaction is that of EZ-Tn5 <T7/KAN-2> Transposon DNA. The sequence of the 19-bp EZ-Tn5 Transposase recognition sequence (ME) found at the junction of the transposon and the target clone insert DNA (present in all insertion clones), is a useful landmark to distinguish vector sequence from target clone insert sequence (see Fig. 3).

8. Troubleshooting

Arcing during transformation by electroporation

- 1) **Excessive salt in target DNA preparation.** Use less sample for electroporation. Ethanol precipitate and 70% ethanol wash, or drop dialyze DNA. Arcing inevitably results in failed transformation. Discard the electroporation reaction and try again with 0.5 µl of the transposition reaction. With the control DNA, no arcing is observed when up to 2 µl of transposition reaction is used for electroporation of 50 µl of electrocompetent *recA*⁻ *E. coli* in a 0.2-cm width electroporation cuvette and using an Eppendorf Multiporator at 2500 V. A voltage gradient of approximately 12,500 V/cm is fairly standard for *E. coli*.

No, or few transposition clones on selective plates

- 1) **Transformation reaction was unsuccessful; low competence.** Test by plating outgrowth using drug resistance marker on target DNA to distinguish between transformation or transposon insertion failure. If competent cells have a transformation efficiency <10⁸ cfu/µg DNA, one may not obtain sufficient clones on a plate. For example, transforming into cells with transformation efficiency <10⁵ cfu/µg DNA results in as few as 2 insertion clones on a plate. Use cells with a transformation efficiency >10⁸ cfu/µg DNA.
- 2) **Transposon insertion reaction was unsuccessful.** Inhibitor contamination in target DNA. Purify target DNA further. Perform procedure with control plasmid provided with kit to assure system components are functional.

DNA sequencing results are ambiguous

- 1) **Two or more transposon insertions into target clone.** Discard the clone. Choose other clone(s) to sequence. The protocol was designed to minimize multiple transposon insertion events. Even so, about 1% of the transposition clones may contain >1 transposon. One can verify single insertion clones by agarose gel electrophoresis of colony minilysates prior to sequencing. A single insertion should be about 1.2 kb larger than the parental target DNA. A double insertion will increase the size by 2.4 kb and also result in “double sequence”.
- 2) **The sequencing primer used has significant homology to the cloning vector or to the DNA being sequenced.** Check homology of primer against the vector. Alter primer annealing conditions or synthesize a new primer with less homology.
- 3) **Components of the DNA sequencing kit and/or of the electrophoresis step are compromised.** Re-verify the integrity of the components of the kit and/or electrophoresis step with appropriate controls. Use a new kit and/or new reagents.

Confluent plates following transformation

- 1) **Target DNA or host cells have the same selective marker (antibiotic resistance) as the transposon used.** Use a different host for transformation and retransform with a portion of your remaining reaction. Some host cells carry Kan^R transposons. Confirm that the genotype of the host strain chosen for the transformation is not Kan^R.

9. Appendix

Primer Data

T7/KAN-2 FP-1 Forward Primer

5' - ACCTACAACAAAGCTCTCATCAACC - 3'

Length: 25 nucleotides

G+C content: 11

Molecular Weight: 7,484 daltons

Temperatures of Dissociation & Melting:

T_d : 68°C (nearest neighbor method)

T_m : 73°C (% G+C method)

T_m : 72°C ([2 (A+T) + 4 (G+C)] method)

T_m : 63°C ((81.5 + 16.6 (log [Na⁺])) + ([41 (#G+C) - 500] / length) method)
where [Na⁺] = 0.1 M

T7/KAN-2 RP-1 Reverse Primer

5' - GCAATGTAACATCAGAGATTTTGAG - 3'

Length: 25 nucleotides

G+C content: 9

Molecular Weight: 7,705 daltons

Temperatures of Dissociation & Melting:

T_d : 65°C (nearest neighbor method)

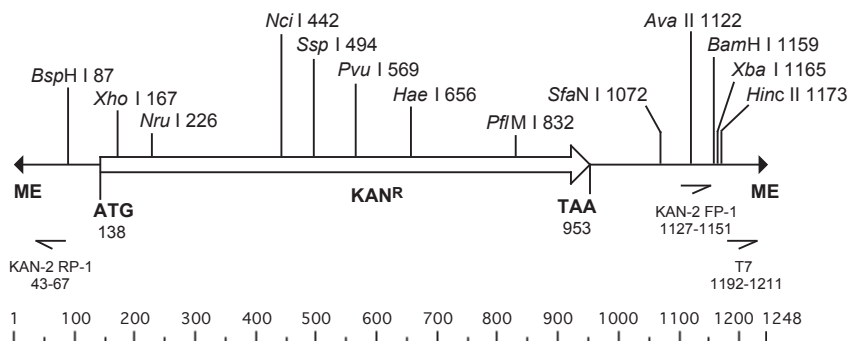
T_m : 69°C (% G+C method)

T_m : 68°C ([2 (A+T) + 4 (G+C)] method)

T_m : 60°C ((81.5 + 16.6 (log [Na⁺])) + ([41 (#G+C) - 500] / length) method)
where [Na⁺] = 0.1 M

EZ-Tn5 <T7/KAN-2> Transposon Schematic Map

EZ-Tn5™ <T7/KAN-2> Transposon (1,248 bp)



Note: Not all restriction enzymes that cut only once are indicated above.

See the following pages for further information.

Primers are not drawn to scale.

| | |
|---------------------------|---------------------------------|
| KAN-2 FP-1 Forward Primer | 5' ACCTACAACAAAGCTCTCATCAACC 3' |
| KAN-2 RP-1 Reverse Primer | 5' GCAATGTAACATCAGAGATTTTGG 3' |
| T7 Promoter Primer | 5' TAATACGACTCACTATAGGG 3' |
| ME = Mosaic End | 5' AGATGTGTATAAGAGACAG 3' |

Figure 4. EZ-Tn5 <T7/KAN-2> Transposon.

EZ-Tn5 <T7/KAN-2> Transposon Restriction Data

Restriction Enzymes that cut the EZ-Tn5 <T7/KAN-2> Transposon one to three times:

| Enzyme | Sites | Location | Enzyme | Sites | Location |
|---------------|--------------|-----------------|---------------|--------------|-----------------|
| Aci I | 1 | 174 | Dde I | 3 | 586, 1041, 1220 |
| Alu I | 1 | 1139 | Dsa I | 2 | 1089, 1150 |
| Apo I | 2 | 182, 366 | Ear I | 1 | 382 |
| Ase I | 2 | 768, 1191 | EcoN I | 1 | 481 |
| AsiS I | 1 | 569 | Fau I | 1 | 1148 |
| Ava I | 1 | 167 | Fnu4H | 2 | 174, 1013 |
| Ava II | 1 | 1122 | Hae I | 1 | 656 |
| BamH I | 1 | 1159 | Hae III | 3 | 173, 656, 1218 |
| Ban II | 1 | 224 | Hinc II | 1 | 1173 |
| Bfa I | 1 | 1166 | HpyCH4 IV | 1 | 159 |
| BfrB I | 2 | 417, 683 | Mae II | 1 | 159 |
| Bpu10 I | 1 | 586 | Mbo II | 3 | 369, 480, 1058 |
| Bsa I | 1 | 1205 | Mly I | 3 | 802, 1178, 1192 |
| BsaW I | 1 | 704 | Mwo I | 3 | 281, 313, 527 |
| BsiE I | 1 | 569 | Nci I | 1 | 442 |
| Bsm I | 2 | 453, 530 | Nla IV | 1 | 1161 |
| BsmB I | 1 | 585 | Nru I | 1 | 226 |
| Bsp1286 I | 1 | 224 | Nsi I | 2 | 419, 685 |
| BspD I | 2 | 29, 260 | PaeR7 I | 1 | 167 |
| BspH I | 1 | 87 | PflM I | 1 | 832 |
| Bsr I | 3 | 360, 984, 1124 | Ple I | 3 | 801, 1177, 1192 |
| BsrD I | 1 | 61 | PspG I | 3 | 457, 814, 1175 |
| BsrF I | 2 | 523, 1214 | Pvu I | 1 | 569 |
| BstDS I | 2 | 1093, 1154 | Rsa I | 1 | 404 |
| BstF5 I | 2 | 200, 826 | Sau96 I | 1 | 1122 |
| BstN I | 3 | 459, 816, 1177 | Sfc I | 1 | 1204 |
| BstU I | 3 | 176, 226, 571 | Sml I | 1 | 167 |
| BstY I | 2 | 818, 1159 | Ssp I | 1 | 494 |
| Bsu36 I | 1 | 1220 | Tli I | 1 | 167 |
| Btg I | 2 | 1089, 1150 | Tsp45 I | 1 | 716 |
| Bts I | 2 | 430, 517 | TspR I | 3 | 442, 517, 989 |
| Cac8 I | 3 | 224, 508, 654 | Xba I | 1 | 1165 |
| Cla I | 2 | 29, 260 | Xho I | 1 | 167 |

Restriction Enzymes that cut the EZ-Tn5 <T7/KAN-2> Transposon four or more times:

| | | | | |
|--------|--------|------------|---------|----------|
| Alw I | Dpn I | Hpy188 I | Mse I | Taq I |
| BsaI | Hha I | HpyCH4 III | Msp I | Tfi I |
| Bsl I | Hinf I | HpyCH4 V | Nla III | Tsp4C I |
| BsmA I | HinP I | Mae III | Sau3A I | Tsp509 I |
| BssK I | Hpa II | Mbo I | ScrF I | |
| CviJ I | Hph I | Mnl I | SfaNI | |

Restriction Enzymes that do not cut the EZ-Tn5 <T7/KAN-2> Transposon:

| | | | | | |
|---------|-----------|-----------|----------|---------|-----------|
| Aat II | Bcl I | BssH II | Hae II | Pci I | Sfi I |
| Acc65 I | Bfu A I | BssS I | Hind III | PflF I | Sfo I |
| Acc I | Bgl I | BstAP I | Hpa I | Pme I | SgrA I |
| Acl I | Bgl II | BstB I | Hpy99 I | Pml I | Sim I |
| Afl II | Blp I | BstE II | Kpn I | PpuM I | Sma I |
| Afl III | Bme1580 I | BstX I | Mfe I | PshA I | SnaB I |
| Age I | BmgB I | BstZ17 I | Mlu I | Psi I | Spe I |
| Ahd I | Bmr I | Dra I | Msc I | PspOM I | Sph I |
| Ale I | BsaA I | Dra III | Msl I | Pst I | Srf I |
| AlwNI | BsaB I | Drd I | MspA1 I | Pvu II | Sse8647 I |
| Apa I | BsaH I | Eae I | Nae I | Rsr II | Stu I |
| ApaB I | BseY I | Eag I | Nar I | Sac I | Sty I |
| ApaL I | BsiHKA I | Eco47 III | Nco I | Sac II | Swa I |
| Asc I | BsiW I | EcoO109 I | Nde I | Sal I | Tat I |
| Avr II | BspE I | EcoR I | NgoM IV | SanD I | Tse I |
| Ban I | BspLU11 I | EcoRV | Nhe I | Sap I | Tth111 I |
| Bbs I | BspM I | Fse I | Not I | Sbf I | Xcm I |
| BbvC I | BsrB I | Fsp I | Nsp I | Sca I | Xma I |
| BciV I | BsrG I | Gdi II | Pac I | SexA I | Xmn I |

EZ-Tn5 <T7/KAN-2> Transposon Sequence**EZ-Tn5™ <T7/KAN-2> Transposon 1,248 bp.**

| | | | | | |
|------|------------|-------------|-------------|-------------|------------|
| 1 | CTGTCTCTTA | TACACATCTC | AACCATCATC | GATGAATTGT | GTCTCAAAAT |
| 51 | CTCTGATGTT | ACATTGCACA | AGATAAAAAAT | ATATCATCAT | GAACAATAAA |
| 101 | ACTGTCTGCT | TACATAAACA | GTAATACAAG | GGGTGTTATG | AGCCATATTC |
| 151 | AACGGGAAAC | GTCTTGCTCG | AGGCCCGCAT | TAAATCCAA | CATGGATGCT |
| 201 | GATTTATATG | GGTATAAATG | GGCTCGCGAT | AATGTGGGGC | AATCAGGTGC |
| 251 | GACAATCTAT | CGATTGTATG | GGAAGCCCGA | TGCGCCAGAG | TTGTTTCTGA |
| 301 | AACATGGCAA | AGGTAGCGTT | GCCAATGATG | TTACAGATGA | GATGGTCAGA |
| 351 | CTAAACTGGC | TGACGGAATT | TATGCCTCTT | CCGACCATCA | AGCATTTTAT |
| 401 | CCGTACTCCT | GATGATGCAT | GGTTACTCAC | CACTGCGATC | CCCGGAAAAA |
| 451 | CAGCATCCA | GGTATTAGAA | GAATATCCTG | ATTCAGGTGA | AAATATTGTT |
| 501 | GATGCGCTGG | CAGTGTTCCT | GCGCCGGTTG | CATTCGATTC | CTGTTTGTAA |
| 551 | TTGTCTTTT | AACAGCGATC | GCGTATTTTCG | TCTCGCTCAG | GCGCAATCAC |
| 601 | GAATGAATAA | CGGTTTGGTT | GATGCGAGTG | ATTTTGTATGA | CGAGCGTAAT |
| 651 | GGCTGCCTG | TTGAACAAGT | CTGGAAAGAA | ATGCATAAAC | TTTTGCCATT |
| 701 | CTCACCGGAT | TCAGTCGTC | CTCATGGTGA | TTTCTCACTT | GATAACCTTA |
| 751 | TTTTTGACGA | GGGGAAATTA | ATAGGTTGTA | TTGATGTTGG | ACGAGTCGGA |
| 801 | ATCGCAGACC | GATACCAGGA | TCTTGCCATC | CTATGGAAC | GCCTCGGTGA |
| 851 | GTTTTCTCCT | TCATTACAGA | AACGGCTTTT | TCAAAAATAT | GGTATTGATA |
| 901 | ATCCTGATAT | GAATAAATG | CAGTTTCATT | TGATGCTCGA | TGAGTTTTTC |
| 951 | TAATCAGAAT | TGGTTAATG | GTTGTAACAC | TGGCAGAGCA | TTACGCTGAC |
| 1001 | TTGACGGGAC | GGCGGCTTTG | TTGAATAAAT | CGAACTTTTG | CTGAGTTGAA |
| 1051 | GGATCAGATC | ACGCATCTTC | CCGACAACGC | AGACCCTTC | GTGGCAAAGC |
| 1101 | AAAAGTTCAA | AATCACC AAC | TGGTCCACCT | ACAACAAAGC | TCTCATCAAC |
| 1151 | CGTGGCGGGG | ATCCTCTAGA | GTCAACCTGG | CTTATCGAAA | TTAATACGAC |
| 1201 | TCACTATAGG | GAGACCGGCC | TCAGGTTGA | GATGTGTATA | AGAGACAG |

The transposon sequence can be downloaded at the URL: <http://www.epicentre.com/sequences>

10. References

Cited:

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

Related:

1. Goryshin, I.Y. and Reznikoff, W.S. (1998) *J. Biol. Chem.* **273**, 7367.
2. Zhou, M. *et al.*, (1998) *J. Mol. Biol.* **276**, 913.
3. Zhou, M. and Reznikoff, W.S. (1997) *J. Mol. Biol.* **271**, 362.
4. Mahnke-Braam, I.A. and Reznikoff, W.S. (1998) *J. Biol. Chem.* **273**, 10908.

These products are intended for research use only.

EZ-Tn5™ Transposon Tools for in vitro transposon insertion are covered by U.S. Patent Nos. 5,925,545; 5,948,622; 5,965,443, and 6,437,109; European Patent No. 0927258, and other patents issued or pending, exclusively licensed or assigned to Epicentre. These products are accompanied by a limited non-exclusive license for the purchaser to use the purchased product(s) solely for in vitro transposon insertion for life science research. Purchase of these products does not grant rights to: (1) offer products, components of products, or any derivatives thereof for resale; or (2) to distribute or transfer the products, components of products, or any derivatives thereof to third parties. Contact Epicentre for information on licenses for uses other than life science research.

AmpliCap, AmpliScribe, EC100, EZ-Tn5, Fast-Screen, MasterPure, SequiTherm EXCEL, TransforMax, and Transposome are trademarks of Epicentre, Madison, Wisconsin.

Triton is a registered trademark of Rohm & Haas, Philadelphia, Pennsylvania.

Visit our technical blog: epicentral.blogspot.com