epicentre®
an Illumina® company

EZ-Tn5™ In-Frame Linker Insertion Kit

Cat. No. EZI04KN

Connect with Epicentre on our blog (**epicentral.blogspot.com**), Facebook (**facebook.com/EpicentreBio**), and Twitter (**@EpicentreBio**).

1. Background

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 Reznikoff[1] 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.

Linker scanning mutagenesis is a powerful technique for introducing codons (peptides) into cloned DNA to evaluate permissive and non-permissive sites in the proteins they encode, as well to introduce "protein tags" into proteins and/or to introduce protease cleavage sites into proteins. The traditional linker scanning mutagenesis method involves ligating an oligonucleotide (linker) into pre-existing restriction endonuclease sites in the cloned DNA. However, this method is limited by the number of restriction sites available and the requirement for a detailed restriction map of the clone. *In vivo* transposon mutagenesis of protein-coding sequences has also been described. This method typically involves cumbersome manipulations and reliance on *in vivo* transposition.

Advantages of the EZ-Tn5 In-Frame Linker Insertion Kit

The EZ-Tn5 In-Frame Linker Insertion Kit is an *in vitro* transposon-based linker insertion method. The kit offers advantages over both traditional linker scanning mutagenesis and transposon-based *in vivo* mutagenesis. The EZ-Tn5 In-Frame Linker Insertion Kit offers:

- A simple *in vitro* enzymatic reaction.
- Random linker insertion into the cloned sequence…not limited to pre-existing restriction endonuclease sites as is the case with linker scanning mutagenesis.
- All three reading frames of the inserted linker are open.
- The protein coding regions on both sides of the inserted linker are conserved.

2. Kit Contents

Desc.	Concentration	Quantity
Reagents included in the kit are sufficient for 10 *in vitro* transposon-linker 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™ <*Not* I/KAN-3> Transposon: 1 pmol in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).	@ 0.1 pmol/µl (0.08 µg/µl)	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
***Not* I/KAN-3 FP-2 Forward Primer:** 1 nmol in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).	@ 50 µM	20 µl
***Not* I/KAN-3 RP-2 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 *Hpa* II fragment of bacteriophage DNA cloned into the *Acc* 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 ml

3. Related Products

The following products are also available:

- TransforMax™ EC100™ Electrocompetent *E. coli*
  - Transformation efficiency >10^9 cfu/µg DNA.
- Fast-Link™ DNA Ligation Kits
- T4 DNA Ligase
- Colony Fast-Screen™ Kits
- 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 In-Frame Linker Insertion Kit is function-tested by performance of the kit's *in vitro* control reaction followed by electroporation into a *rec*A^{-} *E. coli* host strain having a transformation efficiency of $>10^8$ 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 $>10^5$ Kan^R colonies/µg target DNA. Representative insertion clones are tested by *Not* I restriction for liberation of the insertion transposon. 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 <*Not* I/KAN-3> Transposon as template. All components of the EZ-Tn5 In-Frame Linker 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 minilysate 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 <*Not* I/KAN-3> Transposon.** If necessary, dilute the EZ-Tn5 <*Not* I/KAN-3> Transposon with TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

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 <*Not* I/KAN-3> Transposon
x	µl	sterile water to a reaction volume of 9 µl
1	µl	EZ-Tn5 Transposase
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 **Transformation and Recovery** or store the reaction mixture at –20°C.

*Calculation of µmol target DNA:

µmol target DNA = µg target DNA / [(# base pairs in target DNA) x 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] = 0.05×10^{-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 *rec*A– *E. coli* with a transformation efficiency of >10^8 cfu/µg of DNA. A *rec*A– 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 <*Not* I/KAN-3> Transposon. Epicentre's TransforMax™ EC100™ Electrocompetent *E. coli* (available separately) have a transformation efficiency of >1×10^9 cfu/µg and are ideal for this application.

1) Using 1 µl of the insertion reaction mixture, transform *rec*A– *E. coli*. If electrocompetent cells are used, perform electroporation according to the equipment manufacturer's recommended conditions. Use of >1 µl 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 <*Not* I/KAN-3> 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 >5×10^8 cfu/µg DNA, it may be necessary to dilute the cells 1:10 or 1:100 prior to plating. Plate portions (e.g., 100 µl) of cells onto LB plates containing 50 µg/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 µg/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 <*Not* I/KAN-3> Insertion Kit are:

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

The actual number of EZ-Tn5 <*Not* I/KAN-3> 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. Generating the In-Frame 19 Codon Insertion

Transposon Insertion Mapping

The <*Not* I/KAN-3>Transposon will randomly insert into the target DNA. Therefore, the transposon insertion site in each clone should be determined prior to *Not* I digestion by one of three methods:

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 sequencing, depending on the cost-effectiveness and experience of the user.

EZ-Tn5 <*Not* I/KAN-3> 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 *Not* I/KAN-3 FP-2 or *Not* I/KAN-3 RP-2 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 <*Not* I/KAN-3> Transposon provided in the Appendix, for reference.

Not I Digestion

Once the transposon insertion site of the desired clones has been determined, the clones are individually digested with *Not* I according to manufacturer's recommendations. Purify the linearized clone DNA from the 1,100 bp kanamycin-containing fragment (e.g., by agarose gel electrophoresis, column purification, etc.).

Religation and Transformation

Religate the linearized clones using T4 DNA ligase. Successful religation regenerates a single *Not* I restriction site and creates the 57-nucleotide (19 codon) insertion into all three reading frames (see Fig. 1). Transform the religated DNA into desired cells and select using an antibiotic marker present on the original cloning vector (ampicillin for the control DNA).

Analysis of the 19 Codon Insertion Clones

There are two important points to consider when analyzing the clones:

1. *Not* I digestion and religation results in the insertion of 57 nucleotides (19 codons) into all three reading frames of the original clone. Nine of the 57 nucleotides are the result of a 9-bp sequence duplication immediately flanking the transposon insertion site.
2. The amino acid sequence of the protein encoded by the target DNA is conserved on both sides of the 19-codon insertion.

   Fig. 2 shows a representation of the 19 amino acids that are inserted into each of the three reading frames of a hypothetical protein-encoding clone.

8. DNA Sequencing of Transposon insertion Clones

Primer Considerations

The *Not* I/KAN-3 FP-2 Forward and *Not* I/KAN-3 RP-2 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 In-Frame Linker 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 <*Not* I/KAN-3> 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 also Fig. 2).

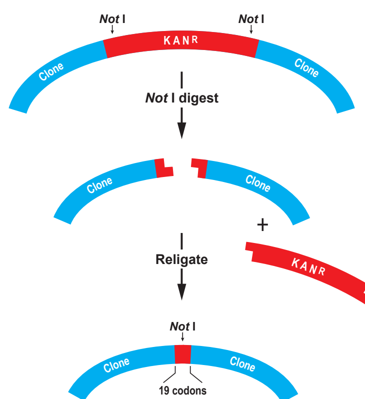


Figure 1. ***Not* I Digestion and Religation of the EZ-Tn5 <*Not* I/KAN-3> Transposon.**

9. 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.

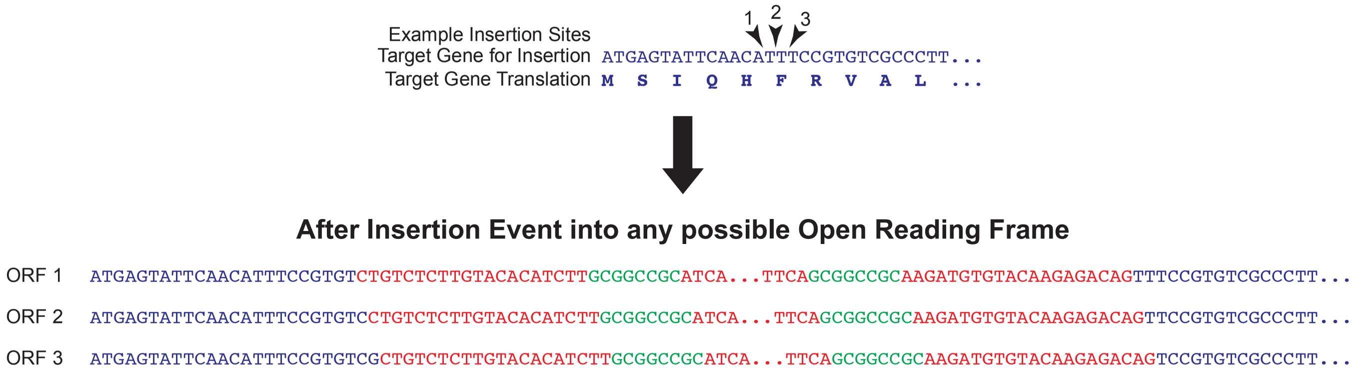
   Arcing inevitably results in failed transformation. Discard the electroporation reaction and try again with 0.5 µl of the transposition reaction. With the control DNA, no arcing is observed when up to 2 µl of transposition reaction is used for electroporation of 50 µl of electrocompetent *rec*A⁻ *E. 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 $<10^8$ cfu/µg DNA, one may not obtain sufficient clones on a plate. For example, transforming into cells with transformation efficiency $<10^5$ cfu/µg DNA results in as few as 2 insertion clones on a plate. Use cells with a transformation efficiency $>10^8$ 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.



After *Not* I Digestion and Religation, Open Reading Frame remains Open

ORF 1 ATGAGTATTCAACATTTCCGTGTCTGTCTCTTGTACACATCTTGCGGCCGCAAGATGTGTACAAGAGACAGTTTCCGTGTCGCCCTT...
Translation M S I Q H F R V C L L Y T S C G R K M C T R D S F R V A L ...

ORF 2 ATGAGTATTCAACATTTCCGTGTCCTGTCTCTTGTACACATCTTGCGGCCGCAAGATGTGTACAAGAGACAGTTCCGTGTCGCCCTT...
Translation M S I Q H F R V L S L V H I L R P Q D V Y K R Q F R V A L ...

ORF 3 ATGAGTATTCAACATTTCCGTGTCGCTGTCTCTTGTACACATCTTGCGGCCGCAAGATGTGTACAAGAGACAGTCCGTGTCGCCCTT...
Translation M S I Q H F R V S V S C T H L A A A R C V Q E T V R V A L ...

Results in the insertion of 19 codons into the open reading frame due to the presence of transposon sequence and insertion site duplication.

Figure 2. Example EZ-Tn5 In-Frame Linker Transposon Insertion

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.** Reverify 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.

10. Appendix

Primer Data

Not I/KAN-3 FP-2 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

Not I/KAN-3 RP-2 Reverse Primer

5′ - TCCCGTTGAATATGGCTCATAAC - 3′

Length: 23 nucleotides

G+C content: 10

Molecular Weight: 8,629 daltons

Temperatures of Dissociation & Melting:

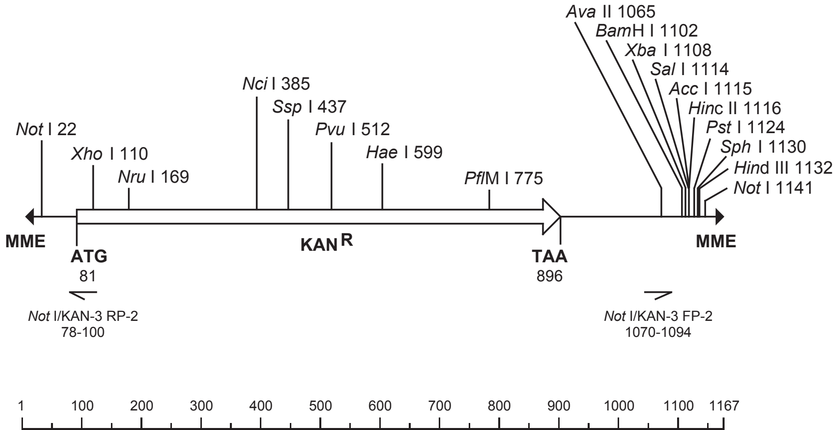
T_d: 68°C (nearest neighbor method)

T_m: 70°C (% G+C method)

T_m: 66°C ([2 (A+T) + 4 (G+C)] method)

T_m: 61°C ((81.5 + 16.6 (log [Na^+])) +
([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

EZ-Tn5 <Not I/KAN-3> Transposon Schematic Map



Note: Not all restriction enzymes that cut only once are indicated above.
See the following pages for further information.
Not I cuts twice in the map above.
Primers are not drawn to scale.

Not I/KAN-3 FP-2 Forward Primer 5' ACCTACAACAAAGCTCTCATCAACC 3'
Not I/KAN-3 RP-2 Reverse Primer 5' TCCCGTTGAATATGGCTCATAAC 3'
MME = Modified Mosaic End 5' AGATGTGTACAAGAGACAG 3'

EZ-Tn5 <Not I/KAN-3> Transposon Restriction Data

Restriction Enzymes that cut the EZ-Tn5 <*Not* I/KAN-3> Transposon 1 to 3 times:

Enzyme	Sites	Location
Acc I	1	1115
Aci I	3	25, 117, 1144
Alu I	2	1082, 1134
Apo I	2	125, 309
Ase I	1	711
AsiS I	1	512
Ava I	1	110
Ava II	1	1065
BamH I	1	1102
Ban II	1	167
Bfa I	1	1109
BfrB I	2	360, 626
BfuA I	1	1127
Bpu10 I	1	529
BsaW I	1	647
BsiE I	3	25, 512, 1144
Bsm I	2	396, 473
BsmA I	3	8, 528, 1155
BsmB I	1	528
Bsp1286 I	1	167
BspD I	1	203
BspH I	1	30
BspM I	1	1127
Bsr I	3	303, 927, 1067
BsrF I	1	466
BsrG I	2	9, 1154
BssK I	3	383, 400, 757
BstDS I	2	1036, 1097
BstF5 I	2	143, 769
BstN I	2	402, 759
BstU I	3	119, 169, 514
BstY I	2	761, 1102
Btg I	2	1032, 1093
Bts I	2	373, 460
Cla I	1	203
Dde I	2	529, 984
Dsa I	2	1032, 1093
Eae I	2	22, 1141
Eag I	2	22, 1141
Ear I	1	325
EcoN I	1	424
Fau I	1	1091
Hae I	1	599
Hinc II	1	1116
Hind III	1	1132
Hpa II	3	385, 467, 648
HpyCH4 IV	1	102
Mae II	1	102
Mbo II	3	312, 423, 1001
Mly I	2	745, 1121
Msp I	3	386, 468, 649
MspA1 I	1	1140
Nci I	1	385
Nla IV	1	1104
Not I	2	22, 1141
Nru I	1	169
Nsi I	2	362, 628
Nsp I	1	1130
PaeR7 I	1	110
PflM I	1	775
Ple I	2	744, 1120
PspG I	2	400, 757
Pst I	1	1124
Pvu I	1	512
Rsa I	3	11, 347, 1156
Sal I	1	1114
Sau96 I	1	1065
Sbf I	1	1124
ScrF I	3	385, 402, 759
Sfc I	1	1120
Sml I	1	110
Sph I	1	1130
Ssp I	1	437
Tat I	2	9, 1154
Tli I	1	110
Tsp45 I	1	659
TspR I	3	385, 460, 932
Xba I	1	1108
Xho I	1	110

Restriction Enzymes that cut the EZ-Tn5 <*Not* I/KAN-3> Transposon 4 or more times:

Alw I
BsaJ I
Bsl I
Cac8 I
CviJ I
Dpn I
Fnu4H I
Gdi II
Hae III
Hha I
Hinf I
HinP I
Hph I
Hpy188 I
HpyCH4 III
HpyCH4 V
Mae III
Mbo I
Mnl I
Mse I
Mwo I
Nla III
Sau3A I
SfaN I
Taq I
Tfi I
Tsp4C I
Tsp509 I

Restriction Enzymes that do not cut the EZ-Tn5 <*Not* I/KAN-3> Transposon:

Aat II
Acc65 I
Acl I
Afe I
Afl II
Afl III
Age I
Ahd I
Ale I
AlwN I
Apa I
ApaB I
ApaL I
Asc I
Avr II
Ban I
Bbs I
BbvC I
BciV I
Bcl I
Bgl I
Bgl II
Blp I
Bme1580 I
BmgB I
Bmr I
Bsa I
BsaA I
BsaB I
BsaH I
BseY I
BsiHKA I
BsiW I
BspE I
BspLU11 I
BsrB I
BsrD I
BssH II
BssS I
BstAP I
BstB I
BstE II
BstX I
BstZ17 I
Bsu36 I
Dra I
Dra III
Drd I
Eco47 III
EcoO109 I
EcoR I
EcoR V
Fse I
Fsp I
Hae II
Hpa I
Hpy99 I
Kpn I
Mfe I
Mlu I
Msc I
Msl I
Nae I
Nar I
Nco I
Nde I
NgoM IV
Nhe I
Pac I
Pci I
PflF I
Pme I
Pml I
PpuM I
PshA I
Psi I
PspOM I
Pvu II
Rsr II
Sac I
Sac II
SanD I
Sap I
Sca I
SexA I
Sfi I
Sfo I
SgrA I
Sim I
Sma I
SnaB I
Spe I
Srf I
Sse8647 I
Stu I
Sty I
Swa I
Tse I
Tth111 I
Xcm I
Xma I
Xmn I

EZ-Tn5 <Not I/KAN-3> Transposon Sequence

EZ-Tn5™ <*Not* I/KAN-3> Transposon 1,167 bp.

1	CTGTCTCTTG	TACACATCTT	GCGGCCGCAT	CATGAACAAT	AAAACTGTCT
51	GCTTACATAA	ACAGTAATAC	AAGGGGTGTT	ATGAGCCATA	TTCAACGGGA
101	AACGTCTTGC	TCGAGGCCGC	GATTAAATTC	CAACATGGAT	GCTGATTTAT
151	ATGGGTATAA	ATGGGCTCGC	GATAATGTCG	GGCAATCAGG	TGCGACAATC
201	TATCGATTGT	ATGGGAAGCC	CGATGCGCCA	GAGTTGTTTC	TGAAACATGG
251	CAAAGGTAGC	GTTGCCAATG	ATGTTACAGA	TGAGATGGTC	AGACTAAACT
301	GGCTGACGGA	ATTTATGCCT	CTTCCGACCA	TCAAGCATTT	TATCCGTACT
351	CCTGATGATG	CATGGTTACT	CACCACTGCG	ATCCCCGGAA	AAACAGCATT
401	CCAGGTATTA	GAAGAATATC	CTGATTCAGG	TGAAAATATT	GTTGATGCGC
451	TGGCAGTGTT	CCTGCGCCGG	TTGCATTCGA	TTCCTGTTTG	TAATTGTCCT
501	TTTAACAGCG	ATCGCGTATT	TCGTCTCGCT	CAGGCGCAAT	CACGAATGAA
551	TAACGGTTTG	GTTGATGCGA	GTGATTTTGA	TGACGAGCGT	AATGGCTGGC
601	CTGTTGAACA	AGTCTGGAAA	GAAATGCATA	AACTTTTGCC	ATTCTCACCG
651	GATTCAGTCG	TCACTCATGG	TGATTTCTCA	CTTGATAACC	TTATTTTTGA
701	CGAGGGGAAA	TTAATAGGTT	GTATTGATGT	TGGACGAGTC	GGAATCGCAG
751	ACCGATACCA	GGATCTTGCC	ATCCTATGGA	ACTGCCTCGG	TGAGTTTTCT
801	CCTTCATTAC	AGAAACGGCT	TTTTCAAAAA	TATGGTATTG	ATAATCCTGA
851	TATGAATAAA	TTGCAGTTTC	ATTTGATGCT	CGATGAGTTT	TTCTAATCAG
901	AATTGGTTAA	TTGGTTGTAA	CACTGGCAGA	GCATTACGCT	GACTTGACGG
951	GACGGCGGCT	TTGTTGAATA	AATCGAACTT	TTGCTGAGTT	GAAGGATCAG
1001	ATCACGCATC	TTCCCGACAA	CGCAGACCGT	TCCGTGGCAA	AGCAAAAGTT
1051	CAAAATCACC	AACTGGTCCA	CCTACAACAA	AGCTCTCATC	AACCGTGGCG
1101	GGGATCCTCT	AGAGTCGACC	TGCAGGCATG	CAAGCTTCAG	CGGCCGCAAG
1151	ATGTGTACAA	GAGACAG			

The transposon sequence can be downloaded at: http://www.epicentre.com/sequences.

11. 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.
3. Mahnke-Braam, l.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.

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**