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EZ-Tn5™ <TET-1> Insertion Kit

Cat. No. EZI921T

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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 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.

2. Kit Contents

Desc.	Concentration	Quantity
Reagents included in the kit are sufficient for 10 *in vitro* transposon insertion reactions.		
EZ-Tn5™ Transposase	10 U @ 1 U/µl	10 µl
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.		
EZ-Tn5™ <TET-1> Transposon: 1 pmol	@ 0.1 pmol/µl (0.11 µg/µl)	10 µl
in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).		
EZ-Tn5™ 10X Reaction Buffer:		100 µl
0.50 M Tris-acetate (pH 7.5), 1.5 M potassium acetate, 100 mM magnesium acetate, and 40 mM spermidine.		
EZ-Tn5™ 10X Stop Solution:		100 µl
1% SDS solution.		
TET-1 FP-1 Forward Primer: 1 nmol	@ 50 µM	20 µl
in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).		
TET-1 RP-1 Reverse Primer: 1 nmol	@ 50 µM	20 µl
in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).		
pUC19/3.4 Control Target DNA: 1 µg	@ 0.1 µg/µl	10 µl
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).		
Sterile Water:		1 µl

3. Related Products

The following products are also available:

- TransforMax™ EC100™ Electrocompetent *E. coli*
    - Transformation efficiency $>10^9$ cfu/µg DNA.
- Colony Fast-Screen™ Kit
- MasterPure™ DNA Purification Kits
- EZ-Tn5™ Tnp Transposome™ Kits
- EZ-Tn5™ Transposase
- EZ-Tn5™ Transposons
- EZ-Tn5™ Insertion Kits

How the EZ-Tn5 <TET-1> Insertion Kit Works.

The EZ-Tn5 <TET-1> Insertion Kit can be used to randomly insert primer binding sites and a Tetracycline resistance selection marker into target DNA *in vitro*. A single 2-hour *in vitro* reaction randomly inserts the <TET-1> Transposon into the target DNA. Use an aliquot of the reaction to transform *E. coli* such as Epicentre's TransforMax™ EC100™ Electrocompetent *E. coli* strain and select on Tetracycline plates. Only those clones harboring DNA containing the <TET-1> Transposon will grow.

The EZ-Tn5 <TET-1> Insertion Kit can be used for:

- 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 Tetracycline resistance selection marker into any DNA.

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

Preparation

- Prepare 0.2 µg of recombinant DNA for the EZ-Tn5 <TET-1> insertion reaction.

Day 1

- Perform the 2-hour *in vitro* EZ-Tn5 <TET-1> insertion reaction.
- Transform competent *rec*A$^-$ *E. coli* with 1 µl of the reaction mix.
- Select for Tetracycline-resistant transposon insertion clones on Tetracycline plates overnight.

Day 2

- Prepare DNA from Tetracycline-resistant colonies.
- (Optional) Map the EZ-Tn5 <TET-1> Transposon insertion sites.
- (Optional) DNA sequence chosen clones bidirectionally using the unlabeled forward and reverse transposon-specific primers supplied in the kit.

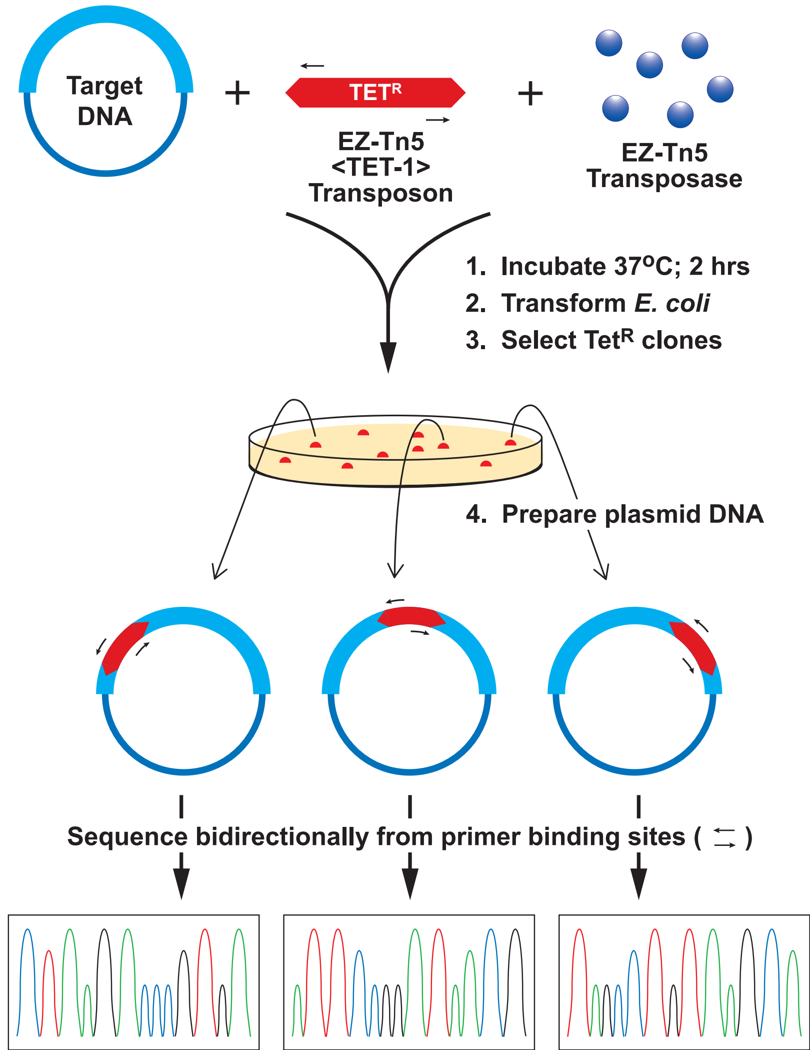

Figure 1. EZ-Tn5 <TET-1> Transposon Insertion Protocol.

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 <TET-1> 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 >10^9 cfu/µg DNA. Transposition frequency, defined as the ratio of the number of Tet^R clones divided by the number of transformants resistant to the antibiotic marker of the target vector, (Tet^R colonies/Amp^R colonies; for the control DNA) must be >0.5% (commonly at 10%) and transposition efficiency must be >10^6 Tet^R colonies/µg target DNA. 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 <TET-1> Transposon as template. All components of the EZ-Tn5 <TET-1> 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 Tetracycline 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 <TET-1> Transposon.** If necessary, dilute the EZ-Tn5 <TET-1> 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 <TET-1> 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 section 4, **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 x10^{-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 >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 tetracycline resistance marker when used with the EZ-Tn5 <TET-1> Transposon. Epicentre's TransforMax EC100 Electrocompetent *E. coli* (available separately) have a transformation efficiency of >1 x 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 medium (***Important:*** *medium must not contain* Mg^{2+}) 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 <TET-1> Transposon insertion clones are selected on tetracycline-containing plates.

Important: *Plate medium must not contain* Mg^{2+}.

Epicentre recommends storing tetracycline-containing plates in the dark, and using plates less than one month old.

1) If transformation was done using cells with an efficiency of >5 x 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 10 µg/ml tetracycline. 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 Tet^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 Tet^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 <TET-1> Insertion Kit are:

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

The actual number of EZ-Tn5 <TET-1> 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. DNA Sequencing of Transposon Insertion Clones

Transposon Insertion Mapping (optional)

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 <TET-1> 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 TET-1 FP-1 or TET-1 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 <TET-1> Transposon provided in the Appendix for reference.

Primer Considerations

The TET-1 FP-1 Forward and TET-1 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: *The sequence of the TET-1 FP-1 Forward and TET-1 RP-1 Reverse Primers do not function well as IRD800-labeled sequencing primers. We recommend using the TET-1 FP-3 Forward and TET-1 RP-3 Reverse Primer sequences for this purpose. See 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 <TET-1> 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 <TET-1> 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. 3).

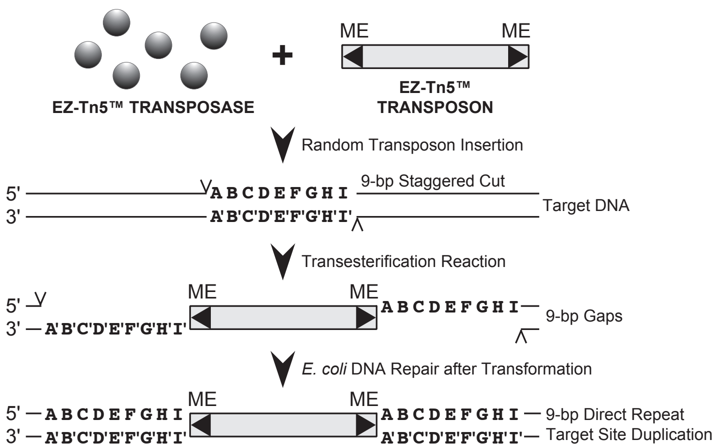

Figure 2. **EZ-Tn5 Transposase Insertion Site Duplication Process.**

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 *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.

3) **Mg^{2+} present in outgrowth or plating media.** Mg^{2+} acts as an antagonist against Tetracycline selection in *E. coli* . Retransform cells and use LB or other Mg^{2+}-free medium for outgrowth and plating.

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".

Figure 3. EZ-Tn5 Transposon Insertion Site Junction.

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 trans-poson used.** Use a different host for transformation and retransform with a portion of your remaining reaction.

   Some host cells carry Tet^R transposons. Confirm that the genotype of the host strain chosen for the transformation is not Tet^R.

9. Appendix

Primer Data

TET-1 FP-1 Forward Primer

5′ - GGGTGCGCATGATCCTCTAGAGT - 3′

Length: 23 nucleotides

G+C content: 13

Molecular Weight: 7,074 daltons

Temperatures of Dissociation & Melting:

T_d: 71°C (nearest neighbor method)

T_m: 75°C (% G+C method)

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

T_m: 66°C ((81.5 + 16.6 (log [Na^+])) + ([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

TET-1 RP-1 Reverse Primer

5′ - TAAATTGCACTGAAATCTAGAAATA - 3′

Length: 25 nucleotides

G+C content: 6

Molecular Weight: 7,657 daltons

Temperatures of Dissociation & Melting:

T_d: 61°C (nearest neighbor method)

T_m: 64°C (% G+C method)

T_m: 62°C ([2 (A+T) + 4 (G+C)] method)

T_m: 55°C ((81.5 + 16.6 (log [Na^+])) + ([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

Note: *Use the following alternative primer sequences for IRD800-labeled sequencing primers.*

TET-1 FP-3 Forward Primer

5′ - GCATCTCGGGCACGTTGGGTCCT - 3′

Length: 23 nucleotides

G+C content: 15

Molecular Weight: 7,905 daltons

Temperatures of Dissociation & Melting:

T_d: 80°C (nearest neighbor method)

T_m: 79°C (% G+C method)

T_m: 76°C ([2 (A+T) + 4 (G+C)] method)

T_m: 70°C ((81.5 + 16.6 (log [Na+])) + ([41 (#G+C) - 500] / length) method)
where [Na+] = 0.1 M

TET-1 RP-3 Reverse Primer

5′ - GGGGCTGACTTCAGGTGCTACATTT - 3′

Length: 25 nucleotides

G+C content: 13

Molecular Weight: 8,561 daltons

Temperatures of Dissociation & Melting:

T_d: 73°C (nearest neighbor method)

T_m: 76°C (% G+C method)

T_m: 76°C ([2 (A+T) + 4 (G+C)] method)

T_m: 66°C ((81.5 + 16.6 (log [Na+])) + ([41 (#G+C) - 500] / length) method)
where [Na+] = 0.1 M

EZ-Tn5 <TET-1> Transposon Sequence

EZ-Tn5™ <TET-1> Transposon 1,674 bp.

```
   1  CTGTCTCTTA  TACACATCTC  AACCATCATC  GATGAATTCG  AGCTCGGTAC  CCGGGGATCA
  61  TCTTATTAAT  CAGATAAAAT  ATTTCTAGAT  TTCAGTGCAA  TTTATCTCTT  CAAATGTAGC
 121  ACCTGAAGTC  AGCCCCATAC  GATATAAGTT  GTAATTCTCA  TGTTTGACAG  CTTATCATCG
 181  ATAAGCTTTA  ATGCGGTAGT  TTATCACAGT  TAAATTGCTA  ACGCAGTCAG  GCACCGTGTA
 241  TGAAATCTAA  CAATGCGCTC  ATCGTCATCC  TCGGCACCGT  CACCCTGGAT  GCTGTAGGCA
 301  TAGGCTTGGT  TATGCCGGTA  CTGCCGGGCC  TCTTGCGGGA  TATCGTCCAT  TCCGACAGCA
 361  TCGCCAGTCA  CTATGGCGTG  CTGCTAGCGC  TATATGCGTT  GATGCAATTT  CTATGCGCAC
 421  CCGTTCTCGG  AGCACTGTCC  GACCGCTTTG  GCCGCCGCCC  AGTCCTGCTC  GCTTCGCTAC
 481  TTGGAGCCAC  TATCGACTAC  GCGATCATGG  CGACCACACC  CGTCCTGTGG  ATCCTCTACG
 541  CCGGACGCAT  CGTGGCCGGC  ATCACCGGCG  CCACAGGTGC  GGTTGCTGGC  GCCTATATCG
 601  CCGACATCAC  CGATGGGGAA  GATCGGGCTC  GCCACTTCGG  GCTCATGAGC  GCTTGTTTCG
 661  GCGTGGGTAT  GGTGGCAGGC  CCCGTGGCCG  GGGGACTGTT  GGGCGCCATC  TCCTTGCATG
 721  CACCATTCCT  TGCGGCGGCG  GTGCTCAACG  GCCTCAACCT  ACTACTGGGC  TGCTTCCTAA
 781  TGCAGGAGTC  GCATAAGGGA  GAGCGTCGAC  CGATGCCCTT  GAGAGCCTTC  AACCCAGTCA
 841  GCTCCTTCCG  GTGGGCGCGG  GGCATGACTA  TCGTCGCCGC  ACTTATGACT  GTCTTCTTTA
 901  TCATGCAACT  CGTAGGACAG  GTGCCGGCAG  CGCTCTGGGT  CATTTTCGGC  GAGGACCGCT
 961  TTCGCTGGAG  CGCGACGATG  ATCGGCCTGT  CGCTTGCGGT  ATTCGGAATC  TTGCACGCCC
1021  TCGCTCAAGC  CTTCGTCACT  GGTCCCGCCA  CCAAACGTTT  CGGCGAGAAG  CAGGCCATTA
1081  TCGCCGGCAT  GGCGGCCGAC  GCGCTGGGCT  ACGTCTTGCT  GGCGTTCGCG  ACGCGAGGCT
1141  GGATGGCCTT  CCCCATTATG  ATTCTTCTCG  CTTCCGGCGG  CATCGGGATG  CCCGCGTTGC
1201  AGGCCATGCT  GTCCAGGCAG  GTAGATGACG  ACCATCAGGG  ACAGCTTCAA  GGATCGCTCG
1261  CGGCTCTTAC  CAGCCTAACT  TCGATCATTG  GACCGCTGAT  CGTCACGGCG  ATTTATGCCG
1321  CCTCGGCGAG  CACATGGAAC  GGGTTGGCAT  GGATTGTAGG  CGCCGCCCTA  TACCTTGTCT
1381  GCCTCCCCGC  GTTGCGTCGC  GGTGCATGGA  GCCGGGCCAC  CTCGACCTGA  ATGGAAGCCG
1441  GCGGCACCTC  GCTAACGGAT  TCACCACTCC  AAGAATTGGA  GCCAATCAAT  TCTTGCGGAG
1501  AACTGTGAAT  GCGCAAACCA  ACCCTTGGCA  GAACATATCC  ATCGCGTCCG  CCATCTCCAG
1561  CAGCGCACGC  GGCGCATCTC  GGGCACGTTG  GGTCCTGGCC  ACGGGTGCGC  ATGATCCTCT
1621  AGAGTCGACC  TGCAGGCATG  CAAGCTTCAG  GGTTGAGATG  TGTATAAGAG  ACAG
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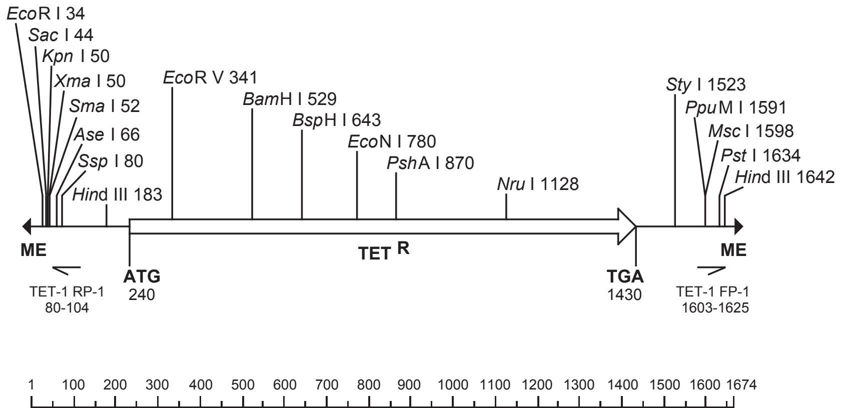
The transposon sequence can be downloaded at the URL: http://www.epicentre.com/sequences

EZ-Tn5 <TET-1> Transposon Schematic Map

EZ-Tn5™ <TET-1> Transposon
(1,674 bp)



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

TET-1 FP-1 Forward Primer	5' GGGTGCGCATGATCCTCTAGAGT 3'
TET-1 RP-1 Reverse Primer	5' TAAATTGCACTGAAATCTAGAAATA 3'
ME = Mosaic End	5' AGATGTGTATAAGAGACAG 3'

Figure 4. EZ-Tn5 <TET-1> Transposon

EZ-Tn5 <TET-1> Transposon Restriction Data

Restriction Enzymes that cut the EZ-Tn5 <TET-1> Transposon one to three times:

Enzyme	Sites	Location
Acc65 I	1	46
Acc I	2	806, 1625
Acl I	1	1055
Afe I	3	388, 650, 931
ApaB I	1	1206
Apo I	1	34
Ase I	1	66
Ava I	2	50, 1578
BamH I	1	529
Ban II	3	44, 629, 643
Bbs I	1	884
Bfa I	3	85, 384, 1619
BfuA I	2	1208, 1637
Bgl I	2	1089, 1323
Bme1580 I	1	1585
Bmr I	3	453, 774, 828
BsaW I	1	847
BseY I	1	1103
BsiE I	3	443, 810, 1096
Bsm I	1	1513
BsmA I	2	8, 1662
BspD I	2	29, 178
BspH I	1	643
BspM I	2	1208, 1637
BstAP I	1	1205
BstDS I	2	686, 1603
BstN I	3	285, 1214, 1595
BstY I	1	529
Btg I	2	682, 1599
Cla I	2	29, 178
Dsa I	2	682, 1599
Eag I	1	1093
Ear I	1	112
Eco47 III	3	388, 650, 931
EcoN I	1	780
EcoO109 I	2	678, 1591
EcoR I	1	34
EcoR V	1	341
Fsp I	3	416, 1512, 1608
Hinc II	2	807, 1626
Hind III	2	183, 1642
HpyCH4 IV	3	1055, 1111, 1585
Kpn I	1	50
Mae II	3	1055, 1111, 1585
Mly I	2	795, 1631
Msc I	1	1598
Mse I	3	66, 188, 210
Msl I	1	1185
MspA1 I	1	1295
Nhe I	1	383
Nru I	1	1128
Nsp I	2	720, 1640
PflM I	2	1475, 1524
Ple I	2	794, 1630
PpuM I	1	1591
PshA I	1	870
PspG I	3	283, 1212, 1593
Pst I	1	1634
Rsa I	2	48, 319
Sac I	1	44
Sal I	2	805, 1624
Sbf I	1	1634
Sfc I	2	292, 1630
SgrA I	1	564
Sim I	2	938, 1591
Sma I	1	52
Sml I	2	818, 1024
Sph I	2	720, 1640
Ssp I	1	80
Sty I	1	1523
Tfi I	3	1006, 1160, 1458
TspR I	3	99, 444, 1048
Xba I	2	84, 1618
Xma I	1	50

Restriction Enzymes that cut the EZ-Tn5 <TET-1> Transposon four or more times:

Aci I
Alu I
Alw I
Ava II
Ban I
BsaH I
BsaJ I
BsiHKA I
Bsl I
Bsp1286 I
Bsr I
BsrF I
BssK I
BstF5 I
BstU I
Cac8 I
CviJ I
Dpn I
Eae I
Fau I
Fnu4H I
Gdi II
Hae I
Hae II
Hae III
Hha I
Hinf I
HinP I
Hpa II
Hph I
Hpy188 I
Hpy99 I
HpyCH4 III
HpyCH4 V
Mae III
Mbo I
Mbo II
Mnl I
Msp I
Mwo I
Nae I
Nar I
Nci I
NgoM IV
Nla III
Nla IV
Sau3A I
Sau96 I
ScrF I
SfaN I
Sfo I
Taq I
Tse I
Tsp45 I
Tsp4C I
Tsp509 I

Restriction Enzymes that do not cut the EZ-Tn5 <TET-1> Transposon:

Aat II
Afl II
Afl III
Age I
Ahd I
Ale I
AlwN I
Apa I
ApaL I
Asc I
AsiS I
Avr II
BbvC I
BciV I
Bcl I
BfrB I
Bgl II
Blp I
BmgB I
Bpu10 I
Bsa I
BsaA I
BsaB I
BsiW I
BsmB I
BspE I
BspLU11 I
BsrB I
BsrD I
BsrG I
BssH II
BssS I
BstB I
BstE II
BstX I
BstZ17 I
Bsu36 I
Bts I
Dde I
Dra I
Dra III
Drd I
Fse I
Hpa I
Mfe I
Mlu I
Nco I
Nde I
Not I
Nsi I
Pac I
PaeR7 I
Pci I
PflF I
Pme I
Pml I
Psi I
PspOM I
Pvu I
Pvu II
Rsr II
Sac II
SanD I
Sap I
Sca I
SexA I
Sfi I
SnaB I
Spe I
Srf I
Sse8647 I
Stu I
Swa I
Tat I
Tli I
Tth111 I
Xcm I
Xho I
Xmn I

10. References

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