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EZ-Tn5™ <DHFR-1> Insertion Kit

Cat. No. EZI912D

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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.	Quantity	
Reagents included in the kit are sufficient for 10 *in vitro* transposon insertion reactions.		
EZ-Tn5™ Transposase: 10 U @ 1 U/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.		10 µl
EZ-Tn5™ <DHFR-1> Transposon: 1 pmol @ 0.1 pmol/µl (0.058 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
DHFR-1 FP-1 Forward Primer: 1 nmol in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).	@ 50 µM	20 µl
DHFR-1 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 *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.
– Colony Fast-Screen™ Kit
– MasterPure™ DNA Purification Kits
– EZ-Tn5™ Tnp Transposome™ Kits
– EZ-Tn5™ Transposase
– EZ-Tn5™ Transposons
– EZ-Tn5™ Insertion Kits

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

The EZ-Tn5 <DHFR-1> Insertion Kit can be used to randomly insert primer binding sites and a trimethoprim resistance selection marker into target DNA *in vitro*. A single 2-hour *in vitro* reaction randomly inserts the <DHFR-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 trimethoprim plates. Only those clones harboring DNA containing the <DHFR-1> Transposon will grow.

The EZ-Tn5 <DHFR-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 trimethoprim resistance selection marker into any DNA.

Figure 1 (below) describes the steps involved when using the EZ-Tn5 <DHFR-1> Insertion Kit. The process can be summarized as follows:

Preparation

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

Day 1

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

Day 2

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

5. 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 <DHFR-1> 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 >10^9 cfu/µg DNA. Transposition frequency, defined as the

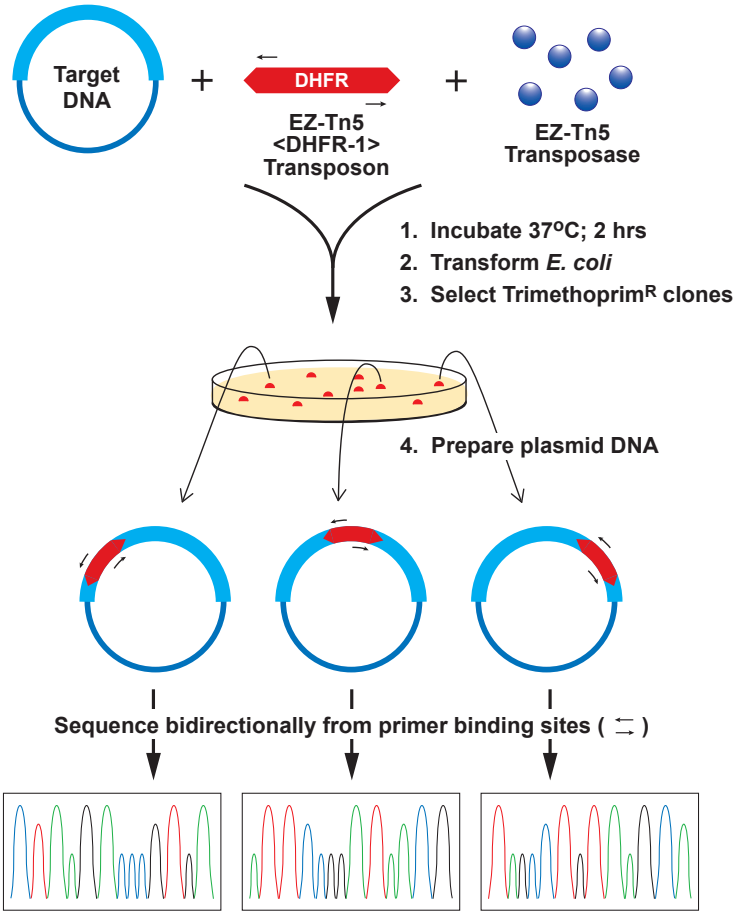


Figure 1. EZ-Tn5 <DHFR-1> Transposon Insertion Protocol.

ratio of the number of trimethoprim^R clones divided by the number of transformants resistant to the antibiotic marker of the target vector, trimethoprim^R colonies/Amp^R colonies; for the control DNA) must be >0.5% (commonly at 10%) and transposition efficiency must be >10^6 trimethoprim^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 <DHFR-1> Transposon as template. All components of the EZ-Tn5 <DHFR-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.

6. Transposon Insertion Reaction

Target DNA Preparation

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 <DHFR-1> Transposon.** If necessary, dilute the EZ-Tn5 <DHFR-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 <DHFR-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 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

7. 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. 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 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 <DHFR-1> Transposon insertion clones are selected on trimethoprim-containing plates. Trimethoprim is an antimetabolite, not an antibiotic, and the sensitivity of dihydrofolate reductase to this compound varies widely among strains of bacteria, including *E. coli*. We have found *E. coli* strain DH10B (Life Technologies, Inc.) to be sensitive to 10 µg/ml of trimethoprim. We recommend testing the sensitivity of your strain by plating on media containing a range of trimethoprim (10-200 µg/ml). Choose a concentration of trimethoprim that results in no visible growth or a light haze of small, pin point colonies. Another strain must be chosen if growth is confluent. A stock of trimethoprim (10 mg/ml) can be made in dimethylformamide.

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. 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 trimethoprim^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 trimethoprim^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 <DHFR-1> Insertion Kit are:

- Transposon insertion frequency = 0.5-20%
- Transposition clones per µg target DNA = 1 x 10^6-1 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 <DHFR-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.

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

Primer Considerations

The DHFR-1 FP-1 Forward and DHFR-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: *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 <DHFR-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 <DHFR-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, below).

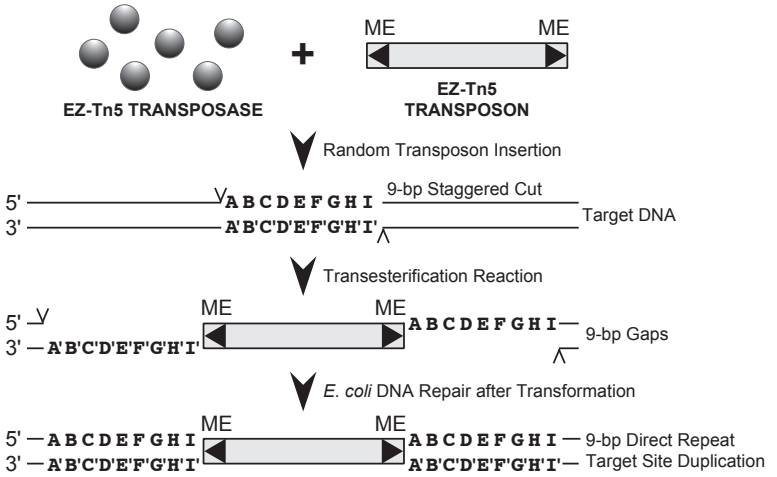


Figure 2. EZ-Tn5 Transposase Insertion Site Duplication Process.

Figure 3. EZ-Tn5 Transposon Insertion Site Junction.

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.

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.

10. Appendix

Primer Data

DHFR-1 FP-1 Forward Primer

5′ - GGCGGAAACATTGGATGCGG - 3′

Length: 20 nucleotides

G+C content: 12

Molecular Weight: 6,228 daltons

Temperatures of Dissociation & Melting:

T_d: 75°C (nearest neighbor method)

T_m: 72°C (% G+C method)

T_m: 64°C ([2 (A+T) + 4 (G+C)] method)

T_m: 64°C ((81.5 + 16.6 (log $[Na^+]$)) + ([41 (#G+C) - 500] / length) method)
where $[Na^+]$ = 0.1 M

DHFR-1 RP-1 Reverse Primer

5′ - GACACTCTGTTATTACAAATCG - 3′

Length: 22 nucleotides

G+C content: 8

Molecular Weight: 6,672 daltons

Temperatures of Dissociation & Melting:

T_d: 56°C (nearest neighbor method)

T_m: 66°C (% G+C method)

T_m: 60°C ([2 (A+T) + 4 (G+C)] method)

T_m: 57°C ((81.5 + 16.6 (log $[Na^+]$)) + ([41 (#G+C) - 500] / length) method)
where $[Na^+]$ = 0.1 M

EZ-Tn5 <DHFR-1> Transposon Sequence

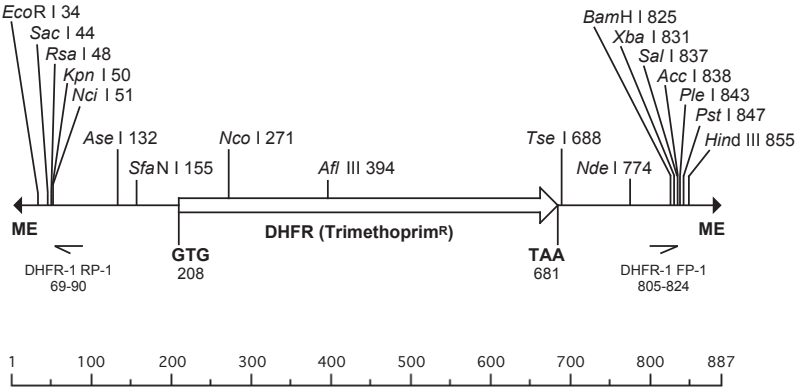
EZ-Tn5™ <DHFR-1> Transposon 887 bp.

1	CTGTCTCTTA	TACACATCTC	AACCATCATC	GATGAATTCG	AGCTCGGTAC
51	CCGGATAGAC	GGCATGCACG	ATTTGTAATA	ACAGAGTGTC	TTGTATTTTT
101	AAAGAAAGTC	TATTTAATAC	AAGTGATTAT	ATTAATTAAC	GGTAAGCATC
151	AGCGGGTGAC	AAAACGAGCA	TGCTTACTAA	TAAAATGTTA	ACCTCTGAGG
201	AAGAATTGTG	AAACTATCAC	TAATGGTAGC	TATATCGAAG	AATGGAGTTA
251	TCGGGAATGG	CCCTGATATT	CCATGGAGTG	CCAAAGGTGA	ACAGCTCCTG
301	TTTAAAGCTA	TTACCTATAA	CCAATGGCTG	TTGGTTGGAC	GCAAGACTTT
351	TGAATCAATG	GGAGCATTAC	CCAACCGAAA	GTATGCGGTC	GTAACACGTT
401	CAAGTTTTAC	ATCTGACAAT	GAGGACGTAT	TGATCTTTCC	ATCAATTAAA
451	GATGCTTTAA	CCAACCTAAA	GAAAATAACG	GATCATGTCA	TTGTTTCAGG
501	TGGTGGGGAG	ATATACAAAA	GCCTGATCGA	TCAAGTAGAT	ACACTACATA
551	TATCTACAAT	AGACATCGAG	CCGGAAGGTG	ATGTTTACTT	TCCTGAAATC
601	CCCAGCAATT	TTAGGCCAGT	TTTTACCCAA	GACTTCGCCT	CTAACATAAA
651	TTATAGTTAC	CAAATCTGGC	AAAAGGGTTA	ACAAGTGGCA	GCAACGGATT
701	CGCAAACCTG	TCACGCCTTT	TGTGCCAAAA	GCCGCGCCAG	GTTTGCGATC
751	CGCTGTGCCA	GGCGTTAGGC	GTCATATGAA	GATTTCGGTG	ATCCCTGAGC
801	AGGTGGCGGA	AACATTGGAT	GCGGGGATCC	TCTAGAGTCG	ACCTGCAGGC
851	ATGCAAGCTT	CAGGGTTGAG	ATGTGTATAA	GAGACAG	

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

EZ-Tn5 <DHFR-1> Transposon Schematic Map

EZ-Tn5™ <DHFR-1> Transposon
(887 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.

DHFR-1 FP-1 Forward Primer	5' GGCGGAAACATTGGATGCGG 3'
DHFR-1 RP-1 Reverse Primer	5' GACACTCTGTTATTACAAATCG 3'
ME = Mosaic End	5' AGATGTGTATAAGAGACAG 3'

EZ-Tn5 <DHFR-1> Transposon Restriction Data

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

Enzyme	Sites	Location	Enzyme	Sites	Location
Acc65 I	1	46	Hinc II	3	189, 679, 839
Acc I1	838		Hind III	1	855
Aci I	2	732, 750	Hinf I	3	352, 697, 835
Afl III	1	394	HinP I	1	734
AlwN I	1	297	Hpa I	2	189, 679
Apo I	1	34	Hpa II	2	51, 571
Ase I1	132		Hpy188 I	2	196, 414
BamH I	1	825	HpyCH4 III	1	141
Ban I	1	46	HpyCH4 IV	2	396, 425
Ban II	1	44	HpyCH4 V	3	66, 845, 853
Bfa I	1	832	Kpn I	1	50
BfuA I	2	790, 850	Mae II	2	396, 425
Bpu10 I	1	795	Mbo II	3	212, 249, 790
BsaH I	1	769	Mly I1	844	
BsaJ I	1	271	Msp I	2	52, 572
BseY I	1	601	MspA1 I	2	152, 752
BsiE I	1	389	Mwo I	2	721, 742
BsiHKA I	1	44	Nci I	1	51
Bsl I	1	799	Nco I	1	271
BsmA I	2	8, 875	Nde I	1	774
Bsp1286 I	1	44	Nla IV	2	48, 827
BspD I	2	29, 527	Nsp I	3	66, 172, 853
BspM I	2	790, 850	Pac I	1	136
Bsr I	1	616	Ple I	1	843
BssK I	3	49, 736, 757	PspG I	2	736, 757
BstDS I	1	275	Pst I	1	847
BstF5 I	1	823	Rsa I 1	48	
BstN I	2	738, 759	Sac I	1	44
BstU I	1	734	Sal I	1	837
BstY I	1	825	Sau96 I	1	259
Btg I	1	271	Sbf I	1	847
Cla I	2	29, 527	ScrF I	3	51, 738, 759
Dde I	2	195, 795	SfaN I	3	155, 441, 808
Dra I2	100, 303		Sfc I	1	843
Dsa I	1	271	Sph I	3	66, 172, 853
EcoR I	1	34	Sty I	1	271
Fau I2	145, 814		Tfi I	2	352, 697
Fnu4H I	2	689, 732	Tse I	1	688
Hae I	1	615	Tsp45 I	2	155, 709
Hae III	2	260, 615	Tsp4C I	1	141
Hha I	1	736	Xba I	1	831

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

Alu I	CviJ I	Mae III	Mse I	Taq I
Alw I	Dpn I	Mbo I	Nla III	Tsp509 I
Cac8 I	Hph I	MnI I	Sau3A I	

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

Aat II	Bme1580 I	BstZ17 I	NgoM IV	Sfi I
Acl I	BmgB I	Bsu36 I	Nhe I	Sfo I
Afe I	Bmr I	Bts I	Not I	SgrA I
Afl II	Bsa I	Dra III	Nru I	Sim I
Age I	BsaA I	Drd I	Nsi I	Sma I
Ahd I	BsaB I	Eae I	PaeR7 I	Sml I
Ale I	BsaW I	Eag I	Pci I	SnaB I
Apa I	BsiW I	Ear I	PflF I	Spe I
ApaB I	Bsm I	Eco47 III	PflM I	Srf I
ApaL I	BsmB I	EcoN I	Pme I	Sse8647 I
Asc I	BspE I	EcoO109 I	Pml I	Ssp I
AsiS I	BspH I	EcoR V	PpuM I	Stu I
Ava I	BspLU11 I	Fse I	PshA I	Swa I
Ava II	BsrB I	Fsp I	Psi I	Tat I
Avr II	BsrD I	Gdi II	PspOM I	Tli I
Bbs I	BsrF I	Hae II	Pvu I	TspR I
BbvC I	BsrG I	Hpy99 I	Pvu II	Tth111 I
BciV I	BssH II	Mfe I	Rsr II	Xcm I
Bcl I	BssS I	Mlu I	Sac II	Xho I
BfrB I	BstAP I	Msc I	SanD I	Xma I
Bgl I	BstB I	Msl I	Sap I	Xmn I
Bgl II	BstE II	Nae I	Sca I	
Blp I	BstX I	Nar I	SexA I	

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