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EZ-Tn5™ <DHFR-1>Tnp Transposome™ Kit

Cat. No. TSM99D1

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1. Introduction

The EZ-Tn5™ <DHFR-1>Tnp Transposome™ is the stable complex formed between the EZ-Tn5 Transposase enzyme and the EZ-Tn5 <DHFR-1> Transposon. The EZ-Tn5 <DHFR-1> Transposon contains the dihydrofolate reductase (DHFR) gene (trimethoprim resistance) that is functional in *E. coli*, flanked by hyperactive 19-basepair Mosaic End (ME) EZ-Tn5 Transposase recognition sequences. The EZ-Tn5 Transposome can be electroporated into living cells where the EZ-Tn5 Transposase is activated by Mg^{2+} in the host's cellular environment resulting in random insertion of the EZ-Tn5 Transposon into the host genomic DNA.[1-4]

Unlabeled forward and reverse transposon-specific primers are supplied in the kit. The primers can be used for bidirectional DNA sequencing or mapping of transposon insertion sites in target genomic DNAs, including direct sequencing from genomic DNA without cloning.[3]

2. Applications

- Create gene "knockouts" by insertional mutagenesis, *in vivo*.†
- Sequence bacterial genomic DNA directly from transposon insertions, without cloning.
- Insert priming sites for mapping or PCR into chromosomal DNA, *in vivo*.
- Insert a trimethoprim-resistance marker into bacteria

3. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: The EZ-Tn5 <DHFR-1>Tnp Transposome is supplied in a 50% glycerol solution containing 27.5 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.3 mM EDTA, 0.05% Triton® X-100, and 0.5 mM dithiothreitol. The DHFR-1 FP-1 Forward and DHFR-1 RP-1 Reverse Primers are supplied in 10 mM Tris-HCl, (pH 7.5), 1 mM EDTA.

Size: Reagents included in the kit are sufficient for 10 *in vivo* transposon insertion reactions.

Quality Control: EZ-Tn5 <DHFR-1>Tnp Transposome activity is assayed by electroporation into a *rec*A$^-$ *E. coli* host strain having a transformation efficiency of $>10^9$ cfu/µg DNA. Assays must yield $>10^6$ trimethoprim^R colonies/µg or $>10^4$ trimethoprim^R colonies/µl of transposome respectively. 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.

Contaminating Activity Assays: All components of the EZ-Tn5 <DHFR-1>Tnp Transposome 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.

4. Kit Contents

Desc.	Concentration	Quantity
EZ-Tn5™ <DHFR-1>Tnp Transposome™	@ 15 ng/µl	10 µl
DHFR-1 FP-1 Forward Primer	@ 50 µM	20 µl
DHFR-1 RP-1 Reverse Primer	@ 50 µM	20 µl
Sterile Water		1 ml

5. Related Products

The following products are also available:

– TransforMax™ EC100™ Electrocompetent *E. coli*
– MasterPure™ DNA Purification Kits
– EZ-Tn5™ Tnp Transposome™ Kits
– EZ-Tn5™ Transposase
– EZ-Tn5™ Transposons
– EZ-Tn5™ Insertion Kits

6. Protocol

Electroporation of Host Cells with EZ-Tn5 <DHFR-1>Tnp Transposome and Selection of Transposition Clones:

Electroporate electrocompetent cells using 1 ml of the EZ-Tn5 <DHFR-1>Tnp Transposome. The electrocompetent cells should have a transformation efficiency of >10^7 cfu/µg of DNA, but use cells of the highest transformation efficiency possible to maximize the number of transposon insertion clones. Perform electroporation according to the equipment manufacturer's recommendations.

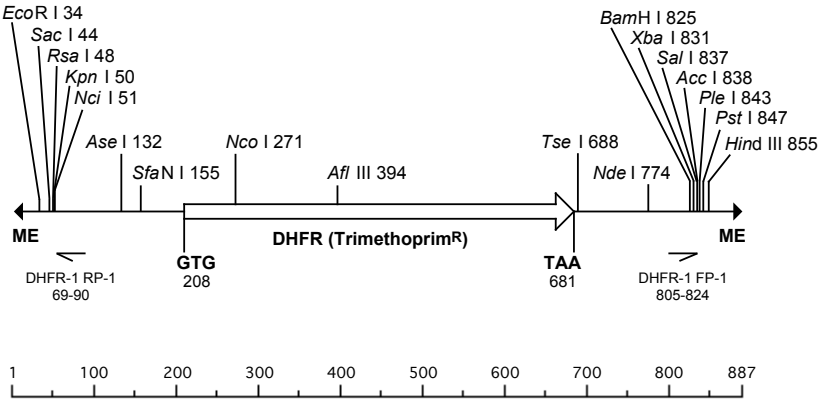
Immediately recover the electroporated cells after electroporation. Even slight delays in initiating the cell recovery process will result in a reduced number of transposition clones. For *E. coli*, add SOC medium to the electroporation cuvette to 1 ml final volume **immediately** after electroporation. 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.

Important Note: *EZ-Tn5 <DHFR-1> transposition clones are selected on trimethoprim 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 (10-200 µg/ml) of trimethoprim. Choose a concentration of that results in no visible growth or a light haze of small, pin point colonies. Another strain must be chosen if growth is confluent.*

If working with *E. coli*, dilute aliquots of the recovered cells (e.g., 1:10 and 1:100). Plate 100 µl of undiluted cells and each cell dilution separately on plates containing trimethoprim (a stock of 10 mg/ml can be made in dimethylformamide). Store the unused portion of the electroporated cells at +4°C for up to 2 days in the event that additional plates need to be prepared. The number of trimethoprim^R colonies/µl of EZ-Tn5 <DHFR-1>Tnp Transposome will be dependent on the transformation efficiency

of the cells used and the level of expression of the DHFR gene in that species. 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.

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'

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

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

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

EZ-Tn5™ <DHFR-1> Transposon 887 bp.

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  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 www.epicentre.com/sequences

Primer Information

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

7. References

1. Hoffman, L.M. *et al.*, (1999) *Current Genetics* **35**, 304.
2. Reznikoff, W. and Goryshin, I.Y. (1999) *Epicentre Forum* **6** (2), 5.
3. Hoffman, L.M. and Jendrisak, J. (1999) *Epicentre Forum* **6** (3), 1.
4. Goryshin, I.Y. *et al.*, (2000) *Nat. Biotechnol.* **18**, 97.

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