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EZ-Tn5™ pMOD™<MCS> Transposon Construction Vectors

Cat. No. MOD0602

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1. Introduction

The EZ-Tn5™ pMOD™<MCS> Transposon Construction Vector* was developed for the preparation of custom EZ-Tn5 Transposons. The vector contains a multiple cloning site (MCS) between the hyperactive 19-bp Mosaic Ends (ME) that are specifically and uniquely recognized by EZ-Tn5 Transposase. Also included between the ME's are primer binding sites for bidirectional sequencing from any custom EZ-Tn5 Transposon. To prepare a transposon, clone any DNA sequence of interest into the MCS and then generate the transposon either by PCR amplification using the Forward and Reverse PCR Primers provided with the vector, or restriction enzyme digestion.

The EZ-Tn5 pMOD-2<MCS> Transposon Construction Vector is a pUC-based vector with a *col*E1 origin of replication (Fig. 3). This vector works well for constructing transposons in most cases. However, if the transposon is prepared by restriction enzyme digestion, there is a chance that the uncut pMOD vector will interfere with downstream applications. Replication from the R6Kγ origin is dependent on the *pir* gene product produced by TransforMax™ EC100D™ *pir*$^+$ and *pir*-116 *E. coli* cells which are available separately. Since most bacterial strains do not contain a *pir* gene, the uncut plasmid DNA that contaminates these transposon preparations can't replicate and background problems are eliminated.

Use the EZ-Tn5 Transposon for random, *in vitro** insertion into a plasmid, cosmid or BAC clone using EZ-Tn5 Transposase or prepare an EZ-Tn5 Transposome™ for *in vivo*† insertions by incubating with EZ-Tn5 Transposase in the absence of Mg^{2+}. Sequence from the transposon bidirectionally using the pMOD<MCS> Forward and Reverse Sequencing Primers which are available separately.

2. Product Specifications

Storage: Store at –20°C.

Quality Control:

The EZ-Tn5 pMOD<MCS> Transposon Construction Vector is function tested for:

a) the presence of each MCS, and each transposon-liberating, restriction site;

b) PCR amplification of the vector with both pMOD Forward and Reverse PCR primers;

c) ME recognition by the EZ-Tn5 Transposase enzyme.

3. Kit Contents

Cat. #	Concentration	Quantity
EZ-Tn5™ pMOD™-2<MCS> Transposon Construction Vector		
MOD0602		
pMOD™-2<MCS> Vector	@ 1 µg/µl	20 µg
pMOD™<MCS> Forward PCR Primer	@ 50 µM	1 nmol
pMOD™<MCS> Reverse PCR Primer	@ 50 µM	1 nmol

*,† *See notes page 12.*

4. Related Products

The following products are also available:

- pMOD™<MCS> Forward and Reverse Sequencing Primers
- TransforMax™ EC100D™ *pir*$^{+}$ Electrocompetent *E. coli*
- TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli*
- APex™ Heat-Labile Alkaline Phosphatase
- Fast-Link™ DNA Ligation Kits
- T4 DNA Ligase
- Colony Fast-Screen™ Kits
- MasterPure™ Nucleic Acid Purification Kits
- End-It™ DNA End-Repair Kit
- EZ-Tn5™ Custom Transposome Construction Kits

5. Protocols

I. Cloning into an EZ-Tn5 Transposon Construction Vector

Creating a custom EZ-Tn5 Transposon requires that you clone your DNA fragment of interest into the MCS of the pMOD Vector. A map of the MCS and sequencing information are provided later in this document to assist in development of a successful cloning strategy.

Please consult a general molecular biology reference [e.g., Maniatis, T., *et al.*, (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y.] for recommendations on restriction digests, dephosphorylation of vector and ligations. Epicentre offers the Fast-Link™ DNA Ligation and Screening Kit for efficient ligation and recombinant screening, APex™ Heat-Labile Alkaline Phosphatase for dephosphorylation of DNA and GELase™ Agarose Gel-Digesting Preparation for recovery of DNA from agarose.

Transform ligation mixtures into a competent bacterial strain and select on media containing 50-100 µg/ml ampicillin or other selective reagents dictated by the transposon insert. Use of a *rec*A^{-}, *end*A^{-} strain is preferable, for target stability and subsequent purification steps (e.g., Epicentre's TransforMax EC100™ Electrocompetent *E. coli**), but not absolutely necessary.

II. Isolation of a Custom EZ-Tn5 Transposon

A functional EZ-Tn5 Transposon can be isolated either by restriction enzyme digestion or PCR amplification.

A. Restriction enzyme digestion with *Pvu* II or *Psh*A I:

Note: *The cloned insert must not contain a recognition site(s) for the restriction enzyme chosen to liberate the EZ-Tn5 Transposon.*

1. Digest the recombinant pMOD<MCS> DNA with either ***Pvu* II** or ***Psh*A I** using conditions recommended by the enzyme supplier.
2. Heat-inactivate the enzyme (if applicable) by incubating at 70°C for 10 minutes.
3. To ensure that false positives are not generated from uncut, parental plasmid DNA, minimize this type of background by purifying the transposon following gel electrophoresis.

B. PCR Amplification:

1. Amplify the transposon region using the pMOD<MCS> Forward and Reverse PCR Primers provided with the vector. A suggested cycling profile is outlined below.
   a. Initially, denature the template at 94°C for 2 minutes.
   b. Perform 30 cycles of:
      Denature at 94°C for 30 seconds.
      Anneal at 60°C for 45 seconds.
      Extend at 72°C for 1 minute for every kb of expected product.
2. We recommend PEG precipitation to remove small molecules (e.g., primers, nucleotides) that may interfere with transposition. Alternatively, a standard ethanol precipitation can be used.
   a. Dilute the PCR reaction to 500 µl with TE.
   b. Add 250 µl of 5 M NaCl and 250 µl of 30% PEG 8000/1.5 M NaCl.
   c. Mix well and incubate at 4°C for at least 30 minutes.
   d. Centrifuge at 4°C for 10 minutes at 10,000 x g. Discard the supernatant, centrifuge again for a few seconds, and discard any remaining supernatant.
   e. Dissolve the DNA in a suitable amount of TE.

III. *In Vitro* Transposon Insertion Reaction

This reaction inserts an EZ-Tn5 Transposon into target DNA, *in vitro*. The target DNA should be free of contaminating chromosomal DNA which is a direct competitor of the target DNA for insertion. Reaction conditions given have been optimized to maximize transposition frequency while minimizing multiple insertion events. **Be sure to calculate the mol of target DNA used in the reaction and add an equimolar amount of the EZ-Tn5 Transposon.**

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

1	µl	EZ-Tn5 10X Reaction Buffer (see Note below)
0.2	µg	target DNA**
x	µl	molar equivalent EZ-Tn5 Transposon
x	µl	sterile water to a reaction volume of 9 µl
1	µl	EZ-Tn5 Transposase (Available in the EZ-Tn5 Custom Transposome Construction Kits)
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.
4. Use 1 µl for electroporation into the appropriate bacterial strain and plate on selective media as dictated by the transposon insert. Use of a *rec*A⁻, *end*A⁻ strain is preferable but not absolutely necessary [e.g., Epicentre's TransforMax EC100 Electrocompetent *E. coli**, (sold separately)]. Store unused 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 a 6,100-bp target clone

= 0.2 µg / [6,100 bp x 660] = 0.05 x 10^{-6} µmol = 0.05 pmol

Note: *EZ-Tn5 10X Reaction Buffer (supplied with the EZ-Tn5 Transposase) is composed of 0.5 M Tris-acetate (pH 7.5), 1.5 M potassium acetate, 100 mM magnesium acetate, and 40 mM spermidine.*

IV. Production of EZ-Tn5 Transposomes

Production of stable EZ-Tn5 Transposomes can only be accomplished in the absence of Mg^{+2}.

Do not use the EZ-Tn5 10X Reaction Buffer provided with the EZ-Tn5 Transposase to prepare EZ-Tn5 Transposomes.

1. Prepare the transposome reaction mixture by adding in the following order:‡

2	µl	EZ-Tn5 Transposon DNA (100 µg/ml in TE Buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA])
4	µl	EZ-Tn5 Transposase (Available in the EZ-Tn5 Custom Transposome Construction Kits)
2	µl	100% glycerol
8	µl	Total reaction volume

2. Mix by vortexing. Incubate for 30 minutes at room temperature.
3. Store the solution at –20°C.
   The solution will not freeze stored at –20°C and is stable for at least one year.
4. Use 1 µl of the EZ-Tn5 Transposome for electroporation into a competent bacterial strain and plate on selective media as dictated by the transposon insert.

‡The EZ-Tn5 Transposome production protocol can be scaled up or scaled down as needed.

V. DNA Sequencing of Transposon Insertion Clones

Information on the Forward and Reverse Sequencing Primers, **available separately**, is given on pages 6 and 7. Since these primers anneal to a region near the ends of the transposon, the first sequence data obtained from each sequencing reaction is that of transposon DNA. The 19-bp EZ-Tn5 Transposase recognition sequence (ME) found at the junction of the inserted transposon and the target DNA is a useful landmark for distinguishing transposon sequence from target sequence (see Fig. 1).

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.

Figure 1. EZ-Tn5 Transposon Insertion Site Junction.

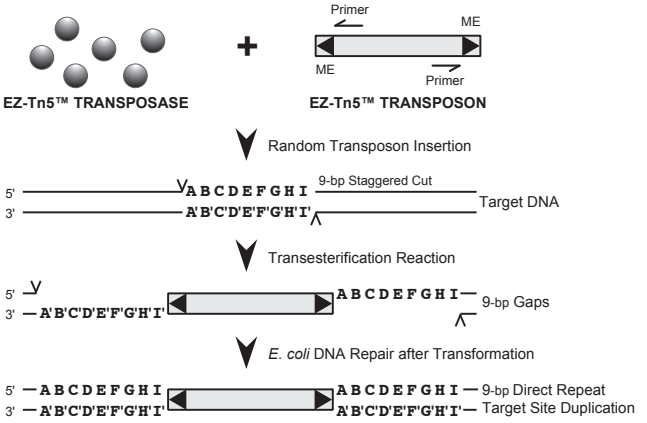


Figure 2. EZ-Tn5 Transposase Insertion Site Duplication Process.

6. Primer Information

pMOD<MCS> Forward PCR Primer

5′ - ATTCAGGCTGCGCAACTGT - 3′

Storage: Store at –20°C.

Concentration: 1 nmol @ 50 µM ..... 20 µl

in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

Length: 19 nucleotides

G+C content: 10

Molecular Weight: 5,786 daltons

Temperatures of Dissociation & Melting:

T_d: 66°C (nearest neighbor method)

T_m: 68°C (% G+C method)

T_m: 58°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

pMOD<MCS> Forward Sequencing Primer

5′ - GCCAACGACTACGCACTAGCCAAC - 3′

Storage: Store at –20°C.

Concentration: 1 nmol @ 50 µM..... 20 µl

in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

Length: 24 nucleotides

G+C content: 14

Molecular Weight: 7,328 daltons

Temperatures of Dissociation & Melting:

T_d: 74°C (nearest neighbor method)

T_m: 77°C (% G+C method)

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

T_m: 68°C ((81.5 + 16.6 (log [Na^+])) +
([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

pMOD<MCS> Reverse PCR Primer

5′ - GTCAGTGAGCGAGGAAGCGGAAG - 3′

Storage: Store at –20°C.

Concentration: 1 nmol @ 50 µM ..... 20 µl

in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

Length: 23 nucleotides

G+C content: 14

Molecular Weight: 7,206 daltons

Temperatures of Dissociation & Melting:

T_d: 74°C (nearest neighbor method)

T_m: 77°C (% G+C method)

T_m: 74°C ([2 (A+T) + 4 (G+C)] method)

T_m: 68°C ((81.5 + 16.6 (log [Na^+])) +
([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

pMOD<MCS> Reverse Sequencing Primer

5′ - GAGCCAATATGCGAGAACACCCGAGAA - 3′

Storage: Store at –20°C.

Concentration: 1 nmol @ 50 µM ..... 20 µl

in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

Length: 27 nucleotides

G+C content: 14

Molecular Weight: 8,294 daltons

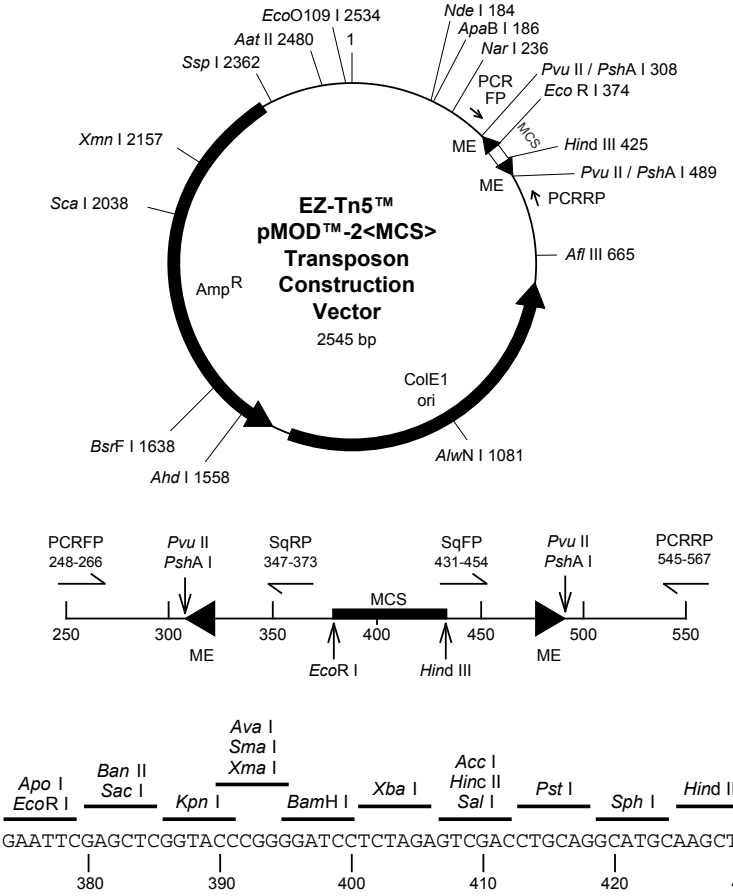
Temperatures of Dissociation & Melting:

T_d: 79°C (nearest neighbor method)

T_m: 78°C (% G+C method)

T_m: 82°C ([2 (A+T) + 4 (G+C)] method)

T_m: 68°C ((81.5 + 16.6 (log [Na^+])) +
([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M



SqFP = pMOD™<MCS> Foward Sequencing Primer	5' GCCAACGACTACGCACTAGCCAAC 3'
SqRP = pMOD™<MCS> Reverse Sequencing Primer	5' GAGCCAATATGCGAGAACACCCGAGAA 3'
PCRFP = pMOD™<MCS> Forward PCR Primer	5' ATTCAGGCTGCGCAACTGT 3'
PCRRP = pMOD™<MCS> Reverse PCR Primer	5' GTCAGTGAGCGAGGAAGCGGAAG 3'
ME = Mosaic End	5' AGATGTGTATAAGAGACAG 3'
MCS = Multiple Cloning Site	

Figure 3. pMOD-2<MCS> Transposon Construction Vector.

The pMOD-2<MCS> Transposon Construction Vector 2,545-bp. sequence can be downloaded at http://http://www.epicentre.com/sequences.

Restriction Enzymes that cut the pMOD-2<MCS> Transposon Construction Vector one to three times:

Enzyme	Sites	Location	Enzyme	Sites	Location
Aat II	1	2480	Drd I	2	97, 773
Acc65 I	1	386	Eae I	2	504, 1946
Acc I	1	408	Ear I	3	296, 549, 2353
Acl I	2	1784, 2157	EcoO109 I	1	2534
Afl III	1	665	EcoR I	1	374
Ahd I	1	1558	Fsp I	2	258, 1780
AlwN I	1	1081	Gdi II	2	514, 1944
ApaB I	1	186	Hae I	3	680, 691, 1143
ApaL I	3	177, 979, 2225	Hae II	3	239, 543, 913
Apo I	2	342, 374	Hinc II	1	409
Ase I	1	1730	Hind III	1	425
Ava I	2	349, 390	Hpy99 I	3	770, 1564, 1827
Ava II	2	1696, 1918	Kpn I	1	390
BamH I	1	395	Msl I	3	1810, 1969, 2328
Ban I	3	235, 386, 1506	Nar I	1	236
Ban II	1	384	Nde I	1	184
BciV I	2	868, 2395	Nsp I	3	41, 423, 669
BfuA I	1	420	Pci I	1	665
Bgl I	2	251, 1678	PshA I	2	308, 489
Bme1580 I	3	181, 983, 2229	PspG I	3	691, 812, 825
Bmr I	1	1598	Pst I	1	417
Bsa I	1	1619	Pvu I	2	279, 1928
BsaH I	3	236, 2095, 2477	Pvu II	2	308, 489
BsaJ I	3	390, 391, 825	Rsa I	3	169, 388, 2038
BsaW I	3	871, 1018, 1849	Sac I	1	384
BseY I	1	969	Sal I	1	407
BsmB I	1	45	Sap I	1	549
BspD I	1	337	Sbf I	1	417
BspH I	3	1385, 2393, 2498	Sca I	1	2038
BspLU11 I	1	665	Sfo I	1	237
BspM I	1	420	Sim I	3	857, 1340, 1626
BsrB I	2	598, 2399	Sma I	1	392
BsrD I	2	1619, 1793	Sph I	1	423
BsrF I	1	1638	Ssp I	1	2362
BssS I	3	838, 2222, 2529	Tat I	2	167, 2036
BstAP I	1	185	Tfi I	2	500, 640
BstN I	3	693, 814, 827	Tsp45 I	3	56, 1814, 2025
Bts I	2	1958, 1978	Xba I	1	401
Cla I	1	337	Xma I	1	390
Dra I	3	1424, 1443, 2135	Xmn I	1	2157

Restriction Enzymes that cut the pMOD-2<MCS> Transposon Construction Vector four or more times:

Aci I	BstF5 I	Hinf I	Mbo II	Sau3A I
Alu I	BstU I	HinP I	Mly I	Sau96 I
Alw I	BstY I	Hpa II	Mnl I	ScrF I
Bfa I	Cac8 I	Hph I	Mse I	SfaN I
BsiE I	CviJ I	Hpy188 I	Msp I	Sfc I
BsiHKA I	Dde I	HpyCH4 III	MspA1 I	Sml I
Bsl I	Dpn I	HpyCH4 IV	Mwo I	Taq I
BsmA I	Fau I	HpyCH4 V	Nci I	Tse I
Bsp1286 I	Fnu4H I	Mae II	Nla III	Tsp4C I
Bsr I	Hae III	Mae III	Nla IV	Tsp509 I
BssK I	Hha I	Mbo I	Ple I	TspR I

Restriction Enzymes that do not cut the pMOD-2<MCS> Transposon Construction Vector:

Afe I	Bpu10 I	Dra III	Not I	SexA I
Afl II	BsaA I	Dsa I	Nru I	Sfi I
Age I	BsaB I	Eag I	Nsi I	SgrA I
Ale I	BsiW I	Eco47 III	Pac I	SnaB I
Apa I	Bsm I	EcoN I	PaeR7 I	Spe I
Asc I	BspE I	EcoR V	PflF I	Srf I
AsiS I	BsrG I	Fse I	PflM I	Sse8647 I
Avr II	BssH II	Hpa I	Pme I	Stu I
Bbs I	BstB I	Mfe I	Pml I	Sty I
BbvC I	BstDS I	Mlu I	PpuM I	Swa I
Bcl I	BstE II	Msc I	Psi I	Tli I
BfrB I	BstX I	Nae I	PspOM I	Tth111 I
Bgl II	BstZ17 I	Nco I	Rsr II	Xcm I
Blp I	Bsu36 I	NgoM IV	Sac II	Xho I
BmgB I	Btg I	Nhe I	SanD I	

7. References

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