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EZ-Tn5™ pMOD™-6 <KAN-2 / MCS> Transposon Construction Vector

Cat. No. MOD7906

The EZ-Tn5™ pMOD™ <MCS> Transposon Construction Vectors* were developed for the preparation of custom EZ-Tn5 Transposons. The vectors contain a multiple cloning site (MCS) between the hyperactive 19 bp Mosaic Ends (ME). The ME sequences 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 generate the transposon either by PCR amplification using the Forward and Reverse PCR Primers provided with the vector, restriction enzyme digestion, or the end-user supplied "ME plus 9" primers.

The EZ-Tn5 pMOD-6 <KAN-2/MCS> Transposon Construction Vector is a pUC-based vector with a *col*E1 origin of replication. The vector also contains two antibiotic resistance markers; the ampicillin resistance marker lies outside of the ME's (not included in the final transposon), while the kanamycin resistance marker lies within the ME's and serves as a marker for transposon insertion.a

Use the EZ-Tn5 Transposon system for random, *in vitro** insertion into plasmid, cosmid or BAC clones 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+}. Sequencing can be performed from the transposon bidirectionally using the pMOD <MCS> Forward and Reverse Sequencing Primers which are available separately.

Product Specifications

Storage: Store at –20°C.

Quality Control:

The EZ-Tn5 pMOD-6 <KAN/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.

Desc.	Concentration	Quantity
EZ-Tn5™ pMOD™-6 <KAN-2/MCS> Transposon Construction Vector		
pMOD™-6 <KAN-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

Additionally, each primer is tested in a DNA cycle sequencing reaction using the SequiTherm EXCEL™ II DNA Sequencing Kit and a pMOD <MCS> vector as template.

Related Products: The following products are also available:

– EZ-Tn5™ Transposase
– pMOD™ <MCS> Forward and Reverse Sequencing Primers
– TypeOne™ Restriction Inhibitor
– TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli*
– pMOD™-2 <MCS> Transposon Construction Vector
– pMOD™-3 <MCS> Transposon Construction Vector
– pMOD™-4 <MCS> Transposon Construction Vector
– pMOD™-5 <MCS> Transposon Construction Vector
– 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

Protocols

1. 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.

2. Isolation of a Custom EZ-Tn5 Transposon

The functional EZ-Tn5 Transposon can be isolated by restriction enzyme digest or PCR amplification. It is important to completely cut the plasmid and remove non-transposon DNA to prevent background colonies from forming. To ensure that transformants are the result of a true transposition event and not vector based background, replica plate the colonies on separate Ampicillin and Kanamycin containing LB Agar plates. Colonies that are a result of true transposition events should be Kanamycin resistant and Ampicillin sensitive.

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. Digestion with PvuII or PshA I generate the necessary 5'-phosphorylated blunt ends.*

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, gel purify the transposon DNA, taking care not to expose it to UV light or ethidium bromide.

B. PCR Amplification:

Method 1 - Using the supplied pMOD <MCS> Forward and reverse primers:

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.
3. Digest the PCR product with *Pvu*II or *Psh*AI restriction enzymes as described in Section 2-A.
4. Purify the PCR product using standard cleanup methods (spin column or gel purification).

Method 2 – Using the "ME Plus 9" PCR Primers (NOT Supplied):

1. Perform PCR using primers which incorporate the reverse complement of the Mosaic Element. We use the reverse complement of the ME plus 9 additional bases to increase the T_m of the primers to improve PCR results. It is VERY important that the ME Plus 9 Forward and Reverse Primers are 5′-phosphorylated for maximal transposition efficiency. If the primers are not phosphorylated, you can easily add the 5-phosphate groups using T4 Polynucleotide Kinase.

Sequences of the two primers to use:

ME Plus 9 – 3′ primer

5′-CTGTCTCTTATACACATCTCAACCATCA-3′

ME Plus 9 – 5′ primer

5′-CTGTCTCTTATACACATCTCAACCCTGA-3′

Cycling protocol is:

a. Initially, denature the template at 94°C for 1 minute.

b. Perform 25-30 cycles of:
   Denature at 94°C for 30 seconds.
   Anneal at 55°C for 1 minute.
   Extend at 72°C for 1 minute for every kb of expected product.

Regarding buffer choice, each template will be different. You will want to use a high fidelity enzyme. Epicentre's FailSafe PCR System enzyme is an excellent choice as it will give you 3X the fidelity of Taq Polymerase but does not have the processivity issues of many pure proofreaders. We strongly recommend to use the FailSafe PCR Selection Kit, use all twelve of the FailSafe 2X PCR PreMixes, and find out the best one to use for each template. Alternatively, a pure proofreader, such as Pfu or Phusion Polymerase, can be used.

After PCR, transposon cleanup may be performed using the methods in Part B, Step 2 or Step 4.

3. *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 moles 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
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 x10^{-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.*

4. 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
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.

5. DNA Sequencing of Transposon Insertion Clones

Information on the Forward and Reverse Sequencing Primers, available separately, is given on pages 7 and 8. 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 below).

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.

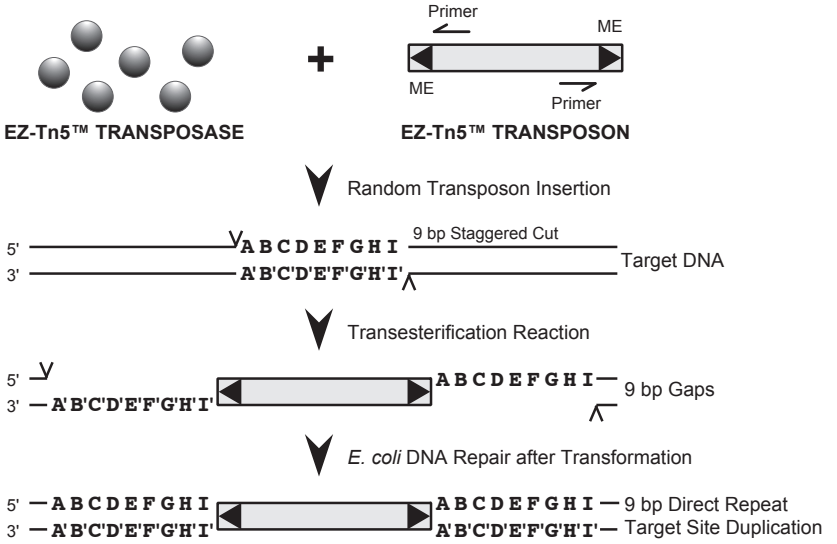


Figure 2. EZ-Tn5™ Transposase Insertion Site Duplication Process.

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> 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> 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 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:

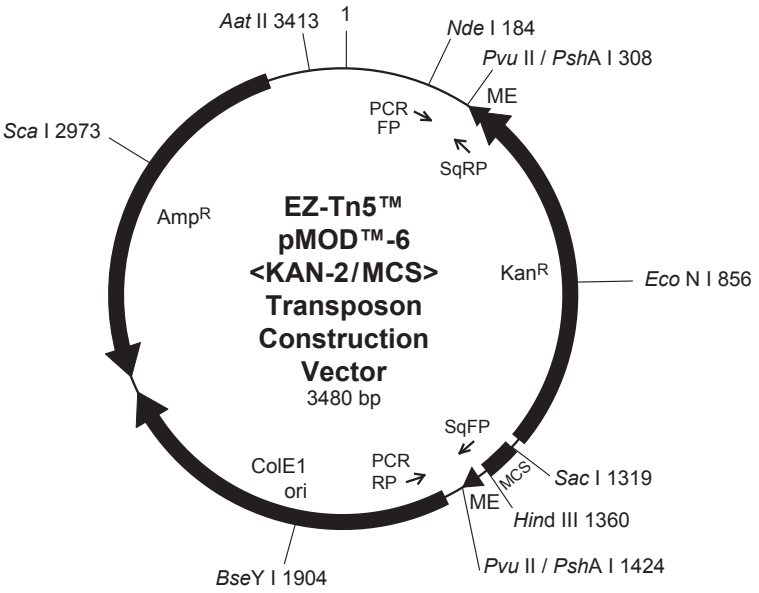
T_d: 79°C (nearest neighbor method)

T_m: 78°C (% G+C method)

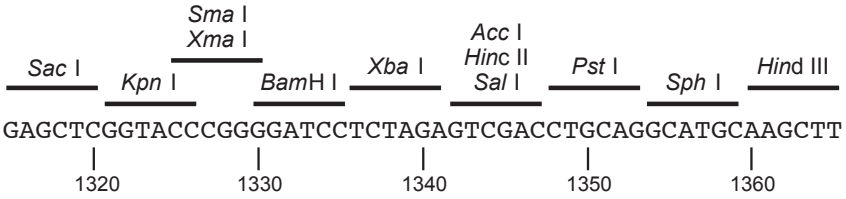
T_m: 82°C ([2 (A+T) + 4 (G+C)] method)

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

The pMOD-6 <KAN-2/MCS> Transposon Construction Vector 3,840 bp. sequence can be downloaded at http://www.epicentre.com/sequences.



Note: Not all restriction enzymes that cut only once are indicated above.
Pvu II / *Psh*A I cut twice in the map above.
Primers are not drawn to scale.



SqFP = pMOD™<MCS> Forward 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™-6<KAN-2MCS> Transposon Construction Vector.

Restriction Enzymes that cut the pMOD-6 <KAN-2/MCS> Transposon Construction Vector 1 to 3 times:

Enzyme	Sites	Location	Enzyme	Sites	Location
Aat II	1	3415	EcoO109 I	1	3469
Acc65 I	1	1321	EcoR I	2	374, 1309
Acc I	1	1343	Fsp I	2	258, 2715
Acl I	2	2719, 3092	Gdi II	2	1449, 2879
Afl III	1	1600	Hae II	3	239, 1478, 1848
Ahd I	1	2493	Hinc II	1	1344
AlwN I	1	2016	Hind III	1	1360
ApaB I	1	186	Hpy99 I	3	1705, 2499, 2762
ApaL I	3	177, 1914, 3160	Kpn I	1	1325
Ase I	2	568, 2665	Msl I	3	2745, 2904, 3263
AsiS I	1	771	Nar I	1	236
Ava I	3	349, 1167, 1325	Nde I	1	184
Ava II	2	2631, 2853	Nru I	1	1112
BamH I	1	1330	Nsi I	2	657, 923
Ban I	3	235, 1321, 2441	Nsp I	3	41, 1358, 1604
Ban II	2	1118, 1319	PaeR7 I	1	1167
Bau I	2	1773, 3157	Pci I	1	1600
BciV I	2	1803, 3330	PflM I	1	509
BfrB I	2	655, 921	Pfo I	1	46
BfuA I	1	1355	PshA I	2	308, 1424
Bgl I	2	251, 2613	PspX I	1	1167
Bme1580 I	3	181, 1918, 3164	Pst I	1	1352
Bmr I	1	2533	Pvu I	3	279, 771, 2863
Bpu10 I	1	749	Pvu II	2	308, 1424
Bsa I	1	2554	Sac I	1	1319
BsaH I	3	236, 3030, 3412	Sal I	1	1342
BseY I	1	1904	Sap I	1	1484
Bsm I	2	810, 887	Sbf I	1	1352
BsmB I	2	45, 749	Sca I	1	2973
BspD I	2	337, 1076	Sfo I	1	237
BspLU11 I	1	1600	Sim I	3	1792, 2275, 2561
BspM I	1	1355	Sma I	1	1327
BspQ I	1	1484	Spe I	1	380
BsrB I	2	1533, 3334	Sph I	1	1358
BsrD I	3	1279, 2554, 2728	Ssp I	2	844, 3297
BsrF I	2	811, 2573	Tat I	2	167, 2971
BssS I	3	1773, 3157, 3464	Tli I	1	1167
BstAP I	1	185	TspM I	1	1325
Cla I	2	337, 1076	Xba I	1	1336
Dra I	3	2359, 2378, 3070	Xho I	1	1167
Drd I	2	97, 1708	Xma I	1	1325
Eae I	2	1439, 2881	Xmn I	1	3092
EcoN I	1	856	Zra I	1	3413

Restriction Enzymes that cut the pMOD-6 <KAN-2/MCS> Transposon Construction Vector ≥4 times:

Aci I	BstF5 I	Hae I	Mbo II	SfaN I
Alu I	BstN I	Hae III	Mly I	Sfc I
Alw I	BstU I	Hha I	Mnl I	Sml I
Apo I	BstY I	Hinf I	Mse I	Taq I
Bcc I	Bts I	HinP I	Msp I	Tfi I
Bfa I	BtsC I	Hpa II	MspA1 I	Tse I
BsaJ I	Cac8 I	Hph I	Mwo I	Tsp45 I
BsaW I	Cvi II	Hpy188 I	Nci I	Tsp4C I
BsiE I	CviJ I	Hpy188 III	Nla III	Tsp509 I
BsiHKA I	CviKI-1	HpyAV	Nla IV	TspR I
Bsl I	CviQ I	HpyCH4 III	Ple I	
BsmA I	Dde I	HpyCH4 IV	PspG I	
Bsp1286 I	Dpn I	HpyCH4 V	Rsa I	
BspH I	Ear I	Mae II	Sau3A I	
Bsr I	Fau I	Mae III	Sau96 I	
BssK I	Fnu4H I	Mbo I	ScrF I	

Restriction Enzymes that do not cut the pMOD-6 <KAN-2/MCS> Transposon Construction Vector:

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

**EZ-Tn5™ Transposon Tools and their use 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.*

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