

EZ-Tn5™ Custom Transposome Construction Kits

Cat. No. TNP10622/TNP10623

The EZ-Tn5™ Custom Transposome Construction Kits provide the reagents needed for construction and use of a custom transposon for *in vivo* or *in vitro* applications. This kit contains EZ-Tn5 Transposase, a hyperactive, mutated form of Tn5 transposase, with an *in vitro* transposition frequency that is 1,000-fold greater than the wild-type transposase.

The kits include a choice of the EZ-Tn5 pMOD™-class of Transposon Construction Vectors for generation of novel Tn5-based transposons.

The EZ-Tn5 Custom Transposome Construction Kit can be used to:

1. Insert any DNA sequence flanked by the 19-bp mosaic ends (MEs) of an EZ-Tn5 Transposon into any target DNA.
2. Prepare Transposomes, in the absence of Mg²⁺, for electroporation into living microorganisms and subsequent random insertion of the transposon into the bacterial chromosome. A list of currently known successful microbial targets *in vivo* may be found on Epicentre's website.

The EZ-Tn5 Custom Transposome Construction Kit contains sufficient reagents for 20 *in vivo* transposition reactions or 10 *in vitro* insertion reactions.

Cat. #	Concentration	Quantity
EZ-Tn5™ Custom Transposome Construction Kit Components		
EZ-Tn5 Transposase 10 U	@ 1 U/μl	10 μl
EZ-Tn5 10X Reaction Buffer		100 μl
EZ-Tn5 10X Stop Solution		100 μl
Sterile Water		1 ml
pMOD™<MCS> Forward PCR Primer	@ 50 μM	1 nmol
pMOD™<MCS> Reverse PCR Primer	@ 50 μM	1 nmol

In addition, the kit contains one of the following vectors:

TNP10622		
pMOD™-2<MCS> Vector	@ 1 μg/μl	20 μg
TNP10623		
pMOD™-3<MCS> Vector	@ 1 μg/μl	20 μg

Product Specifications

Storage: Store only at -20°C in a freezer with-out a defrost cycle.

Storage Buffer: EZ-Tn5 Transposase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1% Triton® X-100, and 1 mM dithiothreitol.

Unit Definition: One unit of EZ-Tn5 Transposase catalyzes the release of the donor backbone fragment from 1 μg of transposed DNA in 1 hour at 37°C , as determined by agarose gel electrophoresis.

Enzyme Structure: Single polypeptide of 55 KDa.

Contaminating Activity Assays: All components of the EZ-Tn5 Transposase are free of detectable DNase and RNase activities as judged by agarose gel electrophoresis following overdigestion assays, with the exception of the inherent endonucleolytic function of the EZ-Tn5 Transposase.

EZ-Tn5 10X Reaction Buffer: 0.5 M Tris-acetate (pH 7.5), 1.5 M potassium acetate, 100 mM magnesium acetate, 40 mM spermidine.

Note: This buffer contains Mg^{2+} . Do not use for the production of Transposomes.

EZ-Tn5 10X Stop Solution: 1% SDS.

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

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 or 1989) 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.

2. Methods for Purifying Transposon DNA

The key to making a good transposon is to make sure that the transposon is “clean” and free of contaminating vector and extraneous DNA.

A functional EZ-Tn5 Transposon can be isolated either by restriction enzyme digestion or PCR amplification. If the transposon is prepared by restriction enzyme digestion, we recommend using pMOD-4 <MCS>, which contains an R6K origin of replication rather than a *colE1* origin of replication. Replication from the R6K origin is dependent on the *pir* gene product produced by TransforMax EC100D *pir*⁺ and *pir*-116 *E. coli* cells (sold separately). Since most bacterial strains do not contain a *pir* gene, the uncut plasmid DNA that contaminates these transposon preparations cannot replicate, and this type of background problem is eliminated.

A. Purification of Transposon DNA by Gel Electrophoresis

The protocol below has been adapted for use with transposon DNA that has been cut out of a pMOD vector using *Pvu* II or *Psh* A I. By eliminating the ethidium bromide and UV exposure, you will note an excellent increase in transformation and transposition efficiency when the transposon DNA is used in either *in vitro* insertion or *in vivo* Transposome mutagenesis.

After digestion of the pMOD-based chimera with *Pvu* II or *Psh* A I, analyze the digested DNA on a 1% agarose gel. If performing standard agarose gel electrophoresis, be certain to cast a long gel (preferably 20 cm). Perform electrophoresis overnight, at 3.0 Volt/cm. This will give optimal resolution of the transposon DNA from the vector backbone.

To resolve the transposon and vector DNA, analyze the digested pMOD clone on a low-melting-point (LMP) 1% agarose gel. It is important to perform this electrophoresis in the absence of ethidium bromide (do not add ethidium bromide to the gel). The DNA that will be cloned should not be exposed to UV light under any circumstances. This can decrease the cloning efficiency by 100-fold or more (see Fig. 1).

Note 1: Even 30 seconds exposure to 302 nm UV light will cause a 100- to 200-fold drop in ligation and cloning efficiency.

Note 2: The protocol below is designed for use with Epicentre's GELase agarose digestion preparation, and thus requires the use of LMP agarose. Standard high-melt agarose can also be used and the DNA can be extracted from the gel slices with other, nonenzymatic methods.

Procedure

1. Prepare a 1% LMP agarose gel in 1X TAE or 1X TBE buffer.

Note: Do not include ethidium bromide in the gel solution.

2. Load the DNA Size Marker into each of the outside lanes of the gel and fill the rest of the wells with *Pvu* II or *Psh* A I-digested pMOD transposon construct.
3. Resolve the samples by gel electrophoresis (e.g., at room temperature overnight) at a constant voltage of 30-35 V. The end-repaired DNA can be visualized and extracted by the method described in the next step.

- Following electrophoresis, cut off the outer lanes of the gel containing the DNA Size Marker, and a small portion of the next lanes that contain the digested DNA (see Fig. 1). Stain the cut-off sides of the gel with ethidium bromide and visualize with UV light. Mark the position of the desired size DNA in the gel using a Pasteur pipet.

Note: Do not expose the sample DNA to UV irradiation! Even short UV exposure can decrease cloning efficiencies by 100- to 200-fold.

Reassemble the gel and excise a 2- to 4-mm wide gel slice containing sample DNA that migrated with and just slightly above (higher MW) the appropriate position of the DNA markers.

Transfer the gel slice to a tared, sterile, 15-ml screw-cap tube for extraction, either by using the GELase method, or other desired method for isolating DNA from agarose gels (see Fig. 1).

B. PCR Amplification of Transposon DNA

Note: Make sure that the PCR primers, no matter which of the three amplification methods you choose, contain 5'-phosphates on both forward and reverse primers.

There are three standard methods for making transposons using PCR:

- Use the pMOD PCR primers provided with the EZ-Tn5 pMOD-series Transposon Construction Vectors.
- Linearize the pMOD transposon construct using an enzyme that will cut only in the vector backbone. An excellent choice, when possible, would be an enzyme that cuts

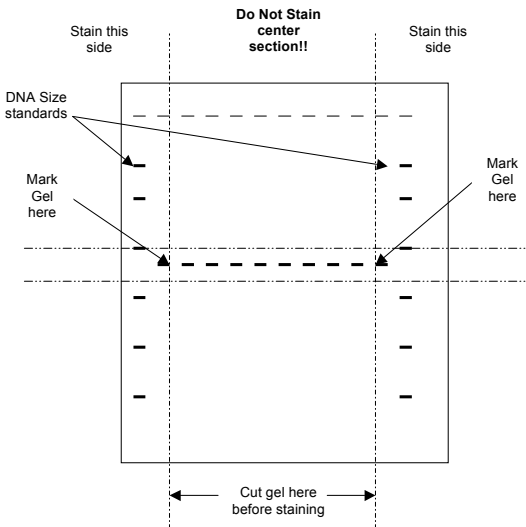


Figure 1. Purification of transposon DNA (PCR product or restricted transposon clone) by gel electrophoresis.

in the origin of replication. Linear DNA is a better template for PCR than circular DNA. A very good enzyme to use is *A/wN* I, as it digests the pMOD vectors only in the *Co/EI* origin of replication.

3. 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:

Denaturation at 94°C for 30 seconds.

Annealing at 60°C for 45 seconds.

Extension at 72°C for 1 minute for every kilobase of expected product.

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 products to 500 µl with TE.

b. Add 250 µl of 5 M NaCl and 250 µl of 30% PEG 8,000/1.5 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.

If you wish to gel-purify their transposons after synthesis by PCR, we strongly recommend following the procedure shown in Fig. 1.

C. PCR Using “ME Plus 9” Primers (User-Provided) with Transposon Constructs Created in the pMOD-Series Vectors

This is a straightforward method that serves as an alternative to *Pvu* II/*Psh* A I digestion. It also serves as an alternative to using the pMOD PCR primers, which require “pruning” of the ends of the PCR product with *Pvu* II or *Psh* A I, for maximum transposition efficiency. The pMOD transposon construct, generated using normal recombinant DNA procedures and growth in *E. coli*, is purified using any alkaline lysis–based plasmid preparation kit.

- 1) Linearize the pMOD transposon construct using an enzyme that will cut only in the vector backbone. An excellent choice, when possible, would be an enzyme that cuts in the origin of replication. Linear DNA is a better template for PCR than circular DNA. A very good enzyme to use is *A/wN* I, since it cuts in the origin of replication on the pMOD vector.
- 2) Perform PCR using primers that incorporate the reverse complement of the ME We use the reverse complement of the ME plus 9 bases.

Note: The ME Plus 9 primers should be purchased containing a 5'-monophosphate.

Sequences of the two primers are:

ME Plus 9 – 3' primer

5'-CTGTCTCTTATACACATCTCAACCATCA-3'

ME Plus 9 – 5' primer

5'-CTGTCTCTTATACACATCTCAACCCTGA-3'

The standard cycling protocol is:

94°C for 1 min, followed by:

25-30 cycles of:

94°C for 30 sec.

55°C for 1 min, and

72°C for 1 minute for every kilobase of expected product.

For best transposition results, we recommend the use of a high-fidelity enzyme. The FailSafe PCR System is an excellent choice, as the fidelity of the FailSafe Polymerase is approximately three times the fidelity of *Taq* DNA Polymerase, but it does not have the slow processivity observed in many pure proofreaders.

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 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.

** Calculation of μmol target DNA:

$$\mu\text{mol target DNA} = \mu\text{g target DNA} / [(\# \text{ base pairs in target DNA}) \times 660]$$

For example: 0.2 μg of a 6,100 bp target clone

$$= 0.2 \mu\text{g} / [6,100 \text{ bp} \times 660] = 0.05 \times 10^{-6} \mu\text{mol} = 0.05 \text{ pmol}$$

1. Prepare the transposon insertion reaction mixture by adding the following, in order:
 - 1 μl EZ-Tn5 10X Reaction Buffer
 - 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 of EZ-Tn5 10X Stop Solution. Mix and heat for 10 minutes at 70°C.
4. Use 1 µl for electroporation into a competent bacterial strain and plate on selective media as dictated by the transposon insert. Use of a *recA*⁻, *endA*⁻ strain is preferable, for target stability and subsequent purification steps (e.g., Epicentre's TransforMax™ EC100™ Electrocompetent *E. coli*), but not absolutely necessary. Store unused reaction mixture at -20°C.

4. Production of EZ-Tn5 Transposomes

Production of stable EZ-Tn5 Transposomes can only be accomplished in the absence of Mg²⁺.

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
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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 when stored at -20°C, and is stable for at least 1 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.

5. Appendices

pMOD 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 and 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 ($[(81.5 + 16.6(\log [Na+])) +$
 $([41(\#G+C) - 500] / \text{length})]$ method)

where $[Na+] = 0.1 \text{ M}$

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 and 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 ($[(81.5 + 16.6(\log [Na+])) +$
 $([41(\#G+C) - 500] / \text{length})]$ method)

where $[Na+] = 0.1 \text{ M}$

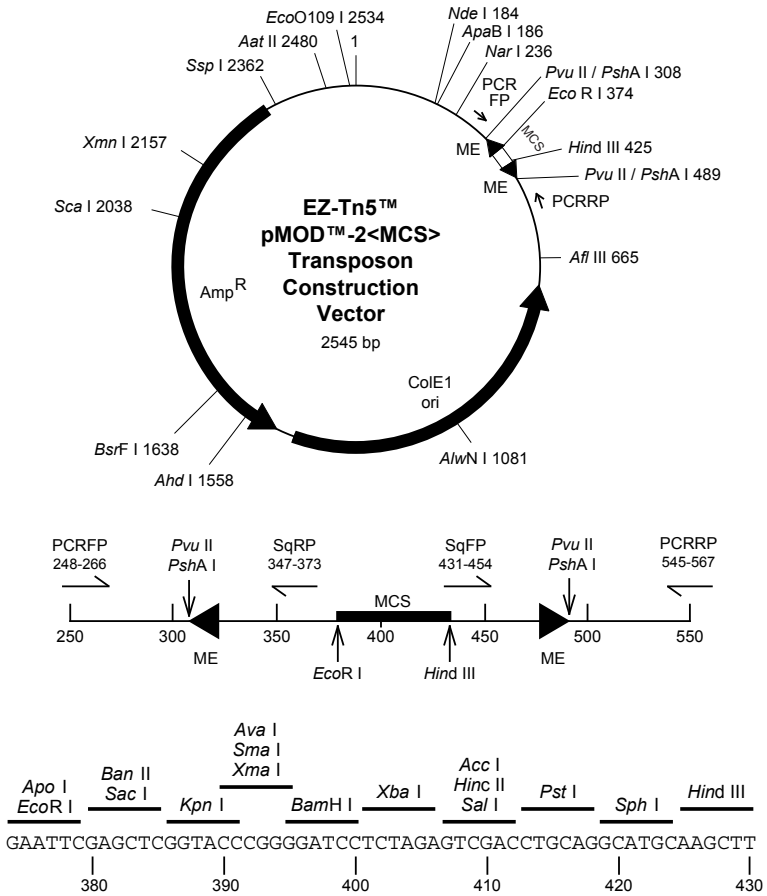
pMOD<MCS> Reverse PCR Primer**5' - GTCAGTGAGCGAGGAAGCGGAAG - 3'****Storage:** Store at -20°C.**Concentration:** 1 nmol @ 50 µM 20 µl

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 and 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 ($((81.5 + 16.6(\log [Na+])) +$
 $([41(\#G+C) - 500] / \text{length})$ 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 and 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 ($((81.5 + 16.6(\log [Na+])) +$
 $([41(\#G+C) - 500] / \text{length})$ method)
where $[Na+] = 0.1$ M



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 2. pMOD™-2<MCS> Transposon Construction Vector.

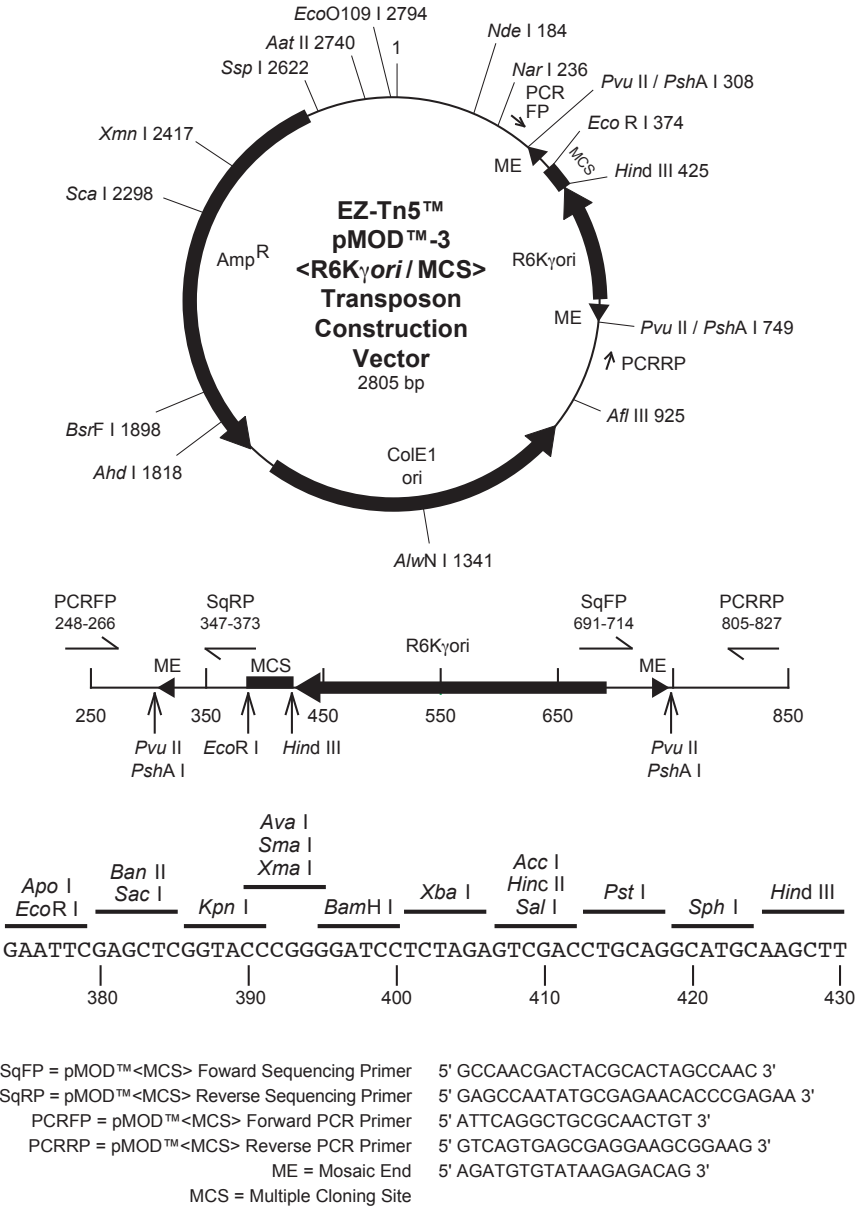


Figure 3. pMOD™-3<R6Kγori/MCS> Transposon Construction Vector.

The pMOD vector sequences can be downloaded from Epicentre's website

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
AlwNI	1	1081	Gdi II	2	514, 1944
ApaBI	1	186	Hae I	3	680, 691, 1143
ApaLI	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
BamHI	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
BciVI	2	868, 2395	Nsp I	3	41, 423, 669
BfuAI	1	420	Pci I	1	665
Bgl I	2	251, 1678	PshA I	2	308, 489
Bme1580 I	3	181, 983, 2229	PspGI	3	691, 812, 825
Bmr I	1	1598	Pst I	1	417
Bsa I	1	1619	Pvu I	2	279, 1928
BsaHI	3	236, 2095, 2477	Pvu II	2	308, 489
BsaJI	3	390, 391, 825	Rsa I	3	169, 388, 2038
BsaWI	3	871, 1018, 1849	Sac I	1	384
BseY I	1	969	Sal I	1	407
BsmBI	1	45	Sap I	1	549
BspDI	1	337	Sbf I	1	417
BspHI	3	1385, 2393, 2498	Sca I	1	2038
BspLU11 I	1	665	Sfo I	1	237
BspMI	1	420	Sim I	3	857, 1340, 1626
BsrBI	2	598, 2399	Sma I	1	392
BsrDI	2	1619, 1793	Sph I	1	423
BsrFI	1	1638	Ssp I	1	2362
BssSI	3	838, 2222, 2529	Tat I	2	167, 2036
BstAP I	1	185	Tfi I	2	500, 640
BstNI	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	EcoRV	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	

Restriction Enzymes that cut the pMOD-3<R6Kyorl/MCS> Transposon Construction Vector one to three times:

Enzyme	Sites	Location			
Aat II	1	2740	Drd I	2	97, 1033
Acc65 I	1	386	Eae I	2	764, 2206
Acc I	1	408	Ear I	3	296, 809, 2613
Acl I	2	2044, 2417	EcoO109 I	1	2794
Afl III	1	925	EcoR I	1	374
Ahd I	1	1818	Fsp I	2	258, 2040
AlwNI	1	1341	Gdi II	2	774, 2204
ApaBI	1	186	Hae I	3	940, 951, 1403
ApaLI	3	177, 1239, 2485	Hae II	3	239, 803, 1173
Apo I	2	342, 374	Hinc II	1	409
Ase I	2	504, 1990	Hind III	1	425
Ava I	2	349, 390	Hpy99 I	3	1030, 1824, 2087
Ava II	2	1956, 2178	Kpn I	1	390
BamHI	1	395	Msl I	3	2070, 2229, 2588
Ban I	3	235, 386, 1766	Nar I	1	236
Ban II	1	384	Nde I	1	184
BciVI	2	1128, 2655	Nsp I	3	41, 423, 929
BfuAI	1	420	Pci I	1	925
Bgl I	2	251, 1938	PshA I	2	308, 749
Bme1580 I	3	181, 1243, 2489	Psi I	1	460
Bmr I	1	1858	PspGI	3	951, 1072, 1085
Bsa I	1	1879	Pst I	1	417
BsaA I	3	537, 647, 669	Pvu I	2	279, 2188
BsaH I	3	236, 2355, 2737	Pvu II	2	308, 749
BsaJ I	3	390, 391, 1085	Sac I	1	384
BsaW I	3	1131, 1278, 2109	Sal I	1	407
BseY I	1	1229	Sap I	1	809
BsmBI	1	45	Sbf I	1	417
BspDI	1	337	Sca I	1	2298
BspHI	3	1645, 2653, 2758	Sfo I	1	237
BspLU11 I	1	925	Sim I	3	1117, 1600, 1886
BspMI	1	420	Sma I	1	392
Bsr I	3	1333, 1346, 2294	SnaBI	1	669
BsrBI	2	858, 2659	Sph I	1	423
BsrDI	2	1879, 2053	Ssp I	1	2622
BsrFI	1	1898	Tat I	2	167, 2296
BssSI	3	1098, 2482, 2789	Tfi I	2	760, 900
BstAP I	1	185	Tsp45 I	3	56, 2074, 2285
BstNI	3	953, 1074, 1087	Xba I	1	401
Bts I	2	2218, 2238	Xma I	1	390
Cla I	1	337	Xmn I	1	2417

Restriction Enzymes that cut the pMOD-3<R6Kyori/MCS> Transposon Construction Vector four or more times:

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

Restriction Enzymes that do not cut the pMOD-3<R6Kyori/MCS> Transposon Construction Vector:

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

References:

1. Goryshin, I. Y. and Reznikoff, W. S. (1998) *J. Biol. Chem.* **273**, 7367.

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Information on the Forward and Reverse Sequencing Primers, available separately, is given on page 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).

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