

## EZ-Tn5<sup>™</sup> <R6K $\gamma$ ori/KAN-2>Tnp Transposome<sup>™</sup> Kit

Cat. No. TSM08KR

The EZ-Tn5<sup>™</sup> <R6K $\gamma$ ori/KAN-2>Tnp Transposome<sup>™</sup> is the stable complex formed between the EZ-Tn5 Transposase enzyme and the EZ-Tn5 <R6K $\gamma$ ori/KAN-2> Transposon. The EZ-Tn5 <R6K $\gamma$ ori/KAN-2> Transposon contains an R6K $\gamma$  conditional origin of replication (R6K $\gamma$ ori) and the Tn903 kanamycin resistance gene (Kan<sup>R</sup>) 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<sup>2+</sup> in the host's cellular environment resulting in random insertion of the EZ-Tn5 Transposon into the genomic DNA of the host.<sup>1,2</sup>

The R6K $\gamma$ ori makes this transposon useful for "rescue cloning" of the region of genomic DNA into which the transposon has been randomly inserted. An overview of the rescue cloning process is presented on page 2. Genomic DNA transposed with the EZ-Tn5 <R6K $\gamma$ ori/KAN-2> Transposon is first purified and then fragmented, self-ligated and finally transformed into an *E. coli* host that expresses the *pir* gene product (II protein).<sup>3</sup> When selected on kanamycin-containing plates, only the cells containing the <R6K $\gamma$ ori/KAN-2> Transposon will grow.

Unlabeled forward and reverse transposon-specific primers are supplied in the kit. These primers can be used for bidirectional DNA sequencing or mapping of transposon insertion sites in target genomic DNAs or rescue clones. Users may also subject sufficiently large rescue clones to transposon insertion reactions using the HyperMu<sup>™</sup> <CHL-1> Insertion Kit and produce sequence from these transposon insertion sites in order to obtain full clone sequence coverage.

### Product Specifications

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

#### EZ-Tn5<sup>™</sup> <R6K $\gamma$ ori/KAN-2>Tnp Transposome<sup>™</sup> Kit Contents

EZ-Tn5 <sup>™</sup> <R6K $\gamma$ ori/KAN-2>Tnp Transposome <sup>™</sup> @ 33 ng/ $\mu$ l.....	10 $\mu$ l
KAN-2 FP-1 Forward Primer @ 50 $\mu$ M.....	20 $\mu$ l
R6KAN-2 RP-1 Reverse Primer @ 50 $\mu$ M.....	20 $\mu$ l
Sterile Water.....	1 ml

**Size:** Reagents included in the kit are sufficient for 10 *in vivo*<sup>†</sup> transposon insertion reactions.

**Storage Buffer:** The EZ-Tn5 <R6K $\gamma$ ori/KAN-2> 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<sup>®</sup>X-100, and 0.5 mM dithiothreitol. The KAN-2 FP-1 Forward and R6KAN-2 RP-1 Reverse Primers are supplied in 10 mM Tris-HCl, (pH 7.5), 1 mM EDTA.

**Quality Control:** EZ-Tn5 <R6K $\gamma$ ori/KAN-2>Tnp Transposome activity is assayed by electroporation into a *recA*<sup>-</sup> *E. coli* host strain having a transformation efficiency of >10<sup>9</sup> cfu/ $\mu$ g DNA. Assays must yield >10<sup>5</sup> Kan<sup>R</sup> colonies/ $\mu$ g or >2.0 x 10<sup>3</sup> Kan<sup>R</sup> colonies/ $\mu$ l of transposome respectively. Primers are function-tested via PCR and in a DNA cycle sequencing reaction using the SequiTherm EXCEL<sup>™</sup> II DNA Sequencing Kit and a plasmid containing an EZ-Tn5 <R6K $\gamma$ ori/KAN-2> Transposon as template.

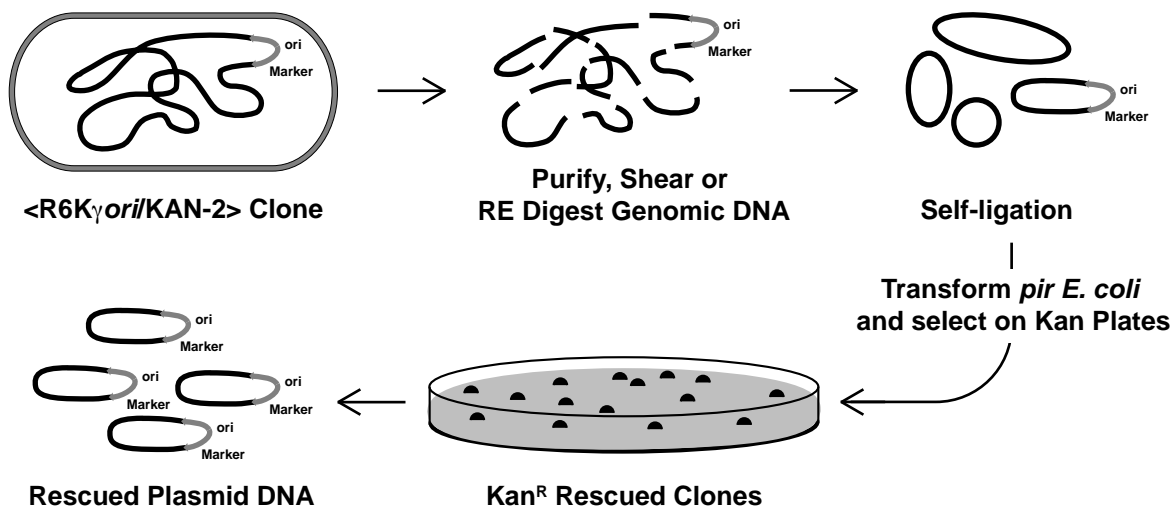
**Contaminating Activity Assays:** All components of the EZ-Tn5 <R6K $\gamma$ ori/KAN-2>Tnp Transposome Kit are free of detectable DNase and RNase activities as judged by agarose gel electrophoresis following over-digestion assays with the exception of the inherent endonucleolytic function of the EZ-Tn5 Transposase.

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Lit. #155

## Rescue Cloning of EZ-Tn5 <R6K $\gamma$ ori/KAN-2> Transposed Genomic DNA

An overview of the process for rescue cloning of the EZ-Tn5 <R6K $\gamma$ ori/KAN-2> Transposon insertion site in genomic DNA is given below.

Figure 1. Rescue Cloning Overview.



## Protocol

- 1. Electroporation of Host Cells with EZ-Tn5 <R6K $\gamma$ ori/KAN-2>Tnp Transposome and Selection of Transposition Clones:** Electroporate electrocompetent cells using 1  $\mu$ l of the EZ-Tn5 <R6K $\gamma$ ori/KAN-2>Tnp Transposome. The electrocompetent cells should have a transformation efficiency of  $>10^7$  cfu/ $\mu$ g 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.

If working with *E. coli*, dilute aliquots of the recovered cells (e.g. 1:10 and 1:100). Plate 100  $\mu$ l of undiluted cells and each cell dilution separately on plates containing 50  $\mu$ g/ml kanamycin. Other species may require plating of undiluted cells on plates containing 25-50  $\mu$ g/ml kanamycin. 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 Kan<sup>R</sup> colonies/ $\mu$ l of EZ-Tn5 <R6K $\gamma$ ori/KAN-2>Tnp Transposome will be dependent on the transformation efficiency of the cells used and the level of expression of the Tn903 kanamycin resistance marker in that species. Select transposition clones for "rescue cloning" by any of a number of methods including observing a desired phenotypic change, Southern Blot analysis or selecting for a desired "gene knockout".

2. **Preparation of Transposed Genomic DNA from Host Cells:** Prepare genomic DNA from chosen clones, for example, using the MasterPure DNA Purification Kit. Fragment 1 µg of the genomic DNA by random shearing or by restriction endonuclease digestion(s) (See p. 4 for the restriction map of the EZ-Tn5 <R6Kγori/KAN-2> Transposon in order to avoid restriction within the transposon). If desired, size-select the fragmented genomic DNA (e.g., by low-melting point agarose gel electrophoresis). Genomic DNA that has been fragmented by random shearing or by digestion with two different restriction endonucleases must be end-repaired (made blunt-ended) and 5'-phosphorylated in order to be self-ligated. End-repair and 5'-phosphorylate the DNA as necessary (e.g., by using the End-It™ DNA End-Repair Kit [EPICENTRE]).
3. **Ligation of Fragmented Genomic DNA:** Self-ligate 0.1-1 µg of DNA using 2 U of T4 DNA Ligase in 10-20 µl total volume for 1 hour at room temperature. The extent of ligation can be quickly monitored by running aliquots of the reaction before and after addition of the T4 DNA Ligase addition, on an agarose gel. Terminate the reaction and inactivate the T4 DNA Ligase by heating at 70°C for 10 minutes.
4. **Transformation and Selection of Rescue Clones:** Electroporate electrocompetent *pir E. coli* (*E. coli* expressing the Π protein, e.g. TransforMax EC100D *pir+* or TransforMax EC100D *pir-116* Electrocompetent *E. coli*) using 1-2 µl of the ligation mix. 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. Plate cells on LB agar containing 50 µg/ml of kanamycin. Select Kan<sup>R</sup> colonies overnight.
5. **DNA Sequencing of Rescue Clones:** Unlabeled forward and reverse EZ-Tn5 <R6Kγori/KAN-2> Transposon-specific primers are supplied in the kit. These primers can be used for bidirectional DNA sequencing or mapping of transposon insertion sites in target genomic DNAs or rescue clones. For DNA sequencing rescue clones containing inserts too large to be completely sequenced using only the EZ-Tn5 <R6Kγori/KAN-2> Transposon-specific primers, users may subject these clones to *in vitro* transposon insertion reactions using the HyperMu <CHL-1> Insertion Kit, and generating DNA sequence from the provided HyperMu <CHL-1> Transposon-specific primers in order to obtain full insert sequence coverage.

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*EZ-Tn5, Transposome, TransforMax, EC100D, End-It, Fast-Link, MasterPure, HyperMu, and SequiTherm EXCEL* are trademarks of EPICENTRE, Madison, Wisconsin.

<sup>†</sup> Use of Transposome™ Complexes for *In Vivo* Transposon Insertion, including, but not limited to EZ-Tn5™ and HyperMu™ Transposome™ complexes, is covered by U.S. Patent Nos. 6,159,736 and 6,294,385; European Patent No. 1115856 and other patents issued or pending, exclusively licensed to EPICENTRE. These products are accompanied by a limited non-exclusive license for nonprofit organizations to use the purchased products solely for life science research. Specifically, researchers at nonprofit organizations may use these products in their research and they may transfer derivatives of the products to colleagues at other nonprofit organizations provided that such colleagues agree in writing to be bound by the terms and conditions of this label license. Researchers may not transfer these products or its derivatives to researchers at other organizations that are not nonprofit organizations without the express written consent of EPICENTRE and without those entities having an express license from EPICENTRE for the use of the products. Other than as specifically provided here, an authorized transferee of these products shall have no right to transfer these products or its derivatives to any other person or entity. A nonprofit organization performing research using this product for a for-profit organization shall also be considered for-profit. For-profit entities purchasing these products shall have three (3) months to evaluate the products in their research. Thereafter, such for-profit entities shall either contact EPICENTRE and take a license for their continued use of the products or destroy the products and all derivatives thereof. Please contact EPICENTRE with respect to licenses for commercial use, including manufacturing, therapeutic or diagnostic use.

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**Primer Information****KAN-2 FP-1 Forward Primer**

5' - ACCTACAACAAAGCTCTCATCAACC - 3'

**Length:** 25 nucleotides**G+C content:** 11**Molecular Weight:** 7484 daltons**Temperatures of Dissociation & Melting:**

T<sub>d</sub>: 68°C (nearest neighbor method)  
T<sub>m</sub>: 73°C (% G+C method)  
T<sub>m</sub>: 72°C ([2 (A+T) + 4 (G+C)] method)  
T<sub>m</sub>: 63°C ((81.5 + 16.6 (log [Na<sup>+</sup>])) +  
([41 (#G+C) - 500] / length) method)  
where [Na<sup>+</sup>] = 0.1 M

**R6KAN-2 RP-1 Reverse Primer**

5' - CTACCCTGTGGAACACCTACATCT - 3'

**Length:** 24 nucleotides**G+C content:** 12**Molecular Weight:** 7210 daltons**Temperatures of Dissociation & Melting:**

T<sub>d</sub>: 66°C (nearest neighbor method)  
T<sub>m</sub>: 74°C (% G+C method)  
T<sub>m</sub>: 72°C ([2 (A+T) + 4 (G+C)] method)  
T<sub>m</sub>: 65°C ((81.5 + 16.6 (log [Na<sup>+</sup>])) +  
([41 (#G+C) - 500] / length) method)  
where [Na<sup>+</sup>] = 0.1 M

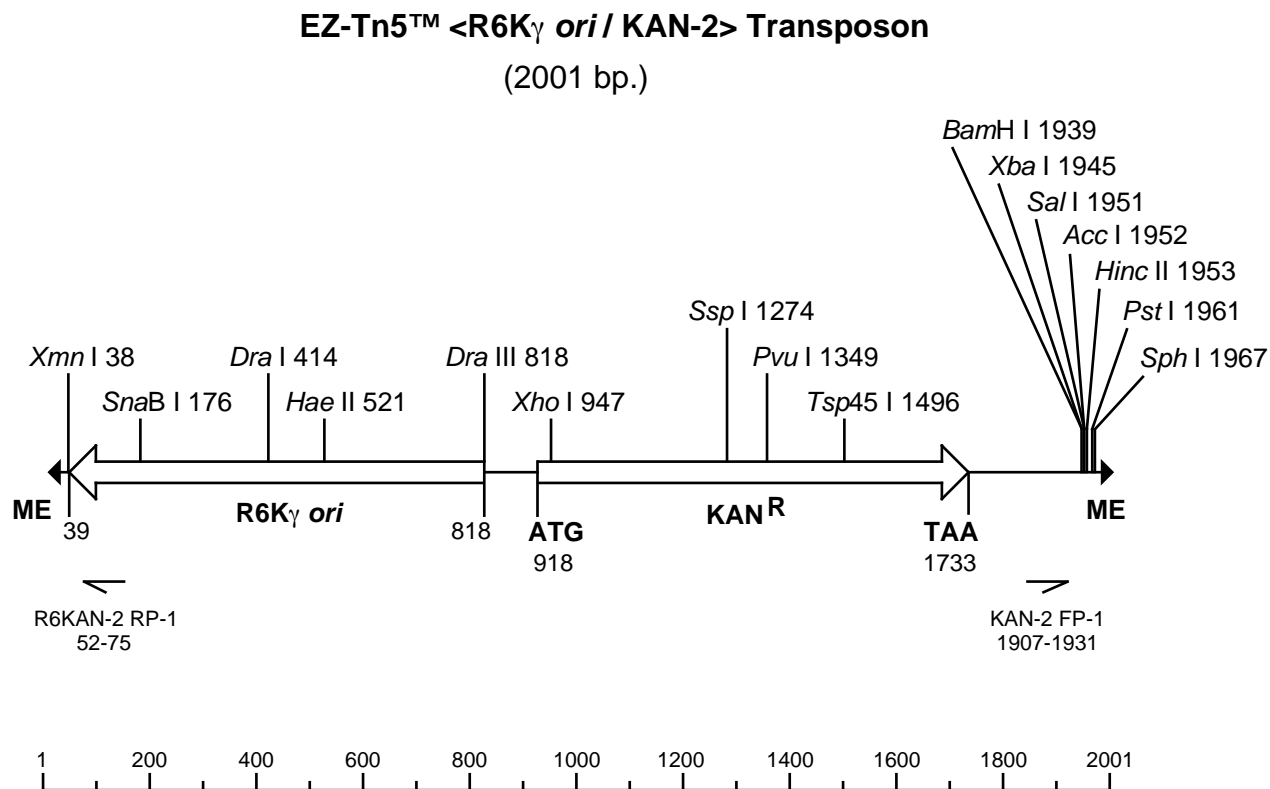
**Related Products:** The following products are also available:

- TransforMax™ EC100D™ *pir*<sup>+</sup> and *pir*-116 Electrocompetent *E. coli*
- End-It™ DNA End-Repair Kit
- Fast-Link™ DNA Ligation Kits
- MasterPure™ DNA Purification Kits
- EZ-Tn5™ Tnp Transposome™ Kits
- EZ-Tn5™ Transposase
- EZ-Tn5™ Transposons and Insertion Kits
- HyperMu™ Transposons and Insertion Kits

**References:**

1. Hoffman, L.M. and Jendrisak, J. (1999) *Epicentre Forum*.**6 (3)**, 1.
2. Goryshin, I. Y. *et al.*, (2000) *Nat. Biotechnol.* **18**, 97.
3. Metcalf, W.W. *et al.*, (1994) *Gene* **138**, 1.

Figure 2. EZ-Tn5 <R6K<sub>γ</sub>ori/KAN-2> Transposon.



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.

R6KAN-2 RP-1 Reverse Primer  
KAN-2 FP-1 Forward Primer  
ME = Mosaic End

5' CTACCCTGTGGAACACCTACATCT 3'  
5' ACCTACAACAAAGCTCTCATCAACC 3'  
5' AGATGTGTATAAGAGACAG 3'

Restriction Enzymes that cut the EZ-Tn5 <R6K<sub>ori</sub>/KAN-2> Transposon 1 to 3 times:

<u>Enzyme</u>	<u>Sites</u>	<u>Location</u>	<u>Enzyme</u>	<u>Sites</u>	<u>Location</u>	<u>Enzyme</u>	<u>Sites</u>	<u>Location</u>
Acc I	1	1952	BsrF I	1	1303	Nsp I	1	1967
Apo I	2	962, 1146	BstDS I	3	718, 1873, 1934	PaeR7 I	1	947
Ase I	2	339, 1548	Btg I	3	714, 1869, 1930	PfiM I	2	798, 1612
AsiS I	1	1349	Bts I	2	1210, 1297	Ple I	2	1581, 1957
Ava I	2	736, 947	Cla I	2	29, 1040	PpuM I	2	681, 723
Ava II	3	681, 723, 1902	Dra I	1	414	Psi I	1	385
BamH I	1	1939	Dra III	1	818	Pst I	1	1961
Ban II	1	1004	Dsa I	3	714, 1869, 1930	Pvu I	1	1349
Bbs I	1	568	Eae I	1	717	Sal I	1	1951
Bfa I	2	674, 1946	Ear I	1	1162	Sau96 I	3	681, 723, 1902
BfrB I	2	1197, 1463	EcoN I	1	1261	Sbf I	1	1961
BfuA I	1	1964	EcoO109 I	2	681, 723	Sfc I	1	1957
Bpu10 I	2	581, 1366	Fau I	2	673, 1928	Sim I	2	681, 723
BsaA I	3	176, 198, 308	Fsp I	2	709, 807	Sml I	1	947
BsaB I	1	95	Hae I	2	719, 1436	SnaB I	1	176
BsaW I	3	99, 497, 1484	Hae II	1	521	Sph I	1	1967
BsiE I	1	1349	Hae III	3	719, 953, 1436	Ssp I	1	1274
BsiHKA I	1	700	Hinc II	1	1953	Sty I	1	792
Bsm I	3	808, 1233, 1310	Hind III	2	416, 1969	Tli I	1	947
BsmB I	1	1365	Mly I	2	1582, 1958	Tsp45 I	1	1496
Bsp1286 I	2	700, 1004	Msc I	1	719	TspR I	3	1222, 1297, 1769
BspD I	2	29, 1040	Msl I	2	508, 703	Xba I	1	1945
BspE I	2	99, 497	Nci I	2	679, 1222	Xho I	1	947
BspH I	1	867	Nla IV	3	682, 725, 1941	Xmn I	1	38
BspM I	1	1964	Nru I	1	1006			
BsrD I	1	841	Nsi I	2	1199, 1465			

Restriction Enzymes that cut the EZ-Tn5 <R6K<sub>ori</sub>/KAN-2> Transposon 4 or more times:

AcI	Bsr I	Cac8 I	Hinf I	HpyCH4 IV	Mnl I	Rsa I	Tse I
Alu I	BssK I	CviJ I	HinP I	HpyCH4 V	Mse I	Sau3A I	Tsp4C I
Alw I	BstF5 I	Dde I	Hpa II	Mae II	Msp I	ScrF I	Tsp509 I
BsaJ I	BstN I	Dpn I	Hph I	Mae III	Mwo I	SfaN I	
Bsl I	BstU I	Fnu4H I	Hpy188 I	Mbo I	Nla III	Taq I	
BsmA I	BstY I	Hha I	HpyCH4 III	Mbo II	PspG I	Tfi I	

Restriction Enzymes that do not cut the EZ-Tn5 <R6K<sub>ori</sub>/KAN-2> Transposon:

Aat II	ApaB I	Bme1580 I	BssH II	EcoR I	Nar I	PshA I	Sfo I	Xma I
Acc65 I	ApaL I	BmgB I	BssS I	EcoR V	Nco I	PspOM I	SgrA I	
Acl I	Asc I	Bmr I	BstAP I	Fse I	Nde I	Pvu II	Sma I	
Afe I	Avr II	Bsa I	BstB I	Gdi II	NgoM IV	Rsr II	Spe I	
Afl II	Ban I	BsaH I	BstE II	Hpa I	Nhe I	Sac I	Srf I	
Afl III	BbvC I	BseY I	BstX I	Hpy99 I	Not I	Sac II	Sse8647 I	
Age I	BciV I	BsiW I	BstZ17 I	Kpn I	Pac I	SanD I	Stu I	
Ahd I	Bcl I	BspLU11 I	Bsu36 I	Mfe I	Pci I	Sap I	Swa I	
Ale I	Bgl I	BsrB I	Drd I	Mlu I	PfiF I	Sca I	Tat I	
AlwN I	Bgl II	BsrD I	Eag I	MspA1 I	Pme I	SexA I	Tth111 I	
Apa I	Blp I	BsrG I	Eco47 III	Nae I	Pml I	Sfi I	Xcm I	

EZ-Tn5™ <R6K<sub>ori</sub>/KAN-2> Transposon 2001 bp.

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1 CTGTCTCTTA TACACATCTC AACCATCATC GATGAATTGC TTCGTTAATA CAGATGTAGG TGTTCCACAG
71 GGTAGCCAGC AGCATCCTGC GATGCAGATC CGGATGCCAT TTCATTACCT CTTTCTCCGC ACCCGACATA
141 GATCCGAAGA TCAGCAGTTC AACCTGTTGA TAGTACGTAC TAAGCTCTCA TGTTTCACGT ACTAAGCTCT
211 CATGTTTAAAC GTECTAAGCT CTCATGTTTA ACGAACTAAA CCCTCATGGC TAACGTAATA AGCTCTCATG
281 GCTAACGTAC TAAGCTCTCA TGTTTCACGT ACTAAGCTCT CATGTTTGAA CAATAAAAT AATATAAATC
351 AGCAACTTAA ATAGCCTCTA AGGTTTTAAG TTTTATAAGA AAAAAAAGAA TATATAAGGC TTTTAAAGCT
421 TTTAAGGTTT AACGGTTGTG GACAACAAGC CAGGGATCTG CCATTTTCATT ACCTCTTTCT CCGCACCCGA
491 CATAGATCCG GAACATAATG GTGCAGGGCG CTGACTTCCG CGTTTCCAGA CTTTACGAAA CACGGAAAAC
561 GAAGACCATT CATGTTGTTG CTCAGGTCGC AGACGTTTTG CAGCAGCAGT CGCTTCACGT TCGCTCGCGT
631 ATCGGTGATT CATCTGCTA ACCAGTAAGG CAACCCCGCC AGCCTAGCCG GGTCTCAAC GACAGGAGCA
701 CGATCATGCG CACCCGTGGC CAGGACCCAA CGCTGCCCGA GATGCGCCGC GTGCGGCTGC TGGAGATGGC
771 GGACGCGATG GATATGTTCT GCCAAGGGT GGTGTCGCA TTCACAGGGT GTCTCAAAT CTCTGATGTT
841 ACATTGCACA AGATAAAAT ATATCATCAT GAACAATAAA ACTGTCTGCT TACATAACA GTAATACAAG
911 GGGTGTATG AGCCATATC AACGGGAAAC GTCTTGCTCG AGGCCGCGAT TAAATCCAA CATGGATGCT
981 GATTTATATG GGTATAAATG GGCTCGCGAT AATGTCGGC AATCAGGTGC GACAATCTAT CGATTGTATG
1051 GGAAGCCCGA TCGCCAGAG TTGTTTCTGA AACATGGCAA AGGTAGCGTT GCCAATGATG TTACAGATGA
1121 GATGGTCAGA CTAACCTGGC TGACGGAAT TATGCCTCTT CCGACCATCA AGCATTATAT CCGTACTCCT
1191 GATGATGCAT GGTACTCAC CACTGCGATC CCCGAAAAA CAGCATTCCA GGTATTAGAA GAATATCCTG
1261 ATTCAGGTGA AAATATTGTT GATGCGCTGG CAGTGTTCCT GCGCCGGTTG CATTCGATTC CTGTTTGTA
1331 TTGTCTTTT AACAGCGATC GCGTATTCG TCTCGCTCAG GCGCAATCAC GAATGAATAA CGGTTTGGTT
1401 GATGCGAGTG ATTTTGATGA CGAGCGTAAT GGCTGGCCTG TTGAACAAGT CTGAAAAGAA ATGCATAAAC
1471 TTTTGCCATT CTCACCGGAT TCAGTCGTCA CTCATGGTGA TTTCTCACTT GATAACCTTA TTTTGGACGA
1541 GGGGAAATTA ATAGGTTGTA TTGATGTTGG ACGAGTCGGA ATCGCAGACC GATACCAGGA TCTTGCCATC
1611 CTATGGAACT GCCTCGGTGA GTTTTCTCCT TCATTACAGA AACGGCTTTT TCAAAAATAT GGTATTGATA
1681 ATCCTGATAT GAATAAATTG CAGTTTCATT TGATGCTCGA TGAGTTTTTC TAATCAGAAT TGTTAATTG
1751 GTTGTAAACAC TGGCAGAGCA TTACGCTGAC TTGACGGGAC GCGGCTTTG TTGAATAAAT CGAACTTTG
1821 CTGAGTTGAA GGATCAGATC ACGCATCTC CCGACAACGC AGACCGTTC GTGGCAAAGC AAAAGTCAA
1891 AATACCAAC TGGTCCACCT ACAACAAAGC TCTCATCAAC CGTGGCGGGG ATCCTCTAGA GTCGACCTGC
1961 AGGCATGCAA GCTTCAGGGT TGAGATGTGT ATAAGAGACA G
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The transposon sequence can be downloaded at the URL: <http://epibio.com/technical.asp>.