Lucigen®

Simplifying Genomics

EZ-Tn5™ <R6K*ori*/KAN-2>Tnp Transposome™ Kit

Cat. No. TSM08KR

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1. Introduction

The EZ-Tn5™ <R6K*ori*/KAN-2>Tnp Transposome™ is the stable complex formed between the EZ-Tn5 Transposase enzyme and the EZ-Tn5 <R6K*ori*/KAN-2> Transposon. The EZ-Tn5 <R6K*ori*/KAN-2> Transposon contains an R6Kγ conditional origin of replication (R6K*ori*) and the Tn903 kanamycin resistance gene (KanR) 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 genomic DNA of the host.[1,2]

The R6K*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*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 (the "pi" protein).[3] When selected on kanamycin-containing plates, only the cells containing the <R6K*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.

2. Product Designations and Kit Components

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
EZ-Tn5 <R6K*ori*/KAN-2> Tnp Transposome Kit	10 Rxns	TSM08KR	EZ-Tn5 <R6K*ori*/KAN-2> Tnp Transposome (0.1 pmol/µL)	SS000590-D	10 µL
			KAN-2 FP-1 Forward Primer (50 µM)	SS000523-D	20 µL
			R6KAN-2 RP-1 Reverse Primer (50 µM)	SS000536-D	20 µL
			Nuclease-Free Water, Sterile	SS000772-D3	1 mL

3. Product Specifications

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

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

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

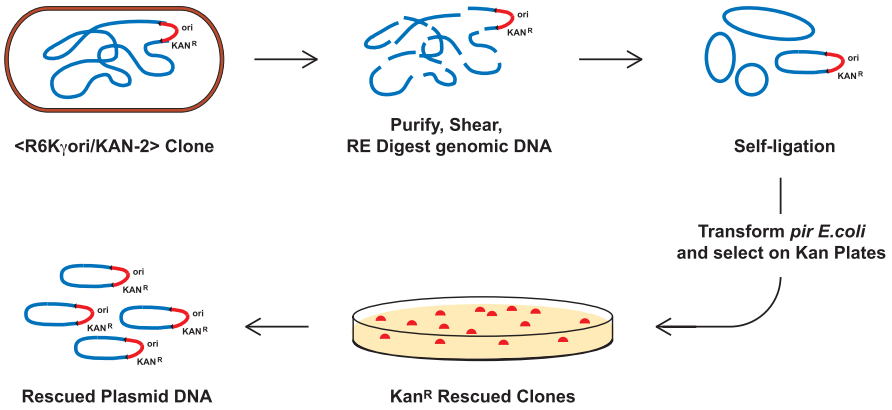


Figure 1. Rescue Cloning Overview.

Quality Control: EZ-Tn5 <R6Kγ*ori*/KAN-2>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 >10^5 Kan^R colonies/µg or >2.0 x 10^3 Kan^R colonies/µL of transposome respectively. Primers are function-tested via PCR and in a DNA cycle sequencing reaction and a plasmid containing an EZ-Tn5 <R6Kγ*ori*/KAN-2> Transposon as template.

Contaminating Activity Assays: All components of the EZ-Tn5 <R6Kγ*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.

4. Rescue Cloning of EZ-Tn5 <R6Kγ*ori*/KAN-2> Transposed Genomic DNA

An overview of the process for rescue cloning of the EZ-Tn5 <R6Kγ*ori*/KAN-2> Transposon insertion site in genomic DNA is given below.

Protocol

1. **Electroporation of Host Cells with EZ-Tn5 <R6Kγ*ori*/KAN-2>Tnp Transposome and Selection of Transposition Clones:** Electroporate electrocompetent cells using 1 µL of the EZ-Tn5 <R6Kγ*ori*/KAN-2>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.

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 50 µg/mL kanamycin. Other species may require plating of undiluted cells on plates containing 25-50 µg/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^R colonies/µL of EZ-Tn5 <R6K*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 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, using a bacterial genomic DNA purification kit or protocol. Fragment 1 µg of the genomic DNA by random shearing or by restriction endonuclease digestion(s) (See page 5 for the restriction map of the EZ-Tn5 <R6K*ori*/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 [Lucigen]).
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^R colonies overnight.
5. **DNA Sequencing of Rescue Clones:** Unlabeled forward and reverse EZ-Tn5 <R6K*ori*/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.

5. Primer Information

KAN-2 FP-1 Forward Primer

5′ - ACCTACAACAAAGCTCTCATCAACC - 3′

Length: 25 nucleotides

G+C content: 11

Molecular Weight: 7,484 daltons

Temperatures of Dissociation & Melting:

T_d: 68°C (nearest neighbor method)

T_m: 73°C (% G+C method)

T_m: 72°C ([2 (A+T) + 4 (G+C)] method)

T_m: 63°C ((81.5 + 16.6 (log [Na^+])) + ([41 (#G+C) - 500] / length) method) where [Na^+] = 0.1 M

R6KAN-2 RP-1 Reverse Primer

5′ - CTACCCTGTGGAACACCTACATCT - 3′

Length: 24 nucleotides

G+C content: 12

Molecular Weight: 7,210 daltons

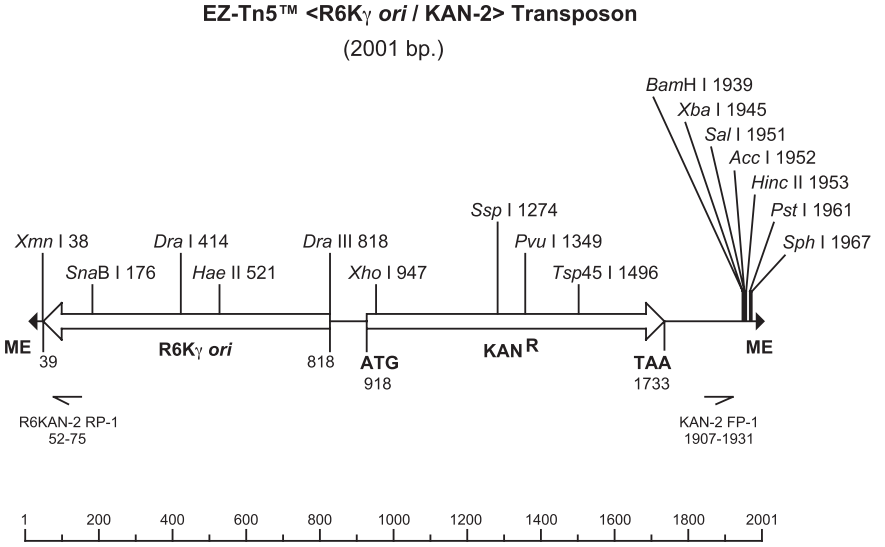
Temperatures of Dissociation & Melting:

T_d: 66°C (nearest neighbor method)

T_m: 74°C (% G+C method)

T_m: 72°C ([2 (A+T) + 4 (G+C)] method)

T_m: 65°C ((81.5 + 16.6 (log [Na^+])) + ([41 (#G+C) - 500] / length) method) where [Na^+] = 0.1 M



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	5' CTACCCTGTGGAACACCTACATCT 3'
KAN-2 FP-1 Forward Primer	5' ACCTACAACAAAGCTCTCATCAACC 3'
ME = Mosaic End	5' AGATGTGTATAAGAGACAG 3'

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

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

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

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

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

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

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

EZ-Tn5™ <R6Kyori/KAN-2> Transposon 2,001 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 CATGTTTAAC GTACTAAGCT CTCATGTTTA ACGAACTAAA CCCTCATGGC TAACGTACTA AGCTCTCATG
 281 GCTAACGTAC TAAGCTCTCA TGTTTCACGT ACTAAGCTCT CATGTTTGAA CAATAAAATT AATATAAATC
 351 AGCAACTTAA ATAGCCTCTA AGGTTTTAAG TTTTATAAGA AAAAAAGAAA TATATAAGGC TTTTAAAGCT
 421 TTTAAGGTTT AACGGTTGTG GACAACAAGC CAGGGATCTG CCATTTCATT ACCTCTTTCT CCGCACCCGA
 491 CATAGATCCG GAACATAATG GTGCAGGGCG CTGACTTCCG CGTTTCCAGA CTTTACGAAA CACGGAAACC
 561 GAAGACCATT CATGTTGTTG CTCAGGTCGC AGACGTTTTG CAGCAGCAGT CGCTTCACGT TCGCTCGCGT
 631 ATCGGTGATT CATTCTGCTA ACCAGTAAGG CAACCCCGCC AGCCTAGCCG GGTCCTCAAC GACAGGAGCA
 701 CGATCATGCG CACCCGTGGC CAGGACCCAA CGCTGCCCGA GATGCGCCGC GTGCGGCTGC TGGAGATGGC
 771 GGACGCGATG GATATGTTCT GCCAAGGGTT GGTTTGCGCA TTCACAGGGT GTCTCAAAAT CTCTGATGTT
 841 ACATTGCACA AGATAAAAAT ATATCATCAT GAACAATAAA ACTGTCTGCT TACATAAACA GTAATACAAG
 911 GGGTGTTATG AGCCATATTC AACGGGAAAC GTCTTGCTCG AGGCCGCGAT TAAATTCCAA CATGGATGCT
 981 GATTTATATG GGTATAAATG GGCTCGCGAT AATGTCGGGC AATCAGGTGC GACAATCTAT CGATTGTATG
1051 GGAAGCCCGA TGCGCCAGAG TTGTTTCTGA AACATGGCAA AGGTAGCGTT GCCAATGATG TTACAGATGA
1121 GATGGTCAGA CTAAACTGGC TGACGGAATT TATGCCTCTT CCGACCATCA AGCATTTTAT CCGTACTCCT
1191 GATGATGCAT GGTTACTCAC CACTGCGATC CCCGGAAAAA CAGCATTCCA GGTATTAGAA GAATATCCTG
1261 ATTCAGGTGA AAATATTGTT GATGCGCTGG CAGTGTTCCT GCGCCGGTTG CATTCGATTC CTGTTTGTAA
1331 TTGTCCTTTT AACAGCGATC GCGTATTTCG TCTCGCTCAG GCGCAATCAC GAATGAATAA CGGTTTGGTT
1401 GATGCGAGTG ATTTTGATGA CGAGCGTAAT GGCTGGCCTG TTGAACAAGT CTGGAAAGAA ATGCATAAAC
1471 TTTTGCCATT CTCACCGGAT TCAGTCGTCA CTCATGGTGA TTTCTCACTT GATAACCTTA TTTTTGACGA
1541 GGGGAAATTA ATAGGTTGTA TTGATGTTGG ACGAGTCGGA ATCGCAGACC GATACCAGGA TCTTGCCATC
1611 CTATGGAACT GCCTCGGTGA GTTTTCTCCT TCATTACAGA AACGGCTTTT TCAAAAATAT GGTATTGATA
1681 ATCCTGATAT GAATAAATTG CAGTTTCATT TGATGCTCGA TGAGTTTTTC TAATCAGAAT TGGTTAATTG
1751 GTTGTAACAC TGGCAGAGCA TTACGCTGAC TTGACGGGAC GGCGGCTTTG TTGAATAAAT CGAACTTTTG
1821 CTGAGTTGAA GGATCAGATC ACGCATCTTC CCGACAACGC AGACCGTTCC GTGGCAAAGC AAAAGTTCAA
1891 AATCACCAAC TGGTCCACCT ACAACAAAGC TCTCATCAAC CGTGGCGGGG ATCCTCTAGA GTCGACCTGC
1961 AGGCATGCAA GCTTCAGGGT TGAGATGTGT ATAAGAGACA G
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The transposon sequence can be downloaded at www.lucigen.com/sequences

6. 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.