Lucigen®

Simplifying Genomics

EZ-Tn5™ <R6Kγ*ori*/KAN-2> Insertion Kit

Cat. No. EZI011RK

Available exclusively thru Lucigen.
lucigen.com/epibio

epicentre®
an Illumina® company

1. Introduction

Transposons are mobile DNA sequences found in the genomes of prokaryotes and eukaryotes. Transposon tagging has long been recognized as a powerful research tool for randomly distributing primer binding sites, creating gene "knockouts", and introducing a physical tag or a genetic tag into large target DNAs. One frequently used transposition system is the Tn5 system isolated from gram-negative bacteria.

Though a naturally occurring transposition system, the Tn5 system can be readily adapted for routine use in research laboratories for the following reasons:

1) Tn5 transposase is a small, single subunit enzyme that has been cloned and purified to high specific activity.
2) Tn5 transposase carries out transposition without the need for host cell factors.
3) Tn5 transposon insertions into target DNA are highly random.
4) Tn5 transposition proceeds by a simple "cut and paste" process. Although the chemistry is unique, the result is similar to using a restriction endonuclease, with random sequence specificity, accompanied by a DNA ligase activity.
5) Tn5 transposase will transpose any DNA sequence contained between its short 19-basepair Mosaic End (ME) Tn5 transposase recognition sequences.

In 1998 Goryshin and Reznikoff[1] demonstrated that a fully functional Tn5 transposition system could be reconstituted *in vitro*. Additionally, the transposition efficiency of this system has been increased more than 1,000 fold compared to wild-type Tn5 by introducing mutations in the transposase gene and in the 19-bp Tn5 ME transposase recognition sequence.

Lucigen's EZ-Tn5 Transposon Tools (kits and reagents) are based on the hyperactive Tn5 transposition system developed by Goryshin and Reznikoff.

2. Kit Contents

Cat. #	Concentration	Quantity
Reagents included in the kit are sufficient for 10 *in vitro* transposon insertion reactions.		
EZ-Tn5™ Transposase: 10 U in 50% glycerol containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, and 0.1% Triton®X-100.	@ 1 U/µl	10 µl
EZ-Tn5™ <R6K*ori*/KAN-2> Transposon: 1 pmol @ 0.1 pmol/µl (0.13 µg/µl) in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).		10 µl
EZ-Tn5™ 10X Reaction Buffer: 0.50 M Tris-acetate (pH 7.5), 1.5 M potassium acetate, 100 mM magnesium acetate, and 40 mM spermidine.		100 µl
EZ-Tn5™ 10X Stop Solution: 1% SDS solution.		100 µl
KAN-2 FP-1 Forward Primer: 1 nmol in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).	@ 50 µM	20 µl
R6KAN-2 RP-1 Reverse Primer: 1 nmol in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).	@ 50 µM	20 µl

pUC19/3.4 Control Target DNA: 1 µg @ 0.1 µg/µl 10 µl
A 3.4-kb Hpa II fragment of bacteriophage DNA cloned into the Acc I site of pUC19. in TE Buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA).

Sterile Water 1 ml

Storage

Store EZ-Tn5 Insertion Kits only at –20°C in a freezer without a defrost cycle. The enzyme solution will not freeze. Other component solutions will freeze. After thawing, be sure to mix the contents of each tube thoroughly before using. After use, return all of the kit components to –20°C for storage.

3. How the EZ-Tn5 <R6Kγori/KAN-2> Insertion Kit Works

The EZ-Tn5 <R6K*γori*/KAN-2> Insertion Kit can be used to randomly insert the conditional *E. coli* R6Kγ origin of replication (R6K*γori*) into target DNA *in vitro*. The EZ-Tn5 <R6K*γori*/KAN-2> Transposon contains the R6K*γori* and a kanamycin resistance selection marker. A single 2-hour *in vitro* reaction randomly inserts the <R6K*γori*/KAN-2> Transposon into the target DNA. Use an aliquot of the reaction to transform *E. coli* that express the *pir* gene product (Π protein) such as Lucigen's TransforMax™ EC100D™ *pir*$^+$ or *pir*-116 Electrocompetent *E. coli* strains and select on kanamycin plates. Only those clones harboring DNA containing the <R6K*γori*/KAN-2> Transposon will grow.

The EZ-Tn5 <R6K*γori*/KAN-2> Insertion Kit can be used to:

- Introduce the R6K*γori* into any plasmid and/or cloning vector.
- Propagate vectors from non-*E. coli* species in *E. coli*.

  Randomly insert the EZ-Tn5 <R6K*γori*/KAN-2> Transposon into any foreign vector, transform an *E. coli pir*$^+$ or *E. coli pir-116* strain and select on kanamycin plates.
- Propagate genomic DNA fragments from any species as independently replicating plasmids in *E. coli* .

  Randomly insert the EZ-Tn5 <R6K*γori*/KAN-2> Transposon *in vitro* into self-ligated genomic DNA fragments, transform an *E. coli pir*$^+$ strain and select on kanamycin plates.

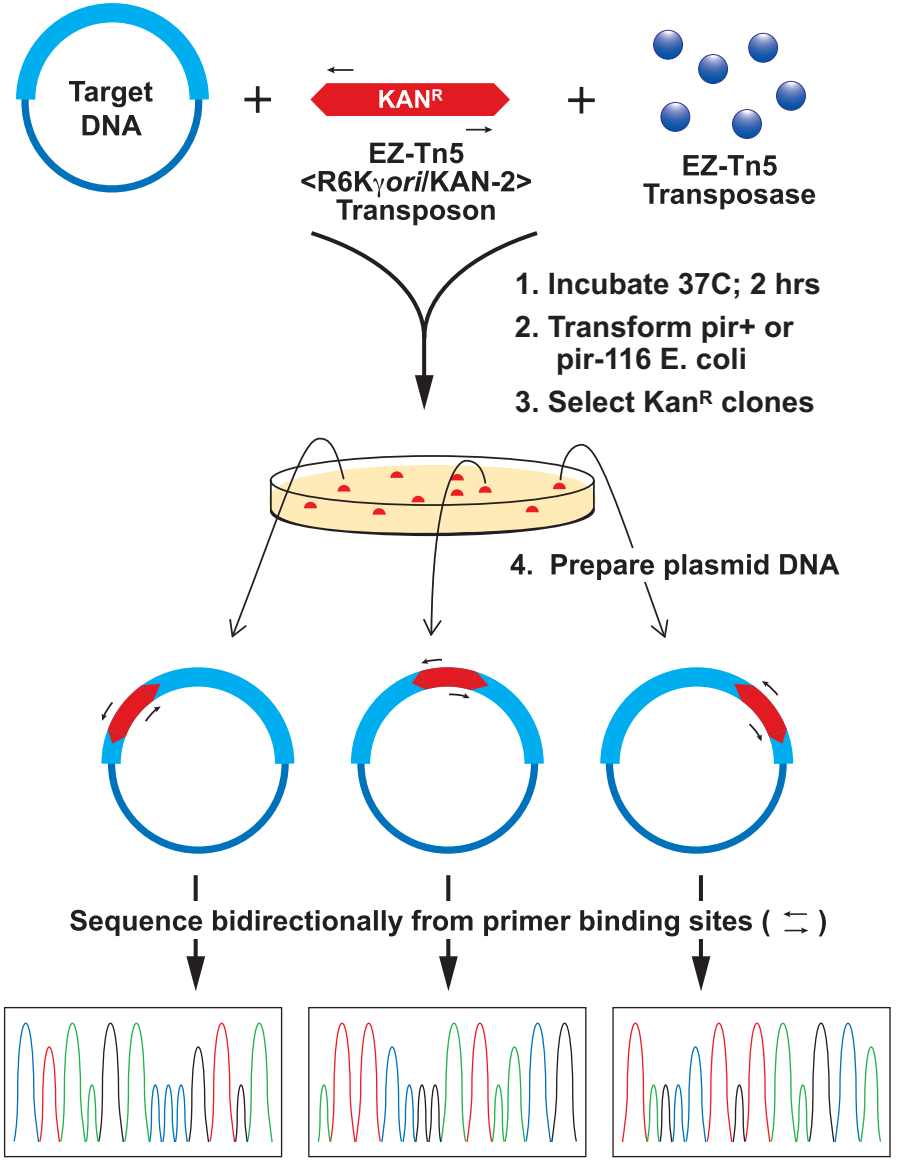


Figure 1. EZ-Tn5 <R6K*γori*/KAN-2> Transposon Insertion Protocol.

Fig. 1 describes the steps involved when using the EZ-Tn5 <R6Kγ*ori*/KAN-2> Insertion Kit. The process can be summarized as follows:

Preparation

- Prepare a minimum of 0.2 µg of recombinant DNA for the EZ-Tn5 <R6Kγ*ori*/KAN-2> insertion reaction.

Day 1

- Perform the 2-hour *in vitro* EZ-Tn5 <R6Kγ*ori*/KAN-2> insertion reaction.
- Transform competent *rec*A$^-$, *pir*$^+$ or *pir-116 E. coli* with 1 µl of the reaction mix.
- Select for kanamycin-resistant transposon insertion clones on kanamycin plates overnight.

Day 2

- Prepare DNA from kanamycin-resistant colonies.
- (Optional) Map the EZ-Tn5 <R6Kγ*ori*/KAN-2> Transposon insertion sites.
- (Optional) DNA sequence chosen clones bidirectionally using the unlabeled forward and reverse transposon-specific primers supplied in the kit.

4. Performance Specifications and Quality Control

The EZ-Tn5 <R6Kγ*ori*/KAN-2> Insertion Kit is function-tested by performance of the kit's *in vitro* control reaction followed by electroporation into a *rec*A$^-$ *E. coli* host strain having a transformation efficiency of >10^9 cfu/µg DNA. Transposition frequency, defined as the ratio of the number of Kan^R clones divided by the number of transformants resistant to the antibiotic marker of the target vector, (Kan^R colonies/Amp^R colonies; for the control DNA) must be >0.5% (commonly at 10%) and transposition efficiency must be >10^6 Kan^R colonies/µg target DNA. The EZ-Tn5 <R6Kγ*ori*/KAN-2> Transposon is tested for selective propagation in TransforMax EC100D *pir*-116 Electrocompetent *E. coli* in the presence of kanamycin. Primers are function-tested in a DNA sequencing reaction and in a PCR reaction using a plasmid containing an EZ-Tn5 <R6Kγ*ori*/KAN-2> Transposon as template. All components of the EZ-Tn5 <R6Kγ*ori*/KAN-2> Insertion 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.

5. Transposon Insertion Reaction

Target DNA Preparation

The target DNA must not contain a kanamycin resistance gene. The transposon insertion reaction is not significantly inhibited by high levels of RNA contamination in target DNA preparations. However, if the target DNA is heavily contaminated with chromosomal DNA, which is a direct competitor for target transposition, the number of clones will be greatly reduced.

Plasmid and cosmid clones can be purified by standard minilysate or plasmid spin column purification procedures and used as target DNA in the insertion reaction without further clean-up. Low copy-number vectors, for example BAC or fosmid clones, are often contaminated with a higher molar proportion of *E. coli* chromosomal DNA thus reducing the transposon insertion frequency. Therefore, BAC and fosmid DNA should be purified, to remove the chromosomal DNA prior to the insertion reaction.

In Vitro Transposon Insertion Reaction

Reaction conditions have been optimized to maximize the efficiency of the EZ-Tn5 Transposon insertion 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 <R6K*y*ori/KAN-2> Transposon.** If necessary, dilute the EZ-Tn5 <R6K*y*ori/KAN-2> Transposon with TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

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

1	µl	EZ-Tn5 10X Reaction Buffer
0.2	µg	target DNA*
x	µl	molar equivalent EZ-Tn5 <R6K*y*ori/KAN-2> 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.
   Proceed **Transformation and Recovery** or store the reaction mixture at –20°C.

*Calculation of µmol target DNA:

µmol target DNA = µg target DNA / [(# base pairs in target DNA) × 660]

For example: 0.2 µg of control pUC19/3.4 DNA which is 6,100 bp

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

6. Selection of Transposon Insertion Clones

Transformation and Recovery

The number of EZ-Tn5 Transposon insertion clones obtained per reaction depends on, among other factors, the transformation efficiency of the competent cells used. An aliquot of the insertion reaction is used to transform competent *E. coli* that express the Π protein (*pir* gene product). We recommend using electrocompetent or chemically competent *rec*A⁻, *pir*⁺ or *pir-116 E. coli* with a transformation efficiency of >10^8 cfu/µg of DNA. The *pir* gene product is required for replication of vectors containing the R6Kγ*ori* (EZ-Tn5 <R6Kγ*ori*/KAN-2> Transposon insertion clones). A *rec*A⁻ and restriction minus (hsd, mcr, mrr-negative) strain of *E. coli* is recommended to eliminate the possibility of generating multimeric forms of the vector. Finally, the host strain used must not have a kanamycin resistance marker when used with the EZ-Tn5 <R6Kγ*ori*/KAN-2> Transposon. Lucigen's TransforMax EC100D *pir*⁺ Electrocompetent *E. coli* or TransforMax EC100D *pir*-116 Electrocompetent *E. coli* (available separately) have a transformation efficiency of >1 × 10^9 cfu/µg and are ideal for this application.

1) Using 1 µl of the insertion reaction mixture, transform *rec*A⁻, *pir*⁺ *E. coli*. If electrocompetent cells are used, perform electroporation according to the equipment manufacturer's recommended conditions. Use of >1 µl of the transposon insertion reaction for transformation may result in arcing. The unused portion of the transposon insertion reaction can be stored at –20°C for future use.
2) 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.

Plating and Selecting Transformants

EZ-Tn5 <R6Kγ*ori*/KAN-2> Transposon insertion clones are selected on kanamycin-containing plates.

1) If transformation was done using cells with an efficiency of >5 × 10^8 cfu/µg DNA, it may be necessary to dilute the cells 1:10 or 1:100 prior to plating. Plate portions (e.g., 100 µl) of cells onto LB plates containing 50 µg/ml kanamycin. Store the unused portion of the recovered cells at +4°C for up to 2 days in the event additional plates need to be prepared.
2) Grow plates overnight at 37°C. Assuming a transposon insertion efficiency of 1% and use of high purity target DNA (i.e., little or no chromosomal DNA contamination), one should see 100-500 Kan^R clones per plate. If too few (or too many) colonies appear, replate the transformed cells at a lower (or higher) dilution.

Typical results obtained with the EZ-Tn5 <R6Kγ*ori*/KAN-2> Insertion Kit are:

- Transposon insertion frequency = 0.5% - 20%
- Transposition clones per µg target DNA = 1 × 10^5 - 2 × 10^8
- Transposition clones per 10 µl transposon insertion reaction = 1 × 10^5 - 1 × 10^7

The actual number of EZ-Tn5 <R6K*γori*/KAN-2> insertion clones obtained will vary depending on factors such as target DNA size and the transformation efficiency of the competent cells used to recover the transposon insertion clones. Users should note that transposons containing the R6Kγ origin of replication, produce an inherent level of background clones by several mechanisms. First, linear transposon DNA can be circularized *in vivo* resulting in a 2-kb plasmid. Second, the transposon itself can serve as its own intramolecular target for transposition.[2] Such events result in: 1) circular transposon segment inversion clones resulting in a 2-kb plasmid, 2) circular transposon clones containing very small deletions resulting in plasmids slightly smaller than 2 kb and 3) circular plasmids consisting of multimers and/or partial multimers of the transposon resulting in plasmids covering a range of sizes all larger than 2 kb.

7. DNA Sequencing of Transposon Insertion Clones

Transposon Insertion Mapping (optional)

EZ-Tn5 Transposon insertion clones can be sequenced bidirectionally using the unlabeled forward and reverse transposon-specific primers provided in the kit using ABI BigDye chemistry and capillary electrophoresis-based sequencers. The insertion site of each clone can also be mapped prior to sequencing, depending on the cost-effectiveness and experience of the user.

EZ-Tn5 <R6K*γori*/KAN-2> Transposon insertion sites can be mapped by size analysis of PCR products using colony minilysate DNA as a template. To map the insertion sites, use the KAN-2 FP-1 or R6KAN-2 RP-1 primers provided with the kit and a vector-specific flanking primers (not provided).

Alternatively, insertion sites can be mapped by restriction endonuclease digest(s). Use the nucleotide sequence and restriction information of the EZ-Tn5 <R6K*γori*/KAN-2> Transposon provided in the Appendix for reference.

Primer Considerations

The KAN-2 FP-1 Forward and R6KAN-2 RP-1 Reverse Primers supplied with the kit have been constructed to minimize homology to commonly used cloning vectors. **However, the sequence of each primer should be compared to that of the user's specific cloning vector to ensure minimal sequence homology to the vector.** The sequence and theoretical melting temperatures for each primer are presented in the Appendix.

Note: *Occasionally a clone will yield the sequence of the cloning vector. This occurs when the EZ-Tn5 Transposon randomly inserts into a non-essential region of the vector rather than into the DNA insert. The frequency of this occurrence is dependent on the size of the DNA insert relative to the size of non-essential regions of vector. The larger the DNA insert, the less frequently an insertion will occur into the vector.*

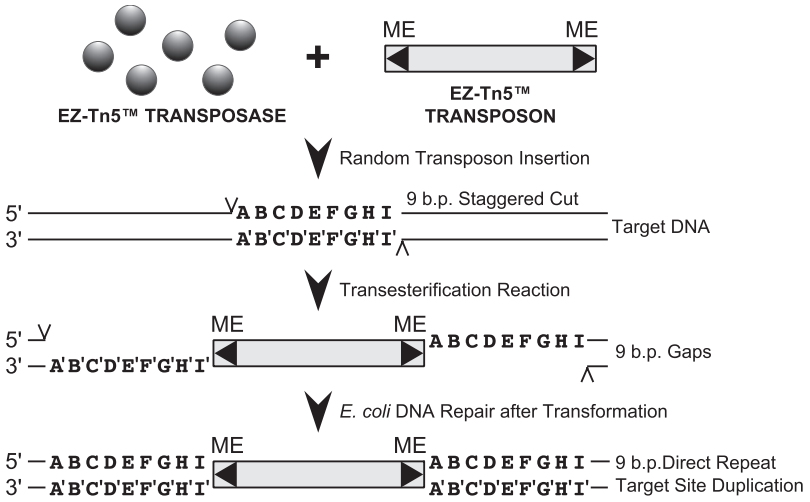


Figure 2. EZ-Tn5 Transposase Insertion Site Duplication Process.

Figure 3. EZ-Tn5 Transposon Insertion Site Junction.

Target Site Duplication

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.

Distinguishing Transposon Sequence from Insert Sequence

Since the primers provided in the EZ-Tn5 <R6K*ori*/KAN-2> Insertion Kit anneal to a region near the ends of the transposon, the first sequence data obtained from each sequencing reaction is that of EZ-Tn5 <R6K*ori*/KAN-2> Transposon DNA. The sequence of the 19-bp EZ-Tn5 Transposase recognition sequence (ME) found at the junction of the transposon and the target clone insert DNA (present in **all** insertion clones), is a useful landmark to distinguish vector sequence from target clone insert sequence (see also Fig. 3).

8. Troubleshooting

Arcing during transformation by electroporation

1) **Excessive salt in target DNA preparation.** Use less sample for electroporation. Ethanol precipitate and 70% ethanol wash, or drop dialyze DNA.

   Arcing inevitably results in failed transformation. Discard the electroporation reaction and try again with 0.5 µl of the transposition reaction. With the control DNA, no arcing is observed when up to 2 µl of transposition reaction is used for electroporation of 50 µl of electrocompetent *rec*A$^-$ *E. coli* in a 0.2-cm width electroporation cuvette and using an Eppendorf Multiporator at 2500 V. A voltage gradient of approximately 12,500 V/cm is fairly standard for *E. coli*.

No, or few transposition clones on selective plates

1) **Transformation reaction was unsuccessful; low competence.** Test by plating outgrowth using drug resistance marker on target DNA to distinguish between transformation or transposon insertion failure.

   If competent cells have a transformation efficiency <10^8 cfu/µg DNA, one may not obtain sufficient clones on a plate. For example, transforming into cells with transformation efficiency <10^5 cfu/µg DNA results in as few as 2 insertion clones on a plate. Use cells with a transformation efficiency >10^8 cfu/µg DNA.

2) **Transposon insertion reaction was unsuccessful.** Inhibitor contamination in target DNA. Purify target DNA further. Perform procedure with control plasmid provided with kit to assure system components are functional.

DNA sequencing results are ambiguous

1) **Two or more transposon insertions into target clone.** Discard the clone. Choose other clone(s) to sequence.

   The protocol was designed to minimize multiple transposon insertion events. Even so, about 1% of the transposition clones may contain >1 transposon. One can verify single insertion clones by agarose gel electrophoresis of colony minilysates prior to sequencing. A single insertion should be about 2.1 kb larger than the parental target DNA. A double insertion will increase the size by 4.2 kb and also result in "double sequence".

2) **The sequencing primer used has significant homology to the cloning vector or to the DNA being sequenced.** Check homology of primer against the vector. Alter primer annealing conditions or synthesize a new primer with less homology.

3) **Components of the DNA sequencing kit and/or of the electrophoresis step are compromised.** Reverify the integrity of the components of the kit and/or electrophoresis step with appropriate controls. Use a new kit and/or new reagents.

Confluent plates following transformation

1) **Target DNA or host cells have the same selective marker (antibiotic resistance) as the transposon used.** Use a different host for transformation and retransform with a portion of your remaining reaction.

   Some host cells carry Kan^R transposons. Confirm that the genotype of the host strain chosen for the transformation is not Kan^R.

9. Appendix

Primer Data

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:

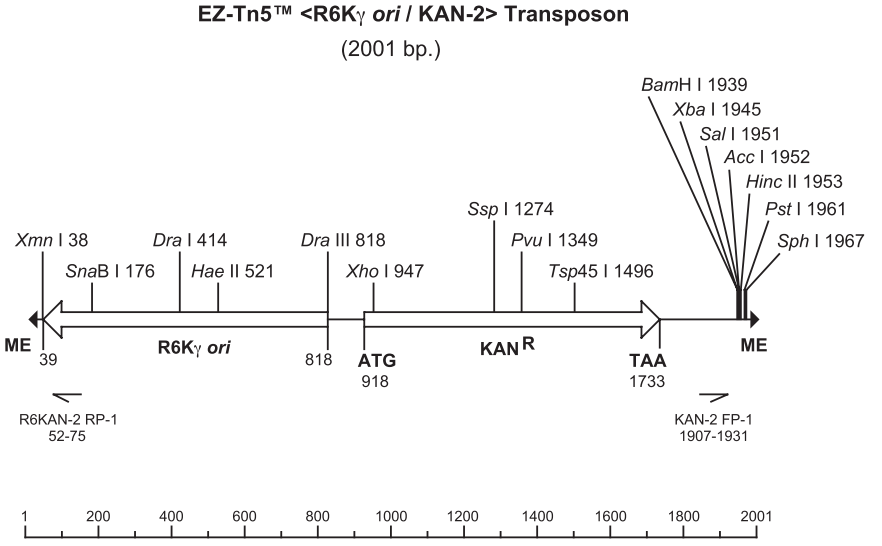
T_d: 66°C (nearest neighbor method)

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

EZ-Tn5 <R6K*ori*/KAN-2> Transposon Schematic Map



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

EZ-Tn5 <R6Kγ*ori*/KAN-2> Transposon Restriction Data Restriction Enzymes that cut the EZ-Tn5 <R6Kγ*ori*/KAN-2> Transposon one to three 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*yori*/KAN-2> Transposon four or more times:**

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

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

EZ-Tn5™ <R6K*y**ori*/KAN-2> Transposon 2,001 bp.

```
   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 AAAAAAAGAA 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
```

The transposon sequence can be downloaded at www.lucigen.com/sequences.

10. References

Cited:

1. Goryshin, I.Y. and Reznikoff, W.S. (1998) *J. Biol. Chem.* **273**, 7367.
2. York, D. *et al.*, (1998) *Nucl. Acids Res.* **26**, 1927.

Related:

1. Goryshin, I.Y. and Reznikoff, W.S. (1998) *J. Biol. Chem.* **273**, 7367.
2. Zhou, M. and Reznikoff, W.S. (1997) *J. Mol. Biol.* **271**, 362.
3. Mahnke-Braam, I.A. and Reznikoff, W.S. (1998) *J. Biol. Chem.* **273**, 10908.

R6K and pir gene product information:

4. Kolter, R. *et al.*, (1978) *Cell* **15**, 1199.
5. York, D. and Filutowicz, M. (1993) *J. Biol. Chem.* **268**, 21854.
6. Metcalf, W.W. *et al.*, (1994) *Gene* **138**, 1.
7. Pillarisetty, V.A., *et al.*, (1996) *Proc. Natl. Acad. Sci USA* **93**, 5522.