

# pRL-TK Vector

Technical Bulletin No. 240

**INSTRUCTIONS FOR USE OF PRODUCT E2241. PLEASE DISCARD PREVIOUS VERSIONS.**

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## I. Description

The pRL-TK Vector<sup>(a,b)</sup> (Figure 1) is intended for use as an internal control reporter and may be used in combination with any experimental reporter vector to cotransfect mammalian cells. All of Promega's pRL Reporter Vectors contain a cDNA (*Rluc*) encoding *Renilla* luciferase, which was originally cloned from the marine organism *Renilla reniformis* (sea pansy; 1). As described below, the *Renilla* luciferase cDNA<sup>(b)</sup> contained within the pRL Vectors has been modified slightly to provide greater utility.

The pRL-TK Vector contains the herpes simplex virus thymidine kinase (HSV-TK) promoter to provide low to moderate levels of *Renilla* luciferase expression in co-transfected mammalian cells. *Renilla* luciferase is a 36kDa monomeric protein that does not require post-translational modification for activity (2). Therefore, like firefly luciferase, the enzyme may function as a genetic reporter immediately following translation. For information about the use of this plasmid in conjunction with a reporter vector containing the firefly luciferase gene, refer to the *Dual-Luciferase® Reporter Assay System*<sup>(c,d)</sup> *Technical Manual* (#TM040).

The pRL Vectors are isolated from a *dam*<sup>-</sup>/*dcm*<sup>-</sup> *E. coli* K host strain, allowing digestion with restriction enzymes that are sensitive to *dam* and *dcm* methylation.

The GenBank®/EMBL Accession number for the pRL-TK Vector is AF025846.



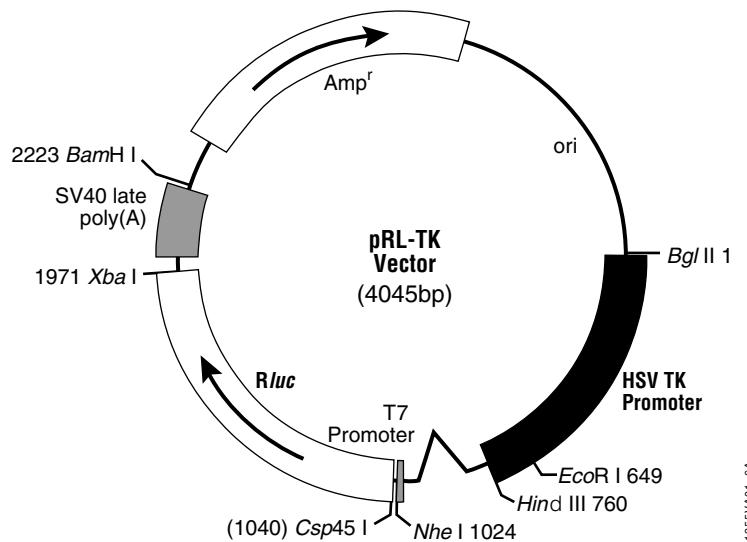
A99TB240 0701TB240

## II. Product Components

Product	Size	Cat.#
pRL-TK Vector	20µg	E2241

All pRL Vectors are supplied in TE buffer (pH 7.4) and are provided with a glycerol stock of bacterial strain JM109. The JM109 cells do not contain vector and are not competent cells.

**Storage Conditions:** Store vector DNA at -20°C and the glycerol stock of JM109 cells at -70°C.



1355VA01\_6A

**Figure 1. The pRL-TK Vector circle map and sequence reference points.**

**Sequence reference points:**

HSV-TK promoter	7–759
Chimeric intron	826–962
T7 RNA polymerase promoter (-17 to +2)	1006–1024
T7 RNA polymerase transcription initiation site	1023
Rluc reporter gene	1034–1969
SV40 late polyadenylation signal	2011–2212
β-lactamase (Amp <sup>r</sup> ) coding region	2359–3219

**In addition:**

- ^— indicates the position of the intron.
  - Rluc is the cDNA encoding the *Renilla luciferase* enzyme.
  - Amp<sup>r</sup> indicates the gene encoding ampicillin resistance in *E. coli*.
  - ori is the origin of replication in *E. coli*.
  - The arrows within the Rluc and Amp<sup>r</sup> genes indicate the direction of transcription.
- Restriction sites shown in parentheses are not unique sites.

### III. Features of the pRL-TK Vector

#### A. TK Promoter Region

The pRL-TK Vector contains the herpes simplex virus thymidine kinase promoter region upstream of *Rluc*. The HSV-TK promoter provides low-level, constitutive expression in cells of both embryonal and mature mammalian tissues (3,4).

#### B. Chimeric Intron

Downstream of the TK promoter region of the pRL-TK Vector is a chimeric intron comprised of the 5'-donor splice site from the first intron of the human  $\beta$ -globin gene, and the branch and 3'-acceptor splice site from an intron preceding an immunoglobulin gene heavy chain variable region (5). The sequences of the donor and acceptor splice sites, along with the branchpoint site, have been modified to match the consensus sequences for optimal splicing (6).

Transfection studies have demonstrated that the presence of an intron flanking a cDNA insert frequently increases the level of gene expression (7–10). In the pRL-SV40 Vector, the intron is positioned 5' to *Rluc* to minimize the utilization of cryptic 5'-donor splice sites that may reside within the reporter gene sequence (11).

#### C. T7 Promoter

A T7 promoter is located downstream of the chimeric intron, immediately preceding the *Rluc* reporter gene. This T7 promoter can be used to synthesize RNA transcripts in vitro using T7 RNA Polymerase (Cat.# P2075). T7 RNA Polymerase can also be used to synthesize active *Renilla* luciferase in a cell-free coupled eukaryotic in vitro transcription/translation reaction (e.g., Promega's TNT® Reticulocyte Lysate [Cat.# L4610]<sup>(c,e,f,g)</sup>, Wheat Germ Extract<sup>(c,e,f,g)</sup> [Cat.# L4140], or TNT® T7 Quick Coupled Transcription/Translation<sup>(c,e,f,g,h)</sup> [Cat.# L1170] Systems).

#### D. *Renilla* Luciferase Reporter Gene (*Rluc*)

The *Renilla* luciferase cDNA inserted into all of the pRL Vectors is derived from the anthozoan coelenterate *Renilla reniformis* (1) but contains nucleotide changes that were engineered during the construction of the individual vectors. The following bases were altered in the pRL-TK Vector: base 1264 (T→C), to eliminate an internal *Bgl* II site; base 1807 (T→C), to eliminate internal *Bam*H I site; base 1840 (C→T), to eliminate internal *Nar*I, *Kas* K, *Ban* K and *Acy* I sites. These nucleotide substitutions do not alter the amino acid sequence of the encoded *Renilla* luciferase reporter enzyme.

#### E. SV40 Late Polyadenylation Signal

Polyadenylation signals cause the termination of transcription by RNA polymerase II and signal the addition of approximately 200–250 adenosine residues to the 3'-end of the RNA transcript (12). Polyadenylation has been shown to enhance RNA stability and translation (13,14). The late SV40 polyadenylation signal, which is extremely efficient and has been shown to increase the steady-state level of RNA approximately five-fold more than the early SV40 polyadenylation signal (15), has been positioned 3' to the *Rluc* gene in the pRL-TK Vector to increase the level of *Renilla* luciferase expression.

**!** Due to sequence differences between the T7 Promoter Primer offered by Promega (Cat.# Q5021) and the T7 promoter used in the pRL family of *Renilla* luciferase co-reporter vectors, Cat.# Q5021 cannot be used for sequencing with this vector.

#### **IV. Transfection of Mammalian Cells with pRL-TK**

The pRL-TK Vector may be used in combination with any experimental reporter vector to cotransfect mammalian cells. However, it is important to realize that *trans* effects between promoters on cotransfected plasmids can potentially affect reporter gene expression (16). This is primarily of concern when either the control or experimental reporter vector, or both, contain very strong promoter/enhancer elements. The occurrence and magnitude of such effects will depend on several factors: i) the combination and activities of the genetic regulatory elements present on the cotransfected vectors, ii) the relative ratio of experimental vector to control vector introduced into the cells, and iii) the type of cell transfected.

To help ensure independent genetic expression between experimental and control reporter genes, preliminary cotransfection experiments should be performed to optimize both the amount of vector DNA and the ratio of the coreporter vectors added to the transfection mixture. Similar to the firefly luciferase assay, the *Renilla* luciferase assay is extremely sensitive, providing accurate measurement of  $\leq 10$  femtograms of *Renilla* luciferase, with linearity over 7 orders of enzyme concentration. Therefore, it is possible to use relatively small quantities of pRL-TK Vector to provide low-level, constitutive coexpression of *Renilla* luciferase control activity. Ratios of 10:1 (or greater) for experimental vector:pRL-TK Vector combinations may aid greatly in suppressing the occurrence of *trans* effects between promoter elements.

The pRL-TK Vector can be used for both transient and stable expression of genes. For stable expression, the pRL-TK Vector must be cotransfected with an expression vector containing a selectable gene in mammalian cells. Transfection of DNA into mammalian cells may be mediated by cationic lipids (17,18), calcium phosphate (19,20), DEAE-Dextran (21–23), polybrene-DMSO (24,25) or electroporation (26,27).

Transfection systems based on cationic lipid compounds (TransFast™ Reagent<sup>(i)</sup>, Tfx™ Reagents<sup>(j)</sup> and Transfectam® Reagent<sup>(k)</sup>), calcium phosphate and DEAE-Dextran are available from Promega. For more information and a protocol for the Transfectam® Reagent, please request the *Transfectam® Reagent Technical Bulletin* (#TB116) and for TransFast™ Reagent, please request the *TransFast™ Transfection Reagent Technical Bulletin* (#TB260). Protocols for the use of the Tfx™ Reagents can be found in the *Tfx™-10, Tfx™-20 and Tfx™-50 Reagent Technical Bulletin* (#TB216). For transfection procedures using calcium phosphate or DEAE-Dextran, please request the *ProFection® Mammalian Transfection Systems Technical Manual* (#TM012).

#### **V. pRL-TK Vector Restriction Sites and Sequence**

##### **A. pRL-TK Vector Restriction Sites**

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3' end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch Office or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526.

**Table 1. Restriction Enzymes That Cut the pRL-TK Vector Between 1 and 5 Times.**

<b>Enzyme</b>	<b># of Sites</b>	<b>Location</b>	<b>Enzyme</b>	<b># of Sites</b>	<b>Location</b>
<b><i>Acc</i> I</b>	1	342	<b><i>Drd</i> I</b>	2	781, 3932
<b><i>Acy</i> I</b>	2	290, 2606	<b><i>Dsa</i> I</b>	5	129, 180, 296, 330
<b><i>Afl</i> II</b>	4	36, 160, 792, 989			543
<b><i>Afl</i> III</b>	2	713, 1216	<b><i>Eae</i> I</b>	4	126, 1384, 1978, 2753
<b><i>Alw44</i> I</b>	2	2474, 3720	<b><i>Eag</i> I</b>	1	1978
<b><i>AlwN</i> I</b>	2	357, 3625	<b><i>Ear</i> I</b>	3	137, 1204, 2347
<b><i>AspH</i> I</b>	3	2478, 2563, 3724	<b><i>Ecl/HK</i> I</b>	1	3146
<b><i>Ava</i> I</b>	3	108, 229, 282	<b><i>Eco52</i> I</b>	1	1978
<b><i>Avr</i> II</b>	1	322	<b><i>EcoR</i> I</b>	1	649
<b><i>Bal</i> I</b>	1	128	<b><i>Ehe</i> I</b>	1	291
<b><i>BamH</i> I</b>	1	2223	<b><i>Fsp</i> I</b>	1	2923
<b><i>Ban</i> I</b>	4	289, 915, 1838, 3193	<b><i>Hae</i> II</b>	3	150, 293, 3794
<b><i>Ban</i> II</b>	1	313	<b><i>Hinc</i> II</b>	1	2121
<b><i>Bbe</i> I</b>	1	293	<b><i>Hind</i> II</b>	1	2121
<b><i>Bbs</i> I</b>	3	6, 900, 1874	<b><i>Hind</i> III</b>	1	760
<b><i>Bcl</i> I</b>	2	1318, 1527	<b><i>Hpa</i> I</b>	1	2121
<b><i>Bgl</i> I</b>	3	298, 301, 3028	<b><i>Hsp92</i> I</b>	2	290, 2606
<b><i>Bgl</i> II</b>	1	1	<b><i>Kas</i> I</b>	1	289
<b><i>Bsa</i> I</b>	3	386, 854, 3080	<b><i>Mlu</i> I</b>	1	713
<b><i>BsaO</i> I</b>	4	1981, 2628, 2777, 3700	<b><i>Nar</i> I</b>	1	290
<b><i>BsaA</i> I</b>	2	346, 1766	<b><i>Nco</i> I</b>	1	330
<b><i>BsaB</i> I</b>	1	2222	<b><i>Nhe</i> I</b>	1	1024
<b><i>BsaH</i> I</b>	2	290, 2606	<b><i>Not</i> I</b>	1	1978
<b><i>BsaM</i> I</b>	2	2042, 2135	<b><i>Nsp</i> I</b>	3	599, 1160, 1220
<b><i>Bsm</i> I</b>	2	2042, 2135	<b><i>PaeR7</i> I</b>	2	882, 4040
<b><i>BspH</i> I</b>	3	1602, 2306, 3314	<b><i>PpuM</i> I</b>	1	319
<b><i>BspM</i> I</b>	1	816	<b><i>Psp5</i> II</b>	1	319
<b><i>BsrBRI</i></b>	1	2222	<b><i>PspA</i> I</b>	1	282
<b><i>BsrG</i> I</b>	1	1732	<b><i>Pst</i> I</b>	3	509, 746, 802
<b><i>BssH</i> II</b>	1	241	<b><i>Pvu</i> I</b>	1	2777
<b><i>BssS</i> I</b>	3	1692, 2477, 3861	<b><i>Pvu</i> II</b>	1	531
<b><i>Bst1107</i> I</b>	1	343	<b><i>Rsa</i> I</b>	4	473, 1002, 1734, 2665
<b><i>Bst98I</i></b>	4	36, 160, 792, 989	<b><i>Sac</i> II</b>	1	299
<b><i>BstZ</i> I</b>	1	1978	<b><i>Sca</i> I</b>	2	1002, 2665
<b><i>Cfr10</i> I</b>	1	3061	<b><i>Sma</i> I</b>	1	284
<b><i>Cla</i> I</b>	1	2216	<b><i>Ssp</i> I</b>	1	2341
<b><i>Csp45</i> I</b>	2	653, 1040	<b><i>Sty</i> I</b>	2	322, 330
<b><i>Dde</i> I</b>	4	2645, 3185, 3351, 3760	<b><i>Vsp</i> I</b>	2	1134, 2971
<b><i>Dra</i> I</b>	4	2182, 2568, 3260, 3279	<b><i>Xba</i> I</b>	1	1971
<b><i>Dra</i> II</b>	1	319	<b><i>Xcm</i> I</b>	1	1683
			<b><i>Xma</i> I</b>	1	282
			<b><i>Xmn</i> I</b>	2	1568, 2546

**Note:** The enzymes listed in boldface type are available from Promega.

**Table 2. Restriction Enzymes That Do Not Cut the pRL-TK Vector.**

<b>Aat</b> II	<i>Bpu</i> 1102 I	<b>Eco</b> ICR I	<b>Nsi</b> I	<b>Sal</b> I	Sse8387 I
<b>AccB7</b> I	<i>Bsp</i> 120 I	<i>Eco</i> N I	<i>Pac</i> I	<b>Sfi</b> I	<b>Stu</b> I
<b>Acc</b> III	<b>BstE</b> II	<b>Eco</b> R V	<i>Pfl</i> M I	<b>Sgf</b> I <sup>(I)</sup>	<i>Swa</i> I
<b>Acc65</b> I	<b>BstX</b> I	<i>Fse</i> I	<i>Pin</i> A I	<i>Sgr</i> A I	<b>Tth</b> 111 I
<b>Age</b> I	<i>Bsu</i> 36 I	<b>I-Ppo</b> I	<i>Pme</i> I	<b>Snab</b> I	<b>Xho</b> I
<b>Apa</b> I	<i>Csp</i> I	<i>Kpn</i> I	<i>Pml</i> I	<b>Spe</b> I	
<b>Asc</b> I	<i>Dra</i> III	<i>Nae</i> I	<i>Ppu</i> 10 I	<b>Sph</b> I	
<i>Bbr</i> P I	<b>Eco</b> 47 III	<i>Nde</i> I	<i>Psh</i> A I	<i>Spl</i> I	
<b>Bbu</b> I	<i>Eco</i> 72 I	<b>Ngo</b> MIV	<i>Rsr</i> II	<i>Srf</i> I	
<i>Blp</i> I	<i>Eco</i> 81 I	<i>Nru</i> I	<b>Sac</b> I		

**Note:** The enzymes listed in boldface type are available from Promega.

**Table 3. Restriction Enzymes That Cut the pRL-TK Vector 6 or More Times.**

<b>Aci</b> I	<b>Bsr</b> S I	<b>Fok</b> I	<i>Mae</i> I	<b>Msp</b> A 1 I	<i>Scr</i> F I
<b>Alu</b> I	<b>Bst</b> 71 I	<b>Hae</b> III	<i>Mae</i> II	<b>Nci</b> I	<i>Sfa</i> N I
<b>Alw26</b> I	<b>Bst</b> O I	<i>Hga</i> I	<i>Mae</i> III	<b>Nde</b> II	<b>Sin</b> I
<b>Ava</b> II	<i>Bst</i> U I	<b>Hha</b> I	<b>Mbo</b> I	<i>Nla</i> III	<b>Taq</b> I
<i>Bbv</i> I	<b>Cfo</b> I	<b>Hinf</b> I	<b>Mbo</b> II	<i>Nla</i> IV	<i>Tfi</i> I
<i>Bsa</i> J I	<b>Dpn</b> I	<b>Hpa</b> II	<i>Mnl</i> I	<i>Ple</i> I	<b>Tru</b> 9 I
<b>Bsp</b> 1286 I	<i>Dpn</i> II	<i>Hph</i> I	<i>Mse</i> I 20	<b>Sau</b> 3A I	<b>Xho</b> II
<i>Bsr</i> I	<i>Fnu</i> 4H I	<b>Hsp</b> 92 II	<b>Msp</b> I	<b>Sau</b> 96 I	

**Note:** The enzymes listed in boldface type are available from Promega.

## B. pRL-TK Vector Sequence

The sequence shown corresponds to the mRNA synthesized from the *Renilla* luciferase gene from the TK promoter. The vector sequence is also available on the Internet at [www.promega.com/vectors](http://www.promega.com/vectors). The GenBank®/EMBL Accession Number for the pRL-TK Vector is AF025846.

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1  AGATCTAAAT  GAGTCCTCGG  ACCTCGCGGG  GGCCGCTTAA  GCGGTGGTTA
51  GGGTTTGTCT  GACGCGGGGG  GAGGGGGAAG  GAACGAAACA  CTCTCATTG
101  GAGGCGGCTC  GGGGTTTGGT  CTTGGTGGCC  ACGGGCACGC  AGAAGAGCGC
151  CGCGATCCTC  TTAAGCACCC  CCCCGCCCTC  CGTGGAGGCG  GGGGTTTGGT
201  CGGCGGGTGG  TAACTGGCGG  GCCGCTGACT  CGGGCGGGTC  GCGCGCCCCA
251  GAGTGTGACC  TTTTCGGTCT  GCTCGCAGAC  CCCCCGGCGG  CGCCGCCGCG
301  GCGGCGACGG  GCTCGCTGGG  TCCTAGGCTC  CATGGGGACC  GTATAACGTGG
351  ACAGGCTCTG  GAGCATCCGC  ACGACTGCGG  TGATATTACC  GGAGACCTTC
401  TGCAGGGACGA  GCCGGGTCAC  GCGGCTGACG  CGGAGCGTCC  GTTGGGCGAC
451  AAACACCAGG  ACGGGGCACA  GGTACACTAT  CTTGTCACCC  GGAGGCGCGA
501  GGGACTGCA  GAGCTTCAGG  GAGTGGCGCA  GCTGCTTCAT  CCCCCGTGGCC
551  CGTTGCTCGC  GTTGCTGGC  GGTGTCCCCG  GAAGAAATAT  ATTTGCATGT
601  CTTTAGTTCT  ATGATGACAC  AAACCCCGCC  CAGCGTCTTG  TCATTGGCGA
651  ATTCAACAC  GCAGATGCAG  TCGGGCGGGC  GCGGTCCCAG  GTCCACTTCG

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**B. pRL-TK Vector Sequence (continued)**

701	CATATTAAGG	TGACGCGTGT	GGCCTCGAAC	ACCGAGCGAC	CCTGCAGCGA
751	CCCGCTTAAA	AGCTTGATTTC	TTCTGACACA	ACAGTCTCGA	ACTTAAGCTG
801	CAGAAGTTGG	TCGTGAGGCA	CTGGGCAGGT	AAGTATCAAG	GTTACAAGAC
851	AGGTTTAAGG	AGACCAATAG	AAACTGGGCT	TGTCGAGACA	GAGAAGACTC
901	TTGCGTTTCT	GATAGGCACC	TATTGGTCTT	ACTGACATCC	ACTTTGCCTT
951	TCTCTCCACA	GGTGTCCACT	CCCAGTTCAA	TTACAGCTCT	TAAGGCTAGA
1001	GTACTTAATA	CGACTCACTA	TAGGCTAGCC	ACCATGACTT	CGAAAGTTA
1051	TGATCCAGAA	CAAAGGAAAC	GGATGATAAC	TGGTCCGCAG	TGGTGGGCCA
1101	GATGTAAACA	AATGAATGTT	CTTGATTATC	TTATTAATTA	TTATGATTCA
1151	GAAAAACATG	CAGAAAATGC	TGTTATTTT	TTACATGGTA	ACGCGGCCCTC
1201	TTCTTATTTA	TGGCGACATG	TTGTGCCACA	TATTGAGCCA	GTAGCGCGGT
1251	GTATTATAACC	AGACCTTATT	GGTATGGCAG	AATCAGGCAA	ATCTGGTAAT
1301	GGTTCTTATA	GGTTACTTGA	TCATTACAAA	TATCTTACTG	CATGGTTG
1351	ACTTCCTTAAT	TTACCAAAGA	AGATCATT	TGTCGGCCAT	GATTGGGGTG
1401	CTTGTGTTGGC	ATTTCATTAT	AGCTATGAGC	ATCAAGATAA	GATCAAAGCA
1451	ATAGTTCACG	CTGAAAAGTGT	AGTAGATGTG	ATTGAATCAT	GGGATGAATG
1501	GCCTGATATT	GAAGAAGATA	TTGCGTTGAT	CAAATCTGAA	GAAGGAGAAA
1551	AAATGGTTTT	GGAGAATAAC	TTCTTCGTGG	AAACCATGTT	GCCATCAAAA
1601	ATCATGAGAA	AGTTAGAAC	AGAAGAATT	GCAGCATATC	TTGAACCATT
1651	CAAAGAGAAA	GGTGAAGTTC	GTCGTCCAAC	ATTATCATGG	CCTCGTGAAA
1701	TCCCGTTAGT	AAAAGGTGGT	AAACCTGACG	TTGTACAAAT	TGTTAGGAAT
1751	TATAATGCTT	ATCTACGTGC	AAGTGATGAT	TTACCAAAAA	TGTTTATTGA
1801	ATCGGACCCA	GGATTCTTT	CCAATGCTAT	TGTTGAAGGT	GCCAAGAAGT
1851	TTCCTAACATC	TGAATTGTC	AAAGTAAAAG	GTCTTCATTT	TTCGCAAGAA
1901	GATGCACCTG	ATGAAATGGG	AAAATATATC	AAATCGTCG	TTGAGCGAGT
1951	TCTCAAAAAT	GAACAATAAT	TCTAGAGCGG	CCGCTTCGAG	CAGACATGAT
2001	AAGATACATT	GATGAGTTG	GACAAACCAC	AACTAGAATG	CAGTAAAAAA
2051	AATGCTTAT	TTGTGAAATT	TGTGATGCTA	TTGCTTATT	TGTAACCATT
2101	ATAAGCTGCA	ATAAACAAAGT	TAACAAACAAC	AATTGCATTC	ATTTTATGTT
2151	TCAGGTTCA	GGGGAGGTGT	GGGAGGTTTT	TTAAAGCAAG	TAAAACCTCT
2201	ACAAATGTGG	TAAAATCGAT	AAGGATCCAG	GTGGCACTTT	TCGGGGAAAT
2251	GTGCGCGGAA	CCCCTATTTG	TTTATTTTC	TAAATACATT	CAAATATGTA
2301	TCCGCTCATG	AGACAATAAC	CCTGATAAAAT	GCTTCATAA	TATTGAAAAAA
2351	GGAAGAGTAT	GAGTATTCAA	CATTTCGTG	TCGCCCTTAT	TCCCTTTTT
2401	GCGGCATTTT	GCCTTCCTGT	TTTGCTCAC	CCAGAAACGC	TGGTGAAAGT
2451	AAAAGATGCT	GAAGATCAGT	TGGGTGCACG	AGTGGGTTAC	ATCGAACTGG
2501	ATCTAACACAG	CGGTAAGATC	CTTGAGAGTT	TTCGCCCCGA	AGAACGTTT

**B. pRL-TK Vector Sequence (continued)**

2551	CCAATGATGA	GCACTTTAA	AGTTCTGCTA	TGTGGCGCGG	TATTATCCCG
2601	TATTGACGCC	GGGCAAGAGC	AACTCGGTG	CCGCATACAC	TATTCTCAGA
2651	ATGACTTGGT	TGAGTACTCA	CCAGTCACAG	AAAAGCATCT	TACGGATGGC
2701	ATGACAGTAA	GAGAATTATG	CAGTGCTGCC	ATAACCATGA	GTGATAACAC
2751	TGCGGCCAAC	TTACTTCTGA	CAACGATCGG	AGGACCGAAG	GAGCTAACCG
2801	CTTTTTGCA	CAACATGGGG	GATCATGTAA	CTCGCCTTGA	TCGTTGGGAA
2851	CCGGAGCTGA	ATGAAGCCAT	ACCAAACGAC	GAGCGTGACA	CCACGATGCC
2901	TGTAGCAATG	GCAACAACGT	TGCGCAAAC	ATTAAC	TGGC GAACTACTTA
2951	CTCTAGCTTC	CCGGCAACAA	TTAATAGACT	GGATGGAGGC	GGATAAAAGTT
3001	GCAGGACAC	TTCTGCGCTC	GGCCCTTCCG	GCTGGCTGGT	TTATTGCTGA
3051	TAAATCTGGA	GCCGGTGAGC	GTGGGTCTCG	CGGTATCATT	GCAGCACTGG
3101	GGCCAGATGG	TAAGCCCTCC	CGTATCGTAG	TTATCTACAC	GACGGGGAGT
3151	CAGGCAACTA	TGGATGAACG	AAATAGACAG	ATCGCTGAGA	TAGGTGCCTC
3201	ACTGATTAAG	CATTGGTAAC	TGTCAGACCA	AGTTTACTCA	TATATACTTT
3251	AGATTGATTT	AAAACCTTCAT	TTTTAATT	AAAGGATCTA	GGTGAAGATC
3301	CTTTTGATA	ATCTCATGAC	CAAAATCCCT	TAACGTGAGT	TTTCGTTCCA
3351	CTGAGCGTCA	GACCCCGTAG	AAAAGATCAA	AGGATCTTCT	TGAGATCCTT
3401	TTTTCTGCG	CGTAATCTGC	TGCTTGCAAA	CAAAAAAAC	ACCGCTACCA
3451	GCGGTGGTT	GTGTTGCCGGA	TCAAGAGCTA	CCAACTCTT	TTCCGAAGGT
3501	AACTGGCTTC	AGCAGAGCGC	AGATACCAAA	TACTGTTCTT	CTAGTGTAGC
3551	CGTAGTTAGG	CCACCACTTC	AAGAACTCTG	TAGCACCGCC	TACATACCTC
3601	GCTCTGCTAA	TCCTGTTACC	AGTGGCTGCT	GCCAGTGGCG	ATAAGTCGTG
3651	TCTTACCGGG	TTGGACTCAA	GACGATAGTT	ACCGGATAAG	GCGCAGCGGT
3701	CGGGCTGAAC	GGGGGGTTCG	TGCACACAGC	CCAGCTTGA	GCGAACGACC
3751	TACACCGAAC	TGAGATACCT	ACAGCGTGAG	CTATGAGAAA	GCGCCACGCT
3801	TCCCCGAAGGG	AGAAAGGCGG	ACAGGTATCC	GGTAAGCGGC	AGGGTGGAA
3851	CAGGAGAGCG	CACGAGGGAG	CTTCCAGGGG	GAAACGCC	GTATCTTAT
3901	AGTCCTGTG	GGTTTCGCCA	CCTCTGACTT	GAGCGTCGAT	TTTTGTGATG
3951	CTCGTCAGGG	GGGCAGGAGCC	TATGGAAAAA	CGCCAGCAAC	GCGGCCTTTT
4001	TACGGTTCC	GGCCTTTGC	TGGCCTTTG	CTCACATGGC	TCGAC

## VI. Related Products

### pRL Family of *Renilla* Luciferase Vectors for Co-Reporter Applications

Product	Size	Cat.#
pRL-CMV Vector <sup>(a,b,m)</sup>	20µg	E2261
pRL-SV40 Vector <sup>(a,b)</sup>	20µg	E2231
pRL-null Vector <sup>(a,b)</sup>	20µg	E2271

To inquire about the availability of bulk packaging and pricing for pRL Vectors, please contact Promega.

For inquiries on the availability of new promoter variations within the pRL family of co-reporter vectors, contact Technical Services or visit our web site at [www.promega.com](http://www.promega.com).

### pGL3 Vectors

Product	Size	Cat.#
pGL3-Control Vector <sup>(a,f,n)</sup>	20µg	E1741
pGL3-Basic Vector <sup>(a,f,n)</sup>	20µg	E1751
pGL3-Promoter Vector <sup>(a,f,n)</sup>	20µg	E1761
pGL3-Enhancer Vector <sup>(a,f,n)</sup>	20µg	E1771

### Luciferase Assay Systems

Product	Size	Cat.#
Dual-Luciferase® Reporter Assay System	100 assays	E1910
Dual-Luciferase® Reporter Assay System 10-pack <sup>(c,d)</sup>	1,000 assays	E1960
Dual-Luciferase® Reporter 1000 Assay System <sup>(c,d)</sup>	1,000 assays	E1980

### Transfection Systems

Product	Size	Cat.#
TransFast™ Transfection Reagents	1.2mg	E2431
Transfectam® Reagent for the Transfection of Eukaryotic Cells	1mg	E1231
	0.5mg	E1232
Tfx™-50 Reagent	2.1mg	E1811
Tfx™-20 Reagent	4.8mg	E2391
Tfx™-10 Reagent	9.3mg	E2381
Tfx™ Reagents Transfection Trio	5.4mg	E2400
ProFection® Mammalian Transfection System —Calcium Phosphate	80 transfections	E1200
ProFection® Mammalian Transfection System —DEAE-Dextran	80 transfections	E1210

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