pRL-CMV Vector

Promega

Technical Bulletin No. 237

INSTRUCTIONS FOR USE OF PRODUCT E2261. *PLEASE DISCARD PREVIOUS VERSIONS.*
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I. Description

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

The pRL-CMV Vector contains the CMV[b] enhancer and early promoter elements to provide high-level expression of *Renilla* luciferase 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*[c,d,e] *Technical Manual* (#TM040).

To avoid DNA methylation, all pRL Vectors are isolated from a *dam*-/*dcm*- *E. coli* K host strain. Therefore, initial propagation of the pRL-CMV Vector should be conducted in an appropriate *E. coli* host strain lacking endogenous restriction endonuclease activity (e.g., JM109).

AF9TB237 07/01TB237

II. Product Components

Product	Size	Cat.#
pRL-CMV Vector	20µg	E2261

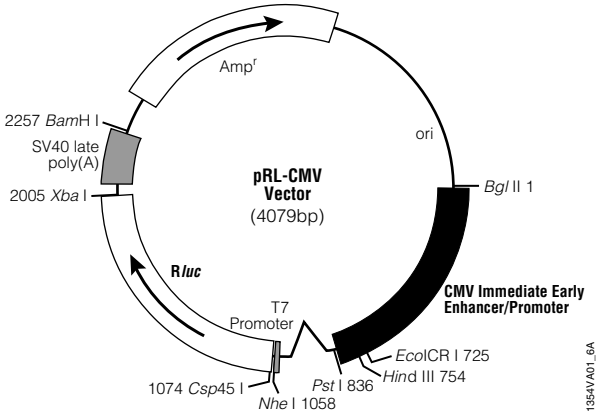
All pRL Vectors are supplied in TE buffer (pH 7.4) and are provided with a glycerol stock of bacterial strain JM109.

Storage Conditions: Store vector DNA at –20°C. Store glycerol stock of JM109 at –70°C.

III. Features of the pRL-CMV Vector

A. CMV Enhancer/Promoter Regions

The pRL-CMV Vector contains the CMV immediate-early enhancer/promoter region, which provides strong, constitutive expression of the *Renilla* luciferase cDNA in a variety of cell types. The promiscuous nature of the CMV enhancer/promoter has been demonstrated in transgenic mice, where its transcriptional activity was observed in 24 of the 28 murine tissues examined (3).



pRL-CMV Vector circle map and sequence reference points:

CMV enhancer and immediate early promoter	7–803
Chimeric intron	860–996
T7 promoter (–17 to +2)	1040–1058
T7 promoter transcription start site	1057
R*luc* reporter gene	1068–2003
SV40 late polyadenylation signal	2045–2246
β-lactamase (Amp^r) coding region	2393–3253

Note: –⌒–, position of intron; R*luc*, cDNA encoding the *Renilla* luciferase enzyme; Amp^r, gene conferring ampicillin resistance in *E. coli*; ori, origin of plasmid replication in *E. coli*. Arrows within the R*luc* and Amp^r gene indicate the direction of transcription.

B. Chimeric Intron

Downstream of the CMV enhancer/promoter region of the pRL-CMV Vector is a chimeric intron comprised of the 5´-donor splice site from the first intron of the human β-globin gene, and the branch and 3´-acceptor splice site from an intron preceding an immunoglobulin gene heavy chain variable region (4). 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 (5).

Transfection studies have demonstrated that the presence of an intron flanking a cDNA insert frequently increases the level of gene expression (6–9). In the pRL-CMV Vector, the intron is positioned 5´ to R*luc* to minimize the utilization of cryptic 5´-donor splice sites that may reside within the reporter gene sequence (10).

C. T7 Promoter

A T7 promoter is located downstream of the chimeric intron, and immediately precedes the R*luc* 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[c,d,f,g] [Cat.# L4610] or Wheat Germ Extract [Cat.# L4140] Systems).

Note: The T7 Promoter Primer offered by Promega (Cat.# Q5021) cannot be used for sequencing this vector because of a mismatch between the 3´ end of the primer and the vector DNA.

D. *Renilla* Luciferase Reporter Gene (R*luc*)

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-CMV Vector: base 1298 (T→C), to eliminate an internal *Bgl* II site; base 1841 (T→C), to eliminate an internal *Bam*H I site; base 1874 (C→T), to eliminate internal *Nar* I, *Kas* I, *Ban* I 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 (11). Polyadenylation has been shown to enhance RNA stability and translation (12,13). The late SV40 polyadenylation signal, which is extremely efficient and has been shown to increase the steady-state level of RNA approximately 5-fold more than the early SV40 polyadenylation signal (14), has been positioned 3´ to the R*luc* gene in the pRL-CMV Vector to increase the level of *Renilla* luciferase expression.

IV. Transfection of Mammalian Cells with pRL-CMV Vector

The pRL-CMV Vector may be used in combination with any experimental reporter vector to co-transfect mammalian cells. However, it is important to realize that *trans* effects between promoters on co-transfected plasmids can potentially affect reporter gene expression (15). Primarily, this is of concern when either the control or experimental reporter vector, or both, contain very strong promoter/enhancer elements (such as CMV). The occurrence and magnitude of such effects will depend on several factors: a) the combination and activities of the genetic regulatory elements present on the co-transfected vectors, b) the relative ratio of experimental vector to control vector introduced into the cells, and c) the cell type transfected.

To help ensure independent genetic expression between experimental and control reporter genes, preliminary co-transfection experiments should be performed to optimize both the **amount** of vector DNA and the **ratio** of the co-reporter vectors added to the transfection mixture. Similar to the firefly luciferase assay, the *Renilla* luciferase assay is extremely sensitive, providing accurate measurement of ≤10 femtograms of *Renilla* luciferase, with linearity over 7 orders of enzyme concentration. Therefore, it is possible to use relatively small quantities of pRL-CMV Vector to provide low-level, constitutive co-expression of *Renilla* luciferase control activity. Ratios of 10:1 to 50:1 (or greater) for experimental vector:pRL-CMV Vector combinations are feasible and may aid greatly in suppressing the occurrence of *trans* effects between promoter elements.

The pRL-CMV Vector can be used for both transient and stable expression of genes. For stable expression, the pRL-CMV Vector must be co-transfected with an expression vector containing a selectable gene in mammalian cells. Transfection of DNA into mammalian cells may be mediated by cationic lipids (16,17), calcium phosphate (18,19), DEAE-Dextran (20–22), polybrene-DMSO (23,24), or electroporation (25,26).

Transfection systems based on cationic lipid compounds (Transfectam® Reagent[h], TransFast™ Transfection Reagent[i] and Tfx™-10, Tfx™-20 and Tfx™-50 Reagents[j]), Calcium Phosphate and DEAE-Dextran are available from Promega. For more information and a protocol for the Transfectam® Reagent, please request the *Transfectam® Reagent for the Transfection of Eukaryotic Cells Technical Bulletin* (#TB116). Information about the TransFast™ Transfection Reagent can be found in 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 Reagents for the Transfection of Eukaryotic Cells Technical Bulletin* (#TB216). For transfection procedures using calcium phosphate or DEAE-Dextran, please request the *ProFection® Mammalian Transfection Systems Technical Manual* (#TM012).

Note: All of Promega's Technical Bulletins and Technical Manuals are available online at www.promega.com.

Note: For assistance in determining transfection conditions for different cell lines, Promega offers the Transfection Assistant available online at www.promega.com/transfectionasst/.

V. pRL-CMV Vector Restriction Sites and Vector Sequence

A. pRL-CMV 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. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AF025843) and on the Internet at www.promega.com/vectors/.

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

Enzyme	# of Sites	Location
***Aat* II**	4	284, 337, 420, 606
***Acy* I**	5	281, 334, 417, 603 2640
Afl II	2	826, 1023
Afl III	1	1250
***Alw*44 I**	2	2508, 3754
*Alw*N I	1	3659
*Asp*H I	4	727, 2512, 2597, 3758
***Ava* II**	4	1116, 1838, 2816, 3038
***Bal* I**	2	16, 70
***Bam*H I**	1	2257
***Ban* I**	4	624, 949, 1872, 3227
***Ban* II**	1	727
Bbs I	2	934, 1908
***Bcl* I**	2	1352, 1561
***Bgl* I**	5	142, 249, 371, 442 3062
***Bgl* II**	1	1
Bsa I	2	888, 3114
***Bsa*O I**	4	2015, 2662, 2811, 3734
*Bsa*A I	2	499, 1800
*Bsa*B I	1	2256
*Bsa*H I	5	281, 334, 417, 603 2640
*Bsa*J I	3	519, 1841, 3908
***Bsa*M I**	2	2076, 2169
Bsm I	2	2076, 2169
***Bsp*1286 I**	4	727, 2512, 2597, 3758
*Bsp*H I	3	1636, 2340, 3348
*Bsp*M I	1	850
***Bsr*BR I**	1	2256
*Bsr*G I	2	102, 1766
*Bss*S I	3	1726, 2511, 3895
***Bst*98 I**	2	826, 1023
***Bst*Z I**	1	2012
***Cla* I**	1	2250
***Csp*45 I**	1	1074
***Dde* I**	4	2679, 3219, 3385, 3794
***Dra* I**	4	2216, 2602, 3294, 3313
Drd I	2	815, 3966
Dsa I	1	519
Eae I	5	14, 68, 1418, 2012 2787
Eag I	1	2012
Ear I	2	1238, 2381
***Ecl*HK I**	1	3180
***Eco*52 I**	1	2012
***Eco*I CR I**	1	725
Fsp I	1	2957
***Hae* II**	1	3828
Hga I	4	685, 2648, 3378, 3956
***Hinc* II**	2	675, 2155
*Hin*d II	2	675, 2155
***Hin*d III**	1	754
***Hpa* I**	1	2155
***Hsp*92 I**	5	281, 334, 417, 603 2640
***Msp*A1 I**	3	2544, 3485, 3730
***Nci* I**	3	2644, 2995, 3691
***Nco* I**	1	519
***Nde* I**	1	393
***Nhe* I**	1	1058
***Not* I**	1	2012
Nsp I	2	1194, 1254
*Pae*R7	2	916, 4074
Ple I	5	563, 924, 1040, 3189, 3692
***Pst* I**	1	836
***Pvu* I**	1	2811
***Sac* I**	1	727

(continued)

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

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Table 1. Restriction Enzymes That Cut the pRL-CMV Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location
***Sca* I**	2	1036, 2699
***Sin* I**	4	1116, 1838, 2816, 3038
***Sna*B I**	1	499
***Spe* I**	1	158
***Ssp* I**	3	11, 58, 2375
***Sty* I**	1	519
Tfi I	5	1158, 1179, 1518, 1833, 1846
***Vsp* I**	3	166, 1168, 3005
***Xba* I**	1	2005
Xcm I	1	1717
***Xmn* I**	2	1602, 2580

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

***Acc*B7 I**	***Bbu* I**	***Bss*H II**	***Eco*R I**	***Nru* I**	***Sgf* I**(k)
***Acc* I**	*Bfr*B I	*Bst*1107 I	***Eco*R V**	***Nsi* I**	*Sgr*A I
***Acc* III**	*Bln* I	*Bst*29 I	*Eco*R124 I	*Pac* I	***Sma* I**
***Acc*65 I**	*Blp* I	***Bst*E II**	*Eco*R124 II	*Pau* I	*Sna* I
Acr I	*Bmg* I	***Bst*X I**	*Eco*prr I	*Pfl*M I	***Sph* I**
*Acs*1371 I	*Bpl* I	***Bsu*36 I**	*Ehe* I	*Pfu* I	*Spl* I
*Afa*24R I	*Bpu*1102 I	***Csp* I**	*Esp*16 I	*Pin*A I	*Srf* I
***Age* I**	*Bpu*10 I	*Dra* II	*Esp*3 I	*Pme* I	*Sse*8387 I
*Ahy*A I	*Bpu*1102 I	*Dra* III	*Fse* I	*Pml* I	*Sse*8647 I
Ama I	*Bpu*1268 I	*Dsa* VI	*Fsu* I	*Ppu*10 I	***Stu* I**
Aos III	*Bsa*X I	*Eci*E I	*Gsp* I	*Ppu*M I	*Sty*SJ
***Apa* I**	*Bsb* I	*Eco*0109 I	*Hin*e I	*Psh*A I	*Sty*SQ
Ape I	*Bsc*E I	***Eco*47 III**	**I-*Ppo* I**	*Psp*5 II	*Swa* I
Asc I	*Bsc*J I	*Eco*72 I	*Kas* I	*Psp*A I	*Tth* I
*Asp*5H I	*Bse*59 I	*Eco*81 I	***Kpn* I**	*Pss* I	***Tth*111 I**
*Asp*78 I	*Bse*R I	*Eco*82 I	*Kpn*2 I	***Pvu* II**	*Uba*1220 I
***Ava* I**	*Bsg* I	*Eco*A I	***Mlu* I**	*Rsr* II	*Uba*1221 I
Ava III	*Bsh*L I	*Eco*B I	*Mlu*1106 I	***Sac* II**	*Uba*1326 I
Avr II	*Bsi*W I	*Eco*D I	*Mlu*113 I	***Sal* I**	*Uba*D I
Bae I	*Bsm*b1	*Eco*DR2	***Nae* I**	*San*D I	*Van*91 I
Bbe I	*Bsp*120 I	*Eco*DR3	***Nar* I**	*Sap* I	***Xho* I**
*Bbe*A I	*Bsp*87 I	*Eco*E I	*Ngo*A IV	*Sci* I	***Xma* I**
*Bbf*7411 I	*Bsp*G I	*Eco*N I	***Ngo*M IV**	*Sex*A I	
*Bbr*P I	*Bsp*J106 I	*Eco*O109 I	*Nli*3877 I	***Sfi* I**	

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

Aci I	***Bst*O I**	***Hae* III**	*Mae* II	***Nde* II**	*Sfa*N I
***Alu* I**	*Bst*U I	***Hha* I**	*Mae* III	*Nla* III	***Taq* I**
***Alw*26 I**	***Cfo* I**	***Hin*f I**	***Mbo* I**	*Nla* IV	***Tru*9 I**
Bbv I	***Dpn* I**	***Hpa* II**	***Mbo* II**	***Rsa* I**	***Xho* II**
Bsr I	*Dpn* II	*Hph* I	*Mnl* I	***Sau*3A I**	
***Bsr*S I**	*Fnu*4H I	***Hsp*92 II**	*Mse* I	***Sau*96 I**	
***Bst*71 I**	***Fok* I**	*Mae* I	***Msp* I**	*Scr*F I	

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

B. pRL-CMV Vector Sequence

The sequence shown corresponds to the mRNA synthesized from the *Renilla* luciferase gene from the CMV promoter.

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   1  AGATCTTCAA TATTGGCCAT TAGCCATATT ATTCATTGGT TATATAGCAT
  51  AAATCAATAT TGGCTATTGG CCATTGCATA CGTTGTATCT ATATCATAAT
 101  ATGTACATTT ATATTGGCTC ATGTCCAATA TGACCGCCAT GTTGGCATTG
 151  ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA
 201  GCCCATATAT GGAGTTCCGC GTTACATAAC TTACGGTAAA TGGCCCGCCT
 251  GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT
 301  TCCCATAGTA ACGCCAATAG GGACTTTCCA TTGACGTCAA TGGGTGGAGT
 351  ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA
 401  AGTCCGCCCC CTATTGACGT CAATGACGGT AAATGGCCCG CCTGGCATTA
 451  TGCCCAGTAC ATGACCTTAC GGGACTTTCC TACTTGGCAG TACATCTACG
 501  TATTAGTCAT CGCTATTACC ATGGTGATGC GGTTTTGGCA GTACACCAAT
 551  GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT
 601  TGACGTCAAT GGGAGTTTGT TTTGGCACCA AAATCAACGG GACTTTCCAA
 651  AATGTCGTAA TAACCCCGCC CCGTTGACGC AAATGGGCGG TAGGCGTGTA
 701  CGGTGGGAGG TCTATATAAG CAGAGCTCGT TTAGTGAACC GTCAGATCAC
 751  TAGAAGCTTT ATTGCGGTAG TTTATCACAG TTAAATTGCT AACGCAGTCA
 801  GTGCTTCTGA CACAACAGTC TCGAACTTAA GCTGCAGAAG TTGGTCGTGA
 851  GGCACTGGGC AGGTAAGTAT CAAGGTTACA AGACAGGTTT AAGGAGACCA
 901  ATAGAAACTG GGCTTGTCGA GACAGAGAAG ACTCTTGCGT TTCTGATAGG
 951  CACCTATTGG TCTTACTGAC ATCCACTTTG CCTTTCTCTC CACAGGTGTC
1001  CACTCCCAGT TCAATTACAG CTCTTAAGGC TAGAGTACTT AATACGACTC
1051  ACTATAGGCT AGCCACCATG ACTTCGAAAG TTTATGATCC AGAACAAAGG
1101  AAACGGATGA TAACTGGTCC GCAGTGGTGG GCCAGATGTA AACAAATGAA
1151  TGTTCTTGAT TCATTTATTA ATTATTATGA TTCAGAAAAA CATGCAGAAA
1201  ATGCTGTTAT TTTTTTACAT GGTAACGCGG CCTCTTCTTA TTTATGGCGA
1251  CATGTTGTGC CACATATTGA GCCAGTAGCG CGGTGTATTA TACCAGACCT
1301  TATTGGTATG GGCAAATCAG GCAAATCTGG TAATGGTTCT TATAGGTTAC
1351  TTGATCATTA CAAATATCTT ACTGCATGGT TTGAACTTCT TAATTTACCA
1401  AAGAAGATCA TTTTTGTCGG CCATGATTGG GGTGCTTGTT TGGCATTTCA
1451  TTATAGCTAT GAGCATCAAG ATAAGATCAA AGCAATAGTT CACGCTGAAA
1501  GTGTAGTAGA TGTGATTGAA TCATGGGATG AATGGCCTGA TATTGAAGAA
1551  GATATTGCGT TGATCAAATC TGAAGAAGGA GAAAAAATGG TTTTGGAGAA
1601  TAACTTCTTC GTGGAAACCA TGTTGCCATC AAAAATCATG AGAAAGTTAG
1651  AACCAGAAGA ATTTGCAGCA TATCTTGAAC CATTCAAAGA GAAAGGTGAA
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1701  GTTCGTCGTC CAACATTATC ATGGCCTCGT GAAATCCCGT TAGTAAAAGG
1751  TGGTAAACCT GACGTTGTAC AAATTGTTAG GAATTATAAT GCTTATCTAC
1801  GTGCAAGTGA TGATTTACCA AAAATGTTTA TTGAATCGGA CCCAGGATTC
1851  TTTTCCAATG CTATTGTTGA AGGTGCCAAG AAGTTTCCTA ATACTGAATT
1901  TGTCAAAGTA AAAGGTCTTC ATTTTTCGCA AGAAGATGCA CCTGATGAAA
1951  TGGGAAAATA TATCAAATCG TTCGTTGAGC GAGTTCTCAA AAATGAACAA
2001  TAATTCTAGA GCGGCCGCTT CGAGCAGACA TGATAAGATA CATTGATGAG
2051  TTTGGACAAA CCACAACTAG AATGCAGTGA AAAAAATGCT TTATTTGTGA
2101  AATTTGTGAT GCTATTGCTT TATTTGTAAC CATTATAAGC TGCAATAAAC
2151  AAGTTAACAA CAACAATTGC ATTCATTTTA TGTTTCAGGT TCAGGGGGAG
2201  GTGTGGGAGG TTTTTTAAAG CAAGTAAAAC CTCTACAAAT GTGGTAAAAT
2251  CGATAAGGAT CCAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA
2301  TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA
2351  TAACCCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT
2401  TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC
2451  CTGTTTTTGC TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT
2501  CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA
2551  GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTTTCCAATG ATGAGCACTT
2601  TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTATTGA CGCCGGGCAA
2651  GAGCAACTCG GTCGCCGCAT ACACTATTCT CAGAATGACT TGGTTGAGTA
2701  CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT
2751  TATGCAGTGC TGCCATAACC ATGAGTGATA ACACTGCGGC CAACTTACTT
2801  CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT
2851  GGGGGATCAT GTAACTCGCC TTGATCGTTG GGAACCGGAG CTGAATGAAG
2901  CCATACCAAA CGACGAGCGT GACACCACGA TGCCTGTAGC AATGGCAACA
2951  ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA
3001  ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC
3051  GCTCGGCCCT TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT
3101  GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC
3151  CTCCCGTATC GTAGTTATCT ACACGACGGG GAGTCAGGCA ACTATGGATG
3201  AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG
3251  TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAAACT
3301  TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA
3351  TGACCAAAAT CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTCAGACCCC
3401  GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT
3451  CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG GTTTGTTTGC
3501  CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA
3551  GCGCAGATAC CAAATACTGT TCTTCTAGTG TAGCCGTAGT TAGGCCACCA
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3601  CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT
3651  TACCAGTGGC TGCTGCCAGT GGCGATAAGT CGTGTCTTAC CGGGTTGGAC
3701  TCAAGACGAT AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG
3751  TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC GAACTGAGAT
3801  ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG
3851  GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG
3901  GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC
3951  GCCACCTCTG ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG
4001  AGCCTATGGA AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT
4051  TTGCTGGCCT TTTGCTCACA TGGCTCGAC
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VI. Related Products

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

Product	Size	Cat.#
pRL-TK Vector[a,c]	20µg	E2241
pRL-SV40 Vector[a,c]	20µg	E2231
pRL-null Vector[a,c]	20µg	E2271

To inquire about the availability of bulk packaging and pricing for individual pRL Vectors, please contact Promega. Please visit our Internet site (www.promega.com) or call Promega Technical Services to inquire about the availability of new promoter variations within the pRL family of co-reporter vectors.

Luciferase Assay

Product	Size	Cat.#
Dual-Luciferase™ Reporter Assay System[c,d,e]	1 each	E1910
Dual-Luciferase™ Reporter Assay 10-Pack[c,d, e]	1,000 assays	E1960
Dual-Luciferase™ Reporter 1000 Assay System[c,d, e]	1,000 assays	E1980

Transfection Systems

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

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VII. References

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