

pRL-SV40 Vector



Technical Bulletin No. 239

INSTRUCTIONS FOR USE OF PRODUCT E2231. PLEASE DISCARD PREVIOUS VERSIONS.

All technical literature is available on the Internet at www.promega.com

Please visit the web site to verify that you are using the most current version of this Technical Bulletin.

I. Description	1
II. Product Components	2
III. Features of the pRL-SV40 Vector	3
A. SV40 Enhancer/Promoter Regions	3
B. Chimeric Intron	3
C. T7 Promoter.....	3
D. <i>Renilla</i> Luciferase Reporter Gene (<i>Rluc</i>).....	3
E. SV40 Late Polyadenylation Signal.....	3
IV. Transfection of Mammalian Cells with pRL-SV40	4
V. pRL-SV40 Vector Restriction Sites and Sequence	4
A. pRL-SV40 Vector Restriction Sites	4
B. pRL-SV40 Vector Sequence	6
VI. Related Products	8
VII. References	9

I. Description

The pRL-SV40 Vector^(a,b) (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^(b) contained within the pRL Vectors has been modified slightly to provide greater utility.

The pRL-SV40 Vector contains the SV40 enhancer and early promoter elements to provide high-level expression of *Renilla* luciferase in cotransfected 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) Technical Manual (#TM040).

The pRL Vectors are isolated from a *dam*⁻/*dcm*⁻ *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-SV40 Vector is AF025845.



AF91BE239 0701TB239

II. Product Components

Product	Size	Cat.#
pRL-SV40 Vector	20µg	E2231

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 .

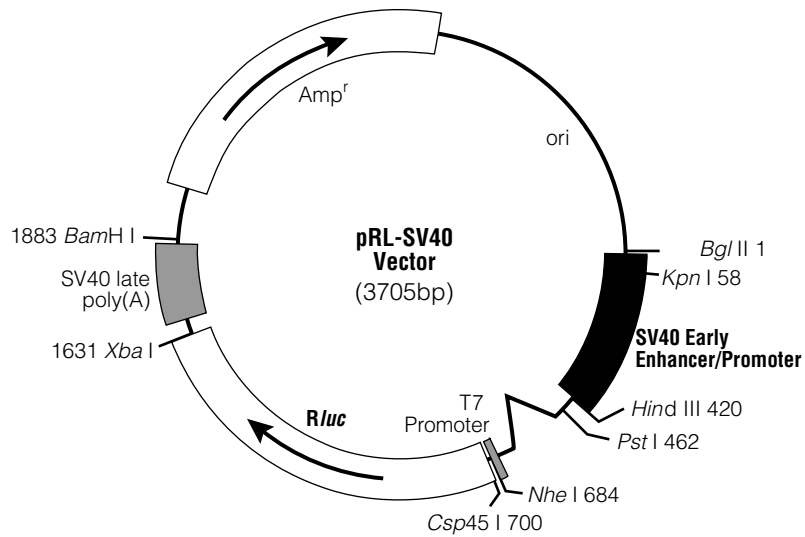


Figure 1. The pRL-SV40 Vector circle map and sequence reference points.

Sequence reference points:

SV40 enhancer and early promoter	7–418
chimeric intron	486–622
T7 RNA polymerase promoter (–17 to +2)	666–684
T7 RNA polymerase transcription initiation site	683
Rluc reporter gene	694–1629
SV40 late polyadenylation signal	1671–1872
β -lactamase (Amp ^r) coding region	2019–2879

In addition:

- \frown indicates the position of the intron.
- Rluc is the cDNA encoding the *Renilla* luciferase enzyme.
- Amp^r 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^r genes indicate the direction of transcription.

III. Features of the pRL-SV40 Vector

A. SV40 Enhancer/Promoter Regions

The pRL-SV40 Vector contains the SV40 enhancer/promoter region, which provides strong, constitutive expression of *Rluc* in a variety of cell types. The vector also contains the SV40 origin of replication, which allows transient, episomal replication in cells expressing the SV40 large T antigen, such as COS-1 or COS-7 cells (3).

B. Chimeric Intron

Downstream of the SV40 enhancer/promoter region of the pRL-SV40 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-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 (10).

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^(c,e,f,g) [Cat.# L4610], Wheat Germ Extract^(c,e,f,g) [Cat.# L4140] or TNT[®] T7 Quick Coupled Transcription/Translation^(c,e,f,g,h) [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-SV40 Vector: base 924 (T→C) to eliminate an internal *Bam*H I site; base 1500 (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 (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 five-fold more than the early SV40 polyadenylation signal (14), has been positioned 3' to the *Rluc* gene in the pRL-SV40 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, this primer cannot be used for sequencing with this vector.

IV. Transfection of Mammalian Cells with pRL-SV40

The pRL-SV40 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 (15). 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 cell type 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 ≥ 10 femtograms of *Renilla* luciferase, with linearity over 7 orders of enzyme concentration. Therefore, it is possible to use relatively small quantities of pRL-SV40 Vector to provide low-level, constitutive coexpression of *Renilla* luciferase control activity. Ratios of 10:1 to 50:1 (or greater) for experimental vector:pRL-SV40 Vector combinations are feasible and may aid greatly in suppressing the occurrence of *trans* effects between promoter elements.

The pRL-SV40 Vector can be used for both transient and stable expression of genes. For stable expression, the pRL-SV40 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 (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 (TransFast™ Reagent⁽ⁱ⁾, Tfx™ Reagents⁽ⁱ⁾ and Transfectam® Reagent^(k)), 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 the 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-SV40 Vector Restriction Sites and Sequence

A. pRL-SV40 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-SV40 Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
Acc65 I	1	54	<i>Eag I</i>	1	1638
Acy I	1	2266	<i>Ear I</i>	2	864, 2007
<i>Afl II</i>	2	452, 649	EclHK I	1	2806
<i>Afl III</i>	1	876	Eco52 I	1	1638
Alw26 I	5	449, 514, 539, 1964, 2740	<i>Fsp I</i>	2	8, 2583
Alw44 I	2	2134, 3380	Hae II	1	3454
<i>AlwN I</i>	1	3285	<i>Hga I</i>	3	2274, 3004, 3582
<i>AspH I</i>	3	2138, 2223, 3384	Hinc II	1	1781
Ava II	4	742, 1464, 2442 2664	<i>Hind II</i>	1	1781
<i>Avr II</i>	1	404	Hind III	1	420
BamH I	1	1883	Hpa I	1	1781
Ban I	4	54, 575, 1498 2853	Hsp92 I	1	2266
<i>Bbs I</i>	2	560, 1534	Kpn I	1	58
Bbu I	2	152, 224	<i>Mae II</i>	5	1388, 1425, 2204, 2577, 2993
Bcl I	2	978, 1187	MspA1 I	4	80, 2170, 3111, 3356
Bgl I	2	357, 2688	<i>Nci I</i>	3	2270, 2621, 3317
Bgl II	1	1	Nco I	2	15, 311
<i>Bsa I</i>	2	514, 2740	Nhe I	1	684
BsaO I	4	1641, 2288, 2437, 3360	Not I	1	1638
<i>BsaA I</i>	1	1426	Nsi I	2	154, 226
<i>BsaB I</i>	1	1882	<i>Nsp I</i>	4	152, 224, 820, 880
<i>BsaH I</i>	1	2266	<i>PaeR7 I</i>	2	542, 3700
BsaM I	2	1702, 1795	<i>Ple I</i>	4	550, 666, 2815, 3318
<i>Bsm I</i>	2	1702, 1795	<i>Ppu10 I</i>	2	150, 222
Bsp1286 I	3	2138, 2223, 3384	Pst I	1	462
<i>BspH I</i>	3	1262, 1966, 2974	Pvu I	1	2437
<i>BspM I</i>	1	476	Pvu II	1	80
BsrBR I	1	1882	Rsa I	4	56, 662, 1394, 2325
<i>BsrG I</i>	1	1392	Sca I	2	662, 2325
<i>BssS I</i>	3	1352, 2137, 3521	Sfi I	1	357
Bst98 I	2	452, 649	Sin I	4	742, 1464, 2442, 2664
BstZ I	1	1638	Sph I	2	152, 224
<i>Cfr10 I</i>	1	2721	Ssp I	1	2001
Cla I	1	1876	Stu I	1	403
Csp45 I	1	700	Sty I	3	15, 311, 404
Dra I	4	1842, 2228, 2920, 2939	Vsp I	2	794, 2631
<i>Drd I</i>	2	441, 3592	Xba I	1	1631
<i>Dsa I</i>	2	15, 311	<i>Xcm I</i>	1	1343
<i>Eae I</i>	3	1044, 1638, 2413	Xmn I	2	1228, 2206

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

Table 2. Restriction Enzymes That Do Not Cut the pRL-SV40 Vector.

Aat II	<i>BbrP I</i>	<i>Dra III</i>	<i>Kas I</i>	<i>Pml I</i>	Sma I
Acc B7 I	<i>Blp I</i>	Eco47 III	Mlu I	<i>PpuM I</i>	SnaB I
Acc I	<i>Bpu 1102 I</i>	<i>Eco72 I</i>	Nae I	<i>PshA I</i>	Spe I
Acc III	<i>Bsp120 I</i>	<i>Eco81 I</i>	Nar I	<i>Psp5 II</i>	<i>Spl I</i>
Age I	BssH II	EcoCR I	Nde I	<i>PspA I</i>	<i>Srf I</i>
Apa I	<i>Bst1107 I</i>	<i>EcoN I</i>	NgoM IV	<i>Rsr II</i>	<i>Sse8387 I</i>
<i>Asc I</i>	BstE II	EcoR I	Nru I	Sac I	<i>Swa I</i>
Ava I	BstX I	EcoR V	<i>Pac I</i>	Sac II	Tth111 I
Bal I	Bsu36 I	<i>Ehe I</i>	<i>PfiM I</i>	Sal I	Xho I
Ban II	Csp I	<i>Fse I</i>	<i>PinA I</i>	Sgf I⁽¹⁾	Xma I
<i>Bbe I</i>	<i>Dra II</i>	I-Ppo I	<i>Pme I</i>	<i>SgrA I</i>	

Table 3. Restriction Enzymes That Cut the pRL-SV40 Vector 6 or More Times.

<i>Aci I</i>	BstO I	Fok I	<i>Mae I</i>	Nde II	Taq I
Alu I	<i>BstU I</i>	Hae III	<i>Mae III</i>	<i>Nla III</i>	<i>Tfi I</i>
<i>Bbv I</i>	Cfo I	Hha I	Mbo I	<i>Nla IV</i>	Tru9 I
<i>BsaJ I</i>	Dde I	Hinf I	Mbo II	Sau3A I	Xho II
<i>Bsr I</i>	Dpn I	Hpa II	<i>Mnl I</i>	Sau96 I	
Bsr S I	<i>Dpn II</i>	<i>Hph I</i>	<i>Mse I</i>	<i>ScrF I</i>	
Bst71 I	<i>Fnu4H I</i>	Hsp92 II	Msp I	<i>SfaN I</i>	

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

B. pRL-SV40 Vector Sequence

The sequence shown corresponds to the mRNA synthesized from the *Renilla* luciferase gene from the SV40 promoter. The vector sequence is also available on the Internet at www.promega.com/vectors. The GenBank®/EMBL Accession number for the pRL-SV40 Vector is AF025845.

```

1  AGATCTGCGC AGCACCATGG CCTGAAATAA CCTCTGAAAG AGGAACTTGG
51  TTAGGTACCT TCTGAGGCGG AAAGAACCAG CTGTGGAATG TGTGTCAGTT
101 AGGGTGTGGA AAGTCCCAG GCTCCCAGC AGGCAGAAGT ATGCAAAGCA
151 TGCATCTCAA TTAGTCAGCA ACCAGGTGTG GAAAGTCCCC AGGCTCCCCA
201 GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCATAGT
251 CCCGCCCTA ACTCCGCCCA TCCCGCCCCT AACTCCGCC AGTTCGCCCC
301 ATTCTCCGCC CCATGGCTGA CTAATTTTTT TTATTTATGC AGAGGCCGAG
351 GCCGCCTCGG CCTCTGAGCT ATTCCAGAAG TAGTGAGGAG GCTTTTTTTGG
401 AGGCCTAGGC TTTTGCAAAA AGCTTGATTC TTCTGACACA ACAGTCTCGA
451 ACTTAAGCTG CAGAAGTTGG TCGTGAGGCA CTGGGCAGGT AAGTATCAAG
501 GTTACAAGAC AGGTTTAAGG AGACCAATAG AACTGGGCT TGTCGAGACA
551 GAGAAGACTC TTGCGTTTCT GATAGGCACC TATTGGTCTT ACTGACATCC
601 ACTTTGCCTT TCTCTCCACA GGTGTCCACT CCCAGTTCAA TTACAGCTCT
651 TAAGGCTAGA GTACTTAATA CGACTCACTA TAGGCTAGCC ACCATGACTT
701 CGAAAGTTTA TGATCCAGAA CAAAGGAAAC GGATGATAAC TGGTCCGCAG
751 TGGTGGGCCA GATGTAAACA AATGAATGTT CTTGATTCAT TTATTAATTA

```

B. Sequence of the pRL-SV40 Vector (continued)

```

801  TTATGATTCA  GAAAAACATG  CAGAAAATGC  TGTTATTTTT  TTACATGGTA
851  ACGCGGCCTC  TTCTTATTTA  TGGCGACATG  TTGTGCCACA  TATTGAGCCA
901  GTAGCGCGGT  GTATTATACC  AGACCTTATT  GGTATGGGCA  AATCAGGCAA
951  ATCTGGTAAT  GGTTCCTTATA  GGTTACTTGA  TCATTACAAA  TATCTTACTG
1001 CATGGTTTGA  ACTTCCTTAAT  TTACCAAAGA  AGATCATTFT  TGTCGGCCAT
1051 GATTGGGGTG  CTTGTTTGGC  ATTTCAATTAT  AGCTATGAGC  ATCAAGATAA
1101 GATCAAAGCA  ATAGTTCACG  CTGAAAGTGT  AGTAGATGTG  ATTGAATCAT
1151 GGGATGAATG  GCCTGATATT  GAAGAAGATA  TTGCGTTGAT  CAAATCTGAA
1201 GAAGGAGAAA  AAATGGTTTT  GGAGAATAAC  TTCTTCGTGG  AAACCATGTT
1251 GCCATCAAAA  ATCATGAGAA  AGTTAGAACC  AGAAGAATTT  GCAGCATATC
1301 TTGAACCATT  CAAAGAGAAA  GGTGAAGTTC  GTCGTCCAAC  ATTATCATGG
1351 CCTCGTGAAA  TCCCGTTAGT  AAAAGGTGGT  AAACCTGACG  TTGTACAAAT
1401 TGTTAGGAAT  TATAATGCTT  ATCTACGTGC  AAGTGATGAT  TTACCAAAAA
1451 TGTTTATTGA  ATCGGACCCA  GGATTCTTTT  CCAATGCTAT  TGTTGAAGGT
1501 GCCAAGAAGT  TTCCTAATAC  TGAATTTGTC  AAAGTAAAAG  GTCTTCATTT
1551 TTCGCAAGAA  GATGCACCTG  ATGAAATGGG  AAAATATATC  AAATCGTTCG
1601 TTGAGCGAGT  TCTCAAAAAT  GAACAATAAT  TCTAGAGCGG  CCGCTTCGAG
1651 CAGACATGAT  AAGATACATT  GATGAGTTTG  GACAAACCAC  AACTAGAATG
1701 CAGTGAAAAA  AATGCTTTAT  TTGTGAAATT  TGTGATGCTA  TTGCTTTATT
1751 TGTAACCATT  ATAAGCTGCA  ATAAACAAGT  TAACAACAAC  AATTGCATTC
1801 ATTTTATGTT  TCAGGTTTCA  GGGGAGGTGT  GGGAGGTTTT  TTAAAGCAAG
1851 TAAAACCTCT  ACAAATGTGG  TAAAATCGAT  AAGGATCCAG  GTGGCACTTT
1901 TCGGGGAAAT  GTGCGCGGAA  CCCCTATTTG  TTTATTTTTT  TAAATACATT
1951 CAAATATGTA  TCCGCTCATG  AGACAATAAC  CCTGATAAAT  GCTTCAATAA
2001 TATTGAAAAA  GGAAGAGTAT  GAGTATTCAA  CATTTCCGTG  TCGCCCTTAT
2051 TCCCTTTTTT  GCGGCATTTT  GCCTTCCTGT  TTTTGCTCAC  CCAGAAACGC
2101 TGGTGAAAGT  AAAAGATGCT  GAAGATCAGT  TGGGTGCACG  AGTGGGTTAC
2151 ATCGAACTGG  ATCTCAACAG  CGGTAAGATC  CTTGAGAGTT  TTCGCCCGA
2201 AGAACGTTTT  CCAATGATGA  GCACTTTTAA  AGTTC TGCTA  TGTGGCGCGG
2251 TATTATCCCG  TATTGACGCC  GGGCAAGAGC  AACTCGGTCT  CCGCATACAC
2301 TATTCTCAGA  ATGACTTGGT  TGAGTACTCA  CCAGTCACAG  AAAAGCATCT
2351 TACGGATGGC  ATGACAGTAA  GAGAATTATG  CAGTGCTGCC  ATAACCATGA
2401 GTGATAACAC  TGCGGCCAAC  TTTACTTCTGA  CAACGATCGG  AGGACCGAAG
2451 GAGCTAACCG  CTTTTTTTGA  CAACATGGGG  GATCATGTAA  CTCGCCTTGA
2501 TCGTTGGGAA  CCGGAGCTGA  ATGAAGCCAT  ACCAAACGAC  GAGCGTGACA
2551 CCACGATGCC  TGTAGCAATG  GCAACAACGT  TGCGCAAAC  ATTAACTGGC
2601 GAACTACTTA  CTCTAGCTTC  CCGGCAACAA  TTAATAGACT  GGATGGAGGC
2651 GGATAAAGTT  GCAGGACCAC  TTCTGCGCTC  GGCCCTTCCG  GCTGGCTGGT

```

B. Sequence of the pRL-SV40 Vector (continued)

```

2701 TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT
2751 GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC
2801 GACGGGGAGT CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA
2851 TAGGTGCCTC ACTGATTAAG CATTGGTAAAC TGTCAGACCA AGTTTACTCA
2901 TATATACTTT AGATTGATTT AAAACTTCAT TTTTAATTTA AAAGGATCTA
2951 GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT
3001 TTTTCGTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT
3051 TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAA CAAAAAACC
3101 ACCGCTACCA GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT
3151 TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAA TACTGTTCTT
3201 CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCGCC
3251 TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT GCCAGTGGCG
3301 ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG
3351 GCGCAGCGGT CGGGCTGAAC GGGGGGTTTCG TGCACACAGC CCAGCTTGGA
3401 GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CTATGAGAAA
3451 GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC
3501 AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCTG
3551 GTATCTTTAT AGTCCTGTCG GGTTCGCCA CCTCTGACTT GAGCGTCGAT
3601 TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGGAAAAA CGCCAGCAAC
3651 GCGGCCTTTT TACGGTTCCT GGCTTTTTC TGGCCTTTTG CTCACATGGC
3701 TCGAC

```

VI. Related Products

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

Product	Size	Cat.#
pRL-TK Vector(a,b)	20µg	E2241
pRL-CMV Vector(a,b,m)	20µg	E2261
pRL-null Vector(a,b)	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.

pGL-3 Vectors

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

VI. Related Products (continued)

Luciferase Assay Systems

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

Transfection Systems

Product	Size	Cat.#
TransFast™ Transfection Reagent	1.2mg	E2431
Transfectam® Reagent for the Transfection of Eukaryotic Cells	1mg	E1231
	0.5mg	E1232
Tfx™-50 Reagent	2.1mg	E1811
Tfx™-10 Reagent	9.3mg	E2381
Tfx™-20 Reagent	4.8mg	E2391
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

VII. References

- Lorenz, W.W. *et al.* (1991) Isolation and expression of a cDNA encoding *Renilla reniformis* luciferase. *Proc. Natl. Acad. Sci. USA* **88**, 4438–42.
- Matthews, J.C. *et al.* (1977) Substrate and substrate analogue binding properties of *Renilla* luciferase. *Biochemistry* **16**, 85–91.
- Gluzman, Y. (1981) SV40-transformed simian cells support the replication of early SV40 mutants. *Cell* **23**, 175–82.
- Bothwell, A.L.M. *et al.* (1981) Heavy chain variable region contribution to the NPb family of antibodies: somatic mutation evident in a gamma 2a variable region. *Cell* **24**, 625–37.
- Senapathy, P., Shapiro, M.B. and Harris, N.L. (1990) Splice junctions, branch point sites, and exons: sequence statistics, identification, and applications to genome project. *Meth. Enzymol.* **183**, 252–78.
- Gross, M.K., Kainz, M.S. and Merrill, G.F. (1987) Introns are inconsequential to efficient formation of cellular thymidine kinase mRNA in mouse L cells. *Mol. Cell. Biol.* **7**, 4576–81.
- Buchman, A.R. and Berg, P. (1988) Comparison of intron-dependent and intron-independent gene expression. *Mol. Cell. Biol.* **8**, 4395–405.
- Evans, M.J. and Scarpulla, R.C. (1989) Introns in the 3′-untranslated region can inhibit chimeric CAT and beta-galactosidase gene expression. *Gene* **84**, 135–42.
- Huang, M.T.F. and Gorman, C.M. (1990) Intervening sequences increase efficiency of RNA 3′ processing and accumulation of cytoplasmic RNA. *Nucl. Acids Res.* **18**, 937–47.
- Huang, M.T.F. and Gorman, C.M. (1990) The simian virus 40 small-t intron, present in many common expression vectors, leads to aberrant splicing. *Mol. Cell. Biol.* **10**, 1805–10.

11. Proudfoot, N.J. (1991) Poly(A) signals. *Cell* **64**, 671–4.
12. Bernstein, P. and Ross, J. (1989) Poly(A), poly(A) binding protein and the regulation of mRNA stability. *Trends Biochem. Sci.* **14**, 373–7.
13. Jackson, R.J. and Standart, N. (1990) Do the poly(A) tail and 3' untranslated region control mRNA translation? *Cell* **62**, 15–24.
14. Carswell, S. and Alwine, J.C. (1989) Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell. Biol.* **9**, 4248–58.
15. Farr, A. and Roman, A. (1991) A pitfall of using a second plasmid to determine transfection efficiency. *Nucl. Acids Res.* **20**, 920.
16. Behr, J.P. *et al.* (1989) Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA. *Proc. Natl. Acad. Sci. USA* **86**, 6982–6.
17. Loeffler, J.P. *et al.* (1990) Lipopolyamine-mediated transfection allows gene expression studies in primary neuronal cells. *J. Neurochem.* **54**, 1812–15.
18. Graham, F.L. and van der Eb, A.J. (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**, 456–67.
19. Wigler, M. *et al.* (1977) Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells. *Cell* **11**, 223–32.
20. McCutchan, J.H. and Pagano, J.S. (1968) Enhancement of the infectivity of simian virus 40 deoxyribonucleic acid with diethylaminoethyl-dextran. *J. Natl. Cancer Inst.* **41**, 351–7.
21. Al-Moslih, M.I. and Dubes, G.R. (1973) The kinetics of DEAE-dextran-induced cell sensitization to transfection. *J. Gen. Virol.* **18**, 189–93.
22. Luthman, H. and Magnusson, G. (1983) High efficiency polyoma DNA transfection of chloroquine treated cells. *Nucl. Acids Res.* **11**, 1295–308.
23. Kawai, S. and Nishizawa, M. (1984) New procedure for DNA transfection with polycation and dimethyl sulfoxide. *Mol. Cell. Biol.* **4**, 1172–4.
24. Aubin, R.J., Weinfeld, M. and Paterson, M.C. (1988) Factors influencing efficiency and reproducibility of polybrene-assisted gene transfer. *Som. Cell Mol. Genet.* **14**, 155–67.
25. Andreason, G.L. and Evans, G.A. (1988) Introduction and expression of DNA molecules in eukaryotic cells by electroporation. *BioTechniques* **6**, 650–60.
26. Neumann, E. *et al.* (1982) Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO J.* **1**, 841–5.

- (a) Certain applications of this product may require licenses from others.
- (b) Licensed under U.S. Pat. Nos. 5,292,658, 5,418,155 and other patents.
- (c) U.S. Pat. No. 5,283,179, Australian Pat. No. 649289 and other patents. Certain applications of this product may require licenses from others.
- (d) U.S. Pat. No. 5,744,320.
- (e) U.S. Pat. Nos. 5,492,817, 5,665,563, Australian Pat. No. 660329 and other patents.
- (f) The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.
- (g) U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.
- (h) U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.
- (i) The cationic lipid component of the TransFast™ Transfection Reagent is covered by U.S. Pat. Nos. 5,824,812, 5,869,715 and pending foreign patents.
- (j) The cationic lipid component of the Tfx™ Reagents is covered by U.S. Pat. Nos. 5,527,928, 5,744,625 and 5,892,071, Australian Pat. No. 704189 and other pending foreign patents.
- (k) Transfectam is a registered trademark of BioSeptra, S.A., the holder of a license from CNRS-ULP Strasbourg under U.S. Pat. No. 5,171,678 to sell the Transfectam® product for research purposes only. The Transfectam® product was developed by J.P. Behr and J.P. Loeffler and is covered by the aforementioned patent.
- (l) U.S. Pat. No. 5,391,487.
- (m) The CMV promoter and its use are covered under U.S. Pat. Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation, Iowa City, Iowa, and licensed FOR RESEARCH USE ONLY. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.
- (n) U.S. Pat. No. 5,670,356.

© 1996–2001 Promega Corporation. All Rights Reserved.

Dual-Luciferase, ProFection, TNT and Transfectam are trademarks of Promega Corporation and are registered with the U.S. Patent and Trademark Office. Tfx and TransFast are trademarks of Promega Corporation.

DNASTAR is a registered trademark of DNASTAR, Inc. GenBank is a registered trademark of the U.S. Department of Health and Human Services.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.



Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399 USA	
Telephone	608-274-4330
Fax	608-277-2516
Internet	www.promega.com
