Technical Manual

pGL2 Luciferase Reporter Vectors

INSTRUCTIONS FOR USE OF PRODUCTS E1611, E1621, E1631 AND E1641.

PRINTED IN USA.
Revised 2/09

Part# TM003

Promega

pGL2 Luciferase Reporter Vectors

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Please visit the web site to verify that you are using the most current version of this Technical Manual. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com**

1. Description

The pGL2 Luciferase Reporter Vectors[a] are designed for the quantitative analysis of factors that potentially regulate mammalian gene expression. These factors may be *cis*-acting, such as promoters and enhancers, or *trans*-acting, such as various DNA-binding factors. The pGL2 Vectors carry the coding region for wildtype firefly (*Photinus pyralis*) luciferase, used to monitor transcriptional activity in transfected eukaryotic cells. The assay of this genetic reporter is rapid, sensitive and quantitative. In addition, the pGL2 Vectors contain numerous features that aid the characterization and mutagenesis of the putative regulatory sequences.

2. Product Components and Storage Conditions

Product	Size	Cat.#
pGL2-Control Vector	20µg	E1611
pGL2-Enhancer Vector	20µg	E1621
pGL2-Promoter Vector	20µg	E1631
pGL2-Basic Vector	20µg	E1641

Information on related products, including the Luciferase Assay System, is provided in Sections 5, 6 and 7.H.

Storage Conditions: Store the vector DNA at –20°C.

3. pGL2 Vector Maps and Sequence Reference Points

This listings of restriction sites for the pGL2 Luciferase Reporter Vectors are provided in Sections 7.C–F.

3.A. pGL2 Basic Vector

The pGL2-Basic Vector lacks eukaryotic promoter and enhancer sequences, allowing maximum flexibility in cloning putative regulatory sequences. Expression of luciferase activity in cells transfected with this plasmid depends on insertion and proper orientation of a functional promoter upstream from *luc*. Potential enhancer elements can also be inserted upstream of the promoter or in the BamHI or SalI sites downstream of the luciferase gene.

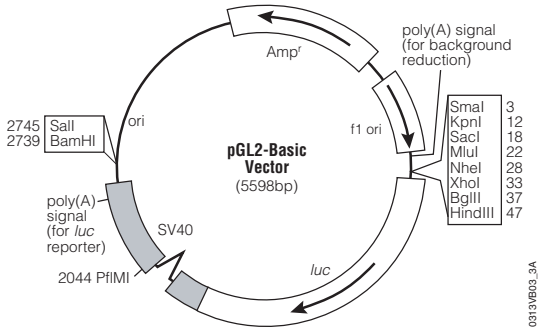

Figure 1. pGL2-Basic Vector map.

pGL2-Basic Vector Sequence Reference Points:

SV40 Promoter	(none)
SV40 Enhancer	(none)
Multiple cloning region	1–53
GLprimer2 binding site	77–99
Luciferase gene (*luc*)	76–1728
SV40 late poly(A) signal	2518–2739
RVprimer4 binding site	2796–2815
β-lactamase (Amp^r) gene	3815–4675
ColE1-derived plasmid replication origin	3053
f1 origin	4807–5262
GLprimer1 binding site	5565–5587

3.B. pGL2-Promoter Vector

The pGL2-Promoter Vector contains the SV40 promoter upstream of the luciferase gene. DNA fragments containing putative enhancer elements can be inserted in either orientation and upstream or downstream of the promoter-*luc* transcriptional unit.

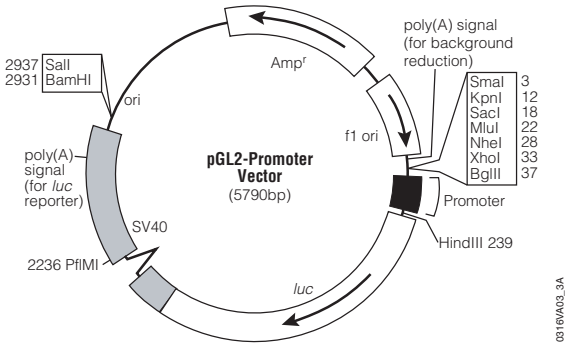

Figure 2. pGL2-Promoter Vector map.

pGL2-Promoter Vector Sequence Reference Points:

Multiple cloning region	1–49
SV40 Promoter	42–244
Luciferase gene (*luc*)	268–1920
GLprimer2 binding site	269–291
RVprimer4 binding site	2988–3007
ColE1-derived plasmid replication origin	3245
β-lactamase (Amp^r) gene	4007–4867
f1 origin	4999–5454
GLprimer1 binding site	5757–5779

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3.C. pGL2-Enhancer Vector

The pGL2-Enhancer Vector contains an SV40 enhancer, derived from the SV40 early promoter, located downstream of the luciferase gene after the poly(A) signal. This aids in the verification of functional promoter elements because the presence of the enhancer in many cases will result in transcription of *luc* at higher levels.

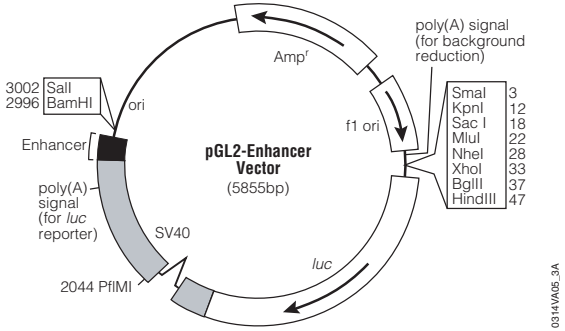

Figure 3. pGL2-Enhancer Vector map.

pGL2-Enhancer Vector Sequence Reference Points:

Multiple cloning region	1–52
Luciferase gene (*luc*)	76–1728
GLprimer2 binding site	77–99
SV40 late poly(A) signal	2518–2739
SV40 Enhancer	2748–2984
RVprimer4 binding site	3053–3072
ColE1-derived plasmid replication origin	3310
β-lactamase (Amp^r) gene	4072–4932
f1 origin	5064–5519
GLprimer1 binding site	5822–5844

3.D. pGL2-Control Vector

The pGL2-Control Vector contains the SV40 promoter and enhancer sequences, resulting in strong *luc* expression in many types of mammalian cells. This plasmid is useful in monitoring transfection efficiency in general and is a convenient internal standard for promoter and enhancer activities expressed by pGL2 recombinants.

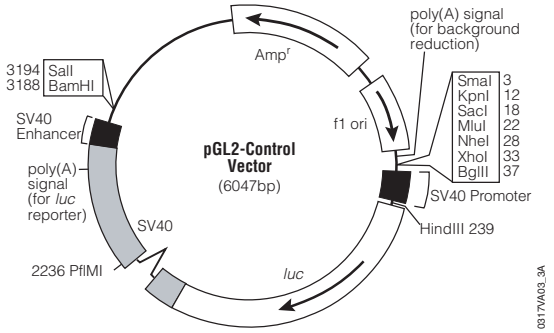

Figure 4. pGL2-Control Vector map.

pGL2-Control Vector Sequence Reference Points:

SV40 Promoter	42–244
Luciferase gene (*luc*)	268–1920
GLprimer2 binding site	269–291
SV40 late poly(A) signal	2710–2931
SV40 Enhancer	2940–3176
RVprimer4 binding site	3245–3264
ColE1-derived plasmid replication origin	3502
β-lactamase (Amp^r) gene	4264–5124
f1 origin	5256–5711
GLprimer1 binding site	6014–6036

3.D. pGL2-Control Vector (continued)

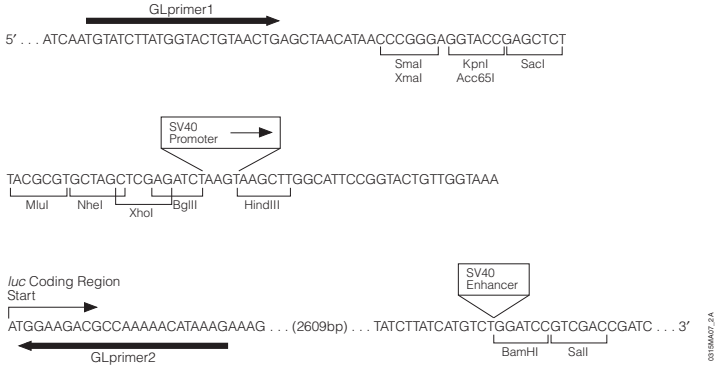

Figure 5. pGL2 Vector multiple cloning regions. Shown are the upstream and downstream cloning regions and the locations of the sequencing primers, GLprimer1 and GLprimer2. The arrows for the primers indicate the direction of sequencing. The positions of the promoter (in the pGL2-Promoter and pGL2-Control Vectors) and the enhancer (in the pGL2-Enhancer and pGL2-Control Vectors) are shown as insertions into the sequence of the pGL2-Basic Vector. (Note that the promoter replaces four bases of the pGL2-Basic Vector.) The sequence shown is of the DNA strand synthesized by the f1 ori.

4. Vector Manipulation

4.A. Cloning Strategies

For most applications, we recommend cloning into the XhoI or BglII sites upstream of *luc* or the SalI and BamHI sites downstream. XhoI and SalI have compatible ends, as do BglII and BamHI, which allow easy interchange of inserts between upstream and downstream positions relative to the luciferase gene. Thus, positional effects of a putative genetic element may be readily tested. Cloning fragments into a single site will generally yield both possible orientations relative to the reporter gene, making these effects also readily testable.

The other upstream restriction sites may be used for cloning; however, note that some of the sites are required for generation of nested deletions (see Section 4.B). Specifically, the KpnI or SacI site is needed to generate a 3′-overhang, and the MluI, NheI or XhoI site is needed to generate a 5′-overhang. The HindIII site may be used for cloning, but it should be noted that this site occurs downstream of the promoter in the pGL2-Promoter and pGL2-Control Vectors.

The SmaI/XmaI site upstream of the restriction sites mentioned above can be used to insert other putative regulatory elements without compromising the ability to make nested deletions. It can also be used to aid in mapping nested deletions.

Because the pGL2 Vectors are supplied as modified DNA, *E. coli* hosts may be either restriction+ or restriction–. The use of a *rec*A host such as JM109 is preferred because this prevents undesirable recombination between the insert and the host chromosomal DNA. A strain that has an F′ episome is required for ssDNA production. Grow JM109 on minimal plates (M-9) supplemented with thiamine-HCl prior to preparation of competent cells and transformation. This selects for the presence of the F′ episome.

Protocols for restriction digestion, alkaline phosphatase treatment, linker ligation and transformation of competent cells can be found in reference 2.

4.B. Mapping Genetic Elements Located within DNA Fragments

The locations of functional elements within a DNA fragment are often determined by making a set of unidirectional deletions following the method of Henikoff (3) and assaying for biological activity. This method takes advantage of the unique properties of Exonuclease III (ExoIII), which will digest 5′-overhangs, but not 3′-overhangs or α-phosphorothioate nucleotide filled-in overhangs. Nested deletions of the insert can be made directly in the promoter regions of the pGL2-Promoter and pGL2-Control Luciferase Reporter Vectors using this method, eliminating the need for subcloning steps. The upstream MluI, NheI and XhoI sites, rare restriction sites in eukaryotic DNA, provide the potential 5′-starting points for ExoIII digestion at the upstream end of the insert; KpnI and SacI generate the 3′-overhangs resistant to ExoIII. After treatment with ExoIII, S1 nuclease is added to remove the resulting ssDNA, and T4 DNA ligase is added to reclose the vectors. Deletion clones can be screened by gel electrophoresis of miniprep DNA, and the precise deletion endpoints within the promoter region can be determined by DNA sequencing using primers designed for the pGL2 Vectors.

4.C. Sequencing

For some applications, it may be desirable to sequence the DNA inserted into the pGL2 Vectors. Two examples of such applications are to determine the exact position of generated deletions (see Section 4.B) and to confirm production of a site-specific mutation. Two primers are available for this purpose: GLprimer1 for sequencing clockwise across the upstream cloning sites, and GLprimer2 for sequencing counterclockwise across the cloning sites.

GLprimer1	5′-TGTATCTTATGGTACTGTAACTG-3′
GLprimer2	5′-CTTTATGTTTTTGGCGTCTTCCA-3′

GLprimer1 is especially useful for identifying positions of nested deletions. Note that both primers can be used for dsDNA sequencing, but GLprimer2 also may be used for ssDNA sequencing.

5. Transfection of Eukaryotic Cells

Transfection of DNA into eukaryotic cells may be mediated by cationic lipid compounds (4,5), calcium phosphate (6,7), DEAE-dextran (6,8) or electroporation (7). Transfection systems based on cationic lipids (TransFast™ Transfection Reagent, Transfectam® Reagent and Tfx™ Reagents), calcium phosphate and DEAE-dextran (Profection® Mammalian Transfection Systems) are available from Promega. For more information on these transfection reagents, please request the *TransFast™ Transfection Reagent Technical Bulletin* #TB260, the *Transfectam® Reagent Technical Bulletin* #TB116, the *Tfx™-Reagents Technical Bulletin* #TB216 or the *ProFection® Mammalian Transfection Systems Technical Manual* #TM012. All of these documents are available on our web site at: **www.promega.com/tbs/**

Note: The specific transcriptional characteristics of the pGL2 Vectors are likely to vary for different cell types. This may be particularly true for COS cells that contain the SV40 large T antigen. The SV40 large T antigen promotes replication of the SV40 origin, found in the promoter region of the pGL2-Promoter and pGL2-Control Vectors. The combination of large T antigen and SV40 origin will result in a higher copy number of these vectors in COS cells, which in turn may result in increased expression of the reporter gene compared to expression levels from vectors lacking the SV40 origin.

6. Assays of Luciferase Activity

Experimental strategies using firefly luciferase may involve the analysis of a few samples per day or as many as several thousand samples per hour, and equipment used to measure luminescence may vary from inexpensive, single-sample luminometers to high-end CCD luminometers. To support this wide range of applications, Promega has developed three luciferase assays with different but complementary characteristics: Luciferase Assay System (Cat.# E1500), Bright-Glo™ Luciferase Assay System (Cat.# E2610), Steady-Glo® Luciferase Assay System (Cat.# E2510), and ONE-Glo™ Luciferase Assay System (Cat.# E6110). Reagent choice depends on the relative importance of experimental format, assay sensitivity and luminescence duration.

Table 1. Characteristics of Promega Luciferase Assay Reagents.

	Bright-Glo™ Reagent	Steady-Glo® Reagent	Luciferase Assay Reagent	ONE-Glo™ Reagent
Format	NH or H	NH or H	NH	NH or H
Process	continuous	batch	bench scale	batch or continuous
Number of Steps	1	1	4	1
Sensitivity	highest	lower	higher	high
Signal Half-Life	~30 minutes	~5 hours	~12 minutes	~50 minutes
Precision	High	High	High	Highest
Cell Lysis Time	~2 minutes maximum	~5 minutes maximum	NA	~3 minutes

NH = nonhomogeneous (first create a lysate); H = homogeneous; NA = not applicable

The Luciferase Assay System has long been the standard reagent for routine laboratory analysis. Before using this reagent, cells from which the luciferase is to be measured must be washed and lysed. This reagent was optimized for high sensitivity in nonhomogeneous, single-sample measurements. The Luciferase Assay System requires a luminometer fitted with injectors to efficiently measure luminescence in 96-well plates.

The Bright-Glo™, Steady-Glo® and ONE-Glo™ Reagents were developed to perform assay reactions within multiwell plates and in the presence of complete cell culture medium: no cell preparation steps such as washing or lysing are required before the luminescence reaction is initiated. Both of these are single-step reagents, requiring only addition of the reagent before measuring luminescence. This makes them ideal reagents for efficient and precise quantitation in 96-, 384- and 1536-well plates.

The Bright-Glo™ and Steady-Glo® Reagents are complementary in their characteristics based on the inverse relationship between luminescence duration and assay sensitivity (9). Generally as the half-life of the luminescence increases, assay sensitivity decreases. The Steady-Glo® Reagent provides long

luminescence duration (changing only about 10% per hour); however, to achieve this long luminescence duration, the assay sensitivity must be reduced. This reagent was designed for experiments in which many microplates are processed as a batch.

In contrast, the Bright-Glo™ Reagent provides high assay sensitivity with shorter luminescence duration (<10% decrease per 5 minutes). This reagent is designed for general research applications and for experiments using robotics for continuous sample processing. Furthermore, as a result of increased sample capacity, the Bright-Glo™ Reagent provides greater assay sensitivity than the Luciferase Assay Reagent in most applications (9).

The ONE-Glo™ Reagent provides the ultimate performance for luciferase assays. It features a high-sensitivity assay with extended duration. The ONE-Glo™ Reagent also demonstrates more robust performance and provides reagent handling enhancements.

The Luciferase Assay System, Bright-Glo™ Reagent and Steady-Glo® Reagent and ONE-Glo™ Reagent provide the highest standards in assay quantitation, sensitivity and convenience. Since these reagents are based on the same underlying design principles, different reagents can be used as experimental needs change. For more information request the *Luciferase Assay System Technical Bulletin* #TB281, the *Steady-Glo® Luciferase Assay System Technical Manual* #TM051, the *Bright-Glo™ Luciferase Assay System Technical Manual* #TM052, or the *ONE-Glo™ Luciferase Assay System Technical Manual* #TM292.

When studying promoter functionalities, it is often desirable to include a second reporter (e.g., *Renilla* luciferase) as an internal control for normalization. Plasmids derived from pGL2, pGL3 or pGL4 vectors can be co-transfected with *Renilla* luciferase vectors, such as phRL-TK, and assayed using the Dual-Luciferase® Reporter Assay System (Cat.# E1910) or the Dual-Glo® Luciferase Assay System (Cat.# E2920).

Table 2. Characteristics of Promega Dual-Luciferase Assays.

	Dual-Luciferase® Assay	Dual-Glo® Assay
Format	NH	H
Process	bench scale	batch
Number of Steps	5	2
Sensitivity	higher	lower
Signal Half-Life—firefly	~9 minutes	~2 hours
Signal Half-Life—*Renilla*	~2 minutes	~2 hours
Precision	high	high
Cell Lysis Time	~10 minutes	~15 minutes

NH = nonhomogeneous (lysis step not part of assay);
H = homogeneous (lysis step included in assay)

7. Appendix

7.A. Structure and Function

Promega supplies four different pGL2 Vectors: pGL2-Basic, pGL2-Promoter, pGL2-Enhancer and pGL2-Control. Each vector carries the luciferase gene (*luc*) followed by the SV40 small t antigen intron and early polyadenylation [poly(A)] signals. A second copy of the poly(A) site, located upstream of the luciferase coding region, limits background transcription from spurious promoters in the plasmids. The vectors also contain a high copy number prokaryotic origin of replication for maintenance in *E. coli*, an ampicillin-resistance gene for selection and a filamentous phage origin of replication (f1 ori) for single-stranded DNA (ssDNA) production. Restriction sites for insertion of DNA fragments are located upstream and downstream of the luciferase gene. Two of the upstream sites (XhoI and BglII) yield cohesive ends compatible with the downstream sites (SalI and BamHI, respectively), allowing the interchange of the DNA insert for rapid analysis of positional effects. Except for the inclusion of promoters and enhancers, the four pGL2 Vectors are structurally identical. Specific features of each plasmid are summarized in Section 3. Maps of the pGL2-Basic, pGL2-Promoter, pGL2-Enhancer and pGL2-Control Vectors are shown in Figures 1–5. The list of restriction sites for these vectors is provided in Section 7.

7.B. pGL3 and pGL4 Vectors

Promega also provides two improved series of luciferase reporter vectors, the pGL3 Luciferase Reporter Vectors and pGL4 Luciferase Reporter Vectors. The pGL3 Vectors provide significant advances over the pGL2 Vectors (1). The pGL3 Vectors contain a modified firefly luciferase cDNA, designated *luc*+, and a redesigned vector backbone. These changes were made to increase luciferase expression, improve in vivo vector stability, and provide greater flexibility in performing genetic manipulations. The modified reporter vectors have resulted in luciferase expression levels dramatically higher than those obtained with the pGL2 Reporter Vectors, while maintaining relatively low background luciferase expression. For further information, see the *pGL3 Luciferase Reporter Vectors Technical Manual* #TM033 available at: **www.promega.com/tbs/**

The pGL4 Luciferase Reporter Vectors are the latest generation of reporter gene vectors optimized for expression in mammalian cells. Numerous configurations of pGL4 Vectors are available, including those with the synthetic firefly *luc2* (*Photinus pyralis*) and *Renilla hRluc* (*Renilla reniformis*) genes, which have been codon optimized for more efficient expression in mammalian cells. Furthermore, both the reporter genes and the vector backbone, including the *bla* (β-lactamase or Amp^r) and mammalian selectable marker genes for hygromycin (Hygro or Hyg^r), neomycin (Neo or Neo^r) and puromycin (Puro or Puro^r), have been engineered to reduce the number of consensus transcription factor binding sites, reducing background and the risk of anomalous transcription. For more information, see the *pGL4 Luciferase Reporter Vectors Technical Manual* #TM259 available at: **www.promega.com/tbs/**

7.C. pGL2-Basic 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 or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. The pGL2-Basic Vector sequence is also available in the GenBank® database (GenBank®/EMBL Accession Number **X65323**) and on the Internet at: **www.promega.com/vectors/**

Table 3. Restriction Enzymes That Cut the pGL2-Basic Vector 1–5 Times.

Enzyme	# of Sites	Location
AccB7I	1	2044
AccI	1	2746
AccIII	2	771, 1287
Acc65I	1	8
AcyI	4	83, 109, 1502, 4425
AflIII	3	22, 569, 2995
Alw26I	4	1099, 1331, 3949, 4725
Alw44I	2	3309, 4555
AlwNI	1	3411
AspHI	5	18, 1541, 3313, 4474, 4559
AvaI	3	1, 33, 1132
AvaII	3	1255, 4026, 4248
BamHI	1	2739
BanII	3	18, 1100, 4966
BbeI	1	112
BbsI	5	86, 1364, 1480, 2192, 2824
BbuI	1	739
BglI	2	4008, 5276
BglII	1	37
BsaI	1	3949
BsaAI	3	230, 5037, 5370
BsaBI	1	2507
BsaHI	4	83, 109, 1502, 4425
BspHI	2	3715, 4723
BspMI	1	1474
BsrGI	1	566
BssSI	2	3168, 4552
BstEII	1	683
Bsu36I	1	689
Cfr10I	5	345, 1504, 2863, 3968, 4932
ClaI	1	1441
Csp45I	2	245, 1033
DraII	1	1255
DraIII	1	5040
DrdI	2	3103, 5084
EaeI	1	4276
EclHKI	1	3888
Eco47III	1	2871
Eco81I	1	689
EcoICRI	1	16
EcoNI	1	1693
EcoRI	1	663
EcoRV	1	1414
EheI	1	110
FspI	2	4110, 5283
HaeII	5	112, 2873, 3243, 4882, 4890
HincII	4	1380, 2608, 2747, 5451
HindII	4	1380, 2608, 2747, 5451
HindIII	1	47
HpaI	2	2608, 5451
Hsp92I	4	83, 109, 1502, 4425
KasI	1	108

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

7.C. pGL2-Basic Vector Restriction Sites (continued)

Table 3. Restriction Enzymes That Cut the pGL2-Basic Vector 1–5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
KpnI	1	12	**SacI**	1	18
MluI	1	22	**SalI**	1	2745
MspA1I	5	139, 1429, 3337, 3582, 4523	**ScaI**	1	4368
			SgrAI	1	1504
NaeI	2	2865, 4934	**SinI**	3	1255, 4026, 4248
NarI	1	109	**SmaI**	1	3
NgoMIV	2	2863, 4932	**SphI**	1	739
NheI	1	28	SplI	1	230
NspI	2	739, 2999	**SspI**	5	2323, 2476, 4692, 5245, 5360
PacI	1	1400			
PaeR7I	1	33	**StyI**	1	2192
PflMI	1	2044	**VspI**	2	2411, 4060
PpuMI	1	1255	**XbaI**	1	123
PshAI	1	2810	XcmI	1	811
Psp5II	1	1255	**XhoI**	1	33
PspAI	1	1	**XmaI**	1	1
PvuI	2	4258, 5304	**XmnI**	1	4487

Table 4. Restriction Enzymes That Do Not Cut the pGL2-Basic Vector.

AatII	Bpu1102I	Eco52I	PmeI	**SpeI**
AflII	Bsp120I	Eco72I	PmlI	SrfI
AgeI	**BssHII**	FseI	Ppu10I	Sse8387I
ApaI	Bst1107I	**I-PpoI**	**PstI**	**StuI**
AscI	**Bst98I**	**NcoI**	**PvuII**	SwaI
AvrII	**BstXI**	**NdeI**	RsrII	**Tth111I**
BalI	**BstZI**	**NotI**	**SacII**	
BbrPI	**CspI**	**NruI**	**SfiI**	
BclI	DsaI	**NsiI**	**SgfI**	
BlpI	EagI	PinAI	**SnaBI**	

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

Table 5. Restriction Enzymes That Cut the pGL2-Basic Vector Six or More Times.

AciI	Bst71I	**HaeIII**	**MboII**	Sau96I
AluI	**BstOI**	HgaI	MnlI	ScrFI
BanI	BstUI	**HhaI**	MseI	SfaNI
BbvI	**CfoI**	**HinfI**	**MspI**	**TaqI**
BsaOI	**DdeI**	**HpaII**	**NciI**	TfiI
BsaJI	**DpnI**	HphI	**NdeII**	**Tru9I**
BsaMI	DpnII	**Hsp92II**	NlaIII	**XhoII**
BsmI	**DraI**	MaeI	NlaIV	
Bsp1286I	EarI	MaeII	PleI	
BsrI	Fnu4HI	MaeIII	**RsaI**	
BsrSI	**FokI**	**MboI**	**Sau3AI**	

7.D. pGL2-Promoter 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 or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. The pGL2-Promoter Vector sequence is also available in the GenBank® database (GenBank®/EMBL Accession Number **X65326**) and on the Internet at: **www.promega.com/vectors/**

Table 6. Restriction Enzymes That Cut the pGL2-Promoter Vector 1–5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AccB7I	1	2236	BbsI	5	278, 1556, 1672, 2384, 3016
AccI	1	2938			
AccIII	2	963, 1479	**BbuI**	1	931
Acc65I	1	8	**BglI**	3	176, 4200, 5468
AcyI	4	275, 301, 1694, 4617	**BglII**	1	37
			BsaI	1	4141
AflIII	3	22, 761, 3187	BsaAI	3	422, 5229, 5562
Alw26I	4	1291, 1523, 4141, 4917	BsaBI	2	42, 2699
			BsaHI	4	275, 301, 1694, 4617
Alw44I	2	3501, 4747			
AlwNI	1	3603	BspHI	2	3907, 4915
AspHI	5	18, 1733, 3505, 4666, 4751	BspMI	1	1666
			BsrGI	1	758
AvaI	3	1, 33, 1324	BssSI	2	3360, 4744
AvaII	3	1447, 4218, 4440	**BstEII**	1	875
AvrII	1	223	**Bsu36I**	1	881
BamHI	1	2931	Cfr10I	5	537, 1696, 3055, 4160, 5124
BanII	3	18, 1292, 5158			
BbeI	1	304	**ClaI**	1	1633

7.D. pGL2-Promoter Vector Restriction Sites (continued)

Table 6. Restriction Enzymes That Cut the pGL2-Promoter Vector 1–5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
Csp45I	2	437, 1225	**NcoI**	1	130
DraII	1	1447	**NgoMIV**	2	3055, 5124
DraIII	1	5232	**NheI**	1	28
DrdI	2	3295, 5276	NspI	2	931, 3191
DsaI	1	130	PacI	1	1592
EaeI	1	4468	PaeR7I	1	33
EclHKI	1	4080	PflMI	1	2236
Eco47III	1	3063	PpuMI	1	1447
Eco81I	1	881	PshAI	1	3002
EcoICRI	1	16	Psp5II	1	1447
EcoNI	1	1885	PspAI	1	1
EcoRI	1	855	**PvuI**	2	4450, 5496
EcoRV	1	1606	**SacI**	1	18
EheI	1	302	**SalI**	1	2937
FspI	2	4302, 5475	**ScaI**	1	4560
HaeII	5	304, 3065, 3435, 5074, 5082	**SfiI**	1	176
			SgrAI	1	1696
HincII	4	1572, 2800, 2939, 5643	**SinI**	3	1447, 4218, 4440
			SmaI	1	3
HindII	4	1572, 2800, 2939, 5643	**SphI**	1	931
			SplI	1	422
HindIII	1	239	**SspI**	5	2515, 2668, 4884, 5437, 5552
HpaI	2	2800, 5643			
Hsp92I	4	275, 301, 1694, 4617	**StuI**	1	222
			StyI	3	130, 223, 2384
KasI	1	300	**VspI**	2	2603, 4252
KpnI	1	12	**XbaI**	1	315
MluI	1	22	XcmI	1	1003
MspA1I	5	331, 1621, 3529, 3774, 4715	**XhoI**	1	33
			XmaI	1	1
NaeI	2	3057, 5126	**XmnI**	1	4679
NarI	1	301			

Table 7. Restriction Enzymes That Do Not Cut the pGL2-Promoter Vector.

AatII	Bpu1102I	Eco52I	PmeI	**SpeI**
AflII	Bsp120I	Eco72I	PmlI	SrfI
AgeI	**BssHII**	FseI	Ppu10I	Sse8387I
ApaI	Bst1107I	**I-PpoI**	**PstI**	SwaI
AscI	**Bst98I**	**NdeI**	**PvuII**	**Tth111I**
BalI	**BstXI**	**NotI**	RsrII	
BbrPI	**BstZI**	**NruI**	**SacII**	
BclI	**CspI**	**NsiI**	**SgfI**	
BlpI	EagI	PinAI	**SnaBI**	

Table 8. Restriction Enzymes That Cut the pGL2-Promoter Vector More Than Six Times.

AciI	Bst71I	**HaeIII**	**MboII**	Sau96I
AluI	**BstOI**	HgaI	MnlI	ScrFI
BanI	BstUI	**HhaI**	MseI	SfaNI
BbvI	**CfoI**	**HinfI**	**MspI**	**TaqI**
BsaOI	**DdeI**	**HpaII**	**NciI**	TfiI
BsaJI	**DpnI**	HphI	**NdeII**	**Tru9I**
BsaMI	DpnII	**Hsp92II**	NlaIII	**XhoII**
BsmI	**DraI**	MaeI	NlaIV	
Bsp1286I	EarI	MaeII	PleI	
BsrI	Fnu4HI	MaeIII	**RsaI**	
BsrSI	**FokI**	**MboI**	**Sau3AI**	

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

7.E. pGL2-Enhancer 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 or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. The pGL2-Enhancer Vector sequence is also available in the GenBank® database (GenBank®/EMBL Accession Number **X65325**) and on the Internet at: **www.promega.com/vectors/**

Table 9. Restriction Enzymes That Cut the pGL2-Enhancer Vector 1–5 Times.

Enzyme	# of Sites	Location
AccB7I	1	2044
AccI	1	3003
AccIII	2	771, 1287
Acc65I	1	8
AcyI	4	83,109,1502, 4682
AflIII	3	22, 569, 3252
Alw26I	4	1099, 1331, 4206, 4982
Alw44I	2	3566, 4812
AlwNI	1	3668
AspHI	5	18, 1541, 3570, 4731, 4816
AvaI	3	1, 33, 1132
AvaII	3	1255, 4283, 4505
BamHI	1	2996
BanII	3	18, 1100, 5223
BbeI	1	112
BbsI	5	86, 1364, 1480, 2192, 3081
BbuI	3	739, 2843, 2915
BglI	2	4265, 5533
BglII	1	37
BsaI	1	4206
BsaAI	3	230, 5294, 5627
BsaBI	1	2507
BsaHI	4	83, 109,1502, 4682
BspHI	2	3972, 4980
BspMI	1	1474
BsrGI	1	566
BssSI	2	3425, 4809
BstEII	1	683
Bsu36I	1	689
Cfr10I	5	345, 1504, 3120, 4225, 5189
ClaI	1	1441
Csp45I	2	245, 1033
DraII	1	1255
DraIII	1	5297
DrdI	2	3360, 5341
DsaI	1	2748
EaeI	1	4533
EclHKI	1	4145
Eco47III	1	3128
Eco81I	1	689
EcoICRI	1	16
EcoNI	1	1693
EcoRI	1	663
EcoRV	1	1414
EheI	1	110
FspI	2	4367, 5540
HaeII	5	112, 3130, 3500, 5139, 5147
HincII	4	1380, 2608, 3004, 5708
HindII	4	1380, 2608, 3004, 5708
HindIII	1	47
HpaI	2	2608, 5708
Hsp92I	4	83, 109, 1502, 4682
KasI	1	108
KpnI	1	12
MluI	1	22
MspA1I	5	139, 1429, 3594, 3839, 4780

Table 9. Restriction Enzymes That Cut the pGL2-Enhancer Vector 1–5 Times (continued).

Enzyme	# of Sites	Location
NaeI	2	3122, 5191
NarI	1	109
NcoI	1	2748
NgoMIV	2	3120, 5189
NheI	1	28
NsiI	2	2841, 2913
NspI	4	739, 2843, 2915, 3256
PacI	1	1400
PaeR7I	1	33
PflMI	1	2044
Ppu10I	2	2837, 2909
PpuMI	1	1255
PshAI	1	3067
Psp5II	1	1255
PspAI	1	1
PvuI	2	4515, 5561
SacI	1	18
SalI	1	3002
ScaI	1	4625
SgrAI	1	1504
SinI	3	1255, 4283, 4505
SmaI	1	3
SphI	3	739, 2843, 2915
SplI	1	230
SspI	5	2323, 2476, 4949 5502, 5617
StyI	2	2192, 2748
VspI	2	2411, 4317
XbaI	1	123
XcmI	1	811
XhoI	1	33
XmaI	1	1
XmnI	1	4744

Table 10. Restriction Enzymes That Do Not Cut the pGL2-Enhancer Vector.

AatII	BlpI	EagI	PmeI	**SpeI**
AflII	Bpu1102I	Eco52I	PmlI	SrfI
AgeI	Bsp120I	Eco72I	**PstI**	Sse8387I
ApaI	**BssHII**	FseI	**PvuII**	**StuI**
AscI	Bst1107I	**I-PpoI**	RsrII	SwaI
AvrII	**Bst98I**	**NdeI**	**SacII**	**Tth111I**
BalI	**BstXI**	**NotI**	**SfiI**	
BbrPI	**BstZI**	**NruI**	**SgfI**	
BclI	**CspI**	PinAI	**SnaBI**	

Table 11. Restriction Enzymes That Cut the pGL2-Enhancer Vector More Than Six Times.

AciI	**BsrSI**	Fnu4HI	MaeII	PleI
AluI	Bst71I	**FokI**	MaeIII	**RsaI**
BanI	**BstOI**	**HaeIII**	**MboI**	**Sau3AI**
BbvI	BstUI	HgaI	**MboII**	Sau96I
BsaOI	**CfoI**	**HhaI**	MnlI	ScrFI
BsaJI	**DdeI**	**HinfI**	MseI	SfaNI
BsaMI	**DpnI**	**HpaII**	**MspI**	**TaqI**
BsmI	DpnII	HphI	**NciI**	TfiI
Bsp1286I	**DraI**	**Hsp92II**	**NdeII**	**Tru9I**
BsrI	EarI	MaeI	NlaIII	**XhoII**

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

7.F. pGL2-Control 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 or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. The pGL2-Control Vector sequence is also available in the GenBank® database (GenBank®/EMBL Accession Number **X65324**) and on the Internet at: **www.promega.com/vectors/**

Table 12. Restriction Enzymes That Cut the pGL2-Control Vector 1–5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AccB7I	1	2236	**BstEII**	1	875
AccI	1	3195	**Bsu36I**	1	881
AccIII	2	963, 1479	Cfr10I	5	537, 1696, 3312, 4417, 5381
Acc65I	1	8			
AcyI	4	275, 301, 1694, 4874	**ClaI**	1	1633
			Csp45I	2	437, 1225
AflIII	3	22, 761, 3444	DraII	1	1447
Alw26I	4	1291, 1523, 4398 5174	DraIII	1	5489
			DrdI	2	3552, 5533
Alw44I	2	3758, 5004	DsaI	2	130, 2940
AlwNI	1	3860	EaeI	1	4725
AspHI	5	18, 1733, 3762, 4923, 5008	**EclHKI**	1	4337
			Eco47III	1	3320
AvaI	3	1, 33, 1324	Eco81I	1	881
AvaII	3	1447, 4475, 4697	**EcoICRI**	1	16
AvrII	1	223	EcoNI	1	1885
BamHI	1	3188	**EcoRI**	1	855
BanII	3	18, 1292, 5415	**EcoRV**	1	1606
BbeI	1	304	EheI	1	302
BbsI	5	278, 1556, 1672, 2384, 3273	**HaeII**	5	304, 3322, 3692, 5331, 5339
BbuI	3	931, 3035, 3107	**HincII**	4	1572, 2800, 3196, 5900
BglI	3	176, 4457, 5725			
BglII	1	37	HindII	4	1572, 2800, 3196, 5900
BsaI	1	4398			
BsaAI	3	422, 5486, 5819	**HindIII**	1	239
BsaBI	2	42, 2699	**HpaI**	2	2800, 5900
BsaHI	4	275, 301, 1694, 4874	**Hsp92I**	4	275 , 301, 1694, 4874
BspHI	2	4164, 5172	KasI	1	300
BspMI	1	1666	**KpnI**	1	12
BsrGI	1	758	**MluI**	1	22
BssSI	2	3617, 5001			

Table 12. Restriction Enzymes That Cut the pGL2-Control Vector 1–5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
MspA1I	5	331, 1621, 3786, 4031, 4972	**SalI**	1	3194
			ScaI	1	4817
NaeI	2	3314, 5383	**SfiI**	1	176
NarI	1	301	SgrAI	1	1696
NcoI	2	130, 2940	**SinI**	3	1447, 4475, 4697
NgoMIV	2	3312, 5381	**SmaI**	1	3
NheI	1	28	**SphI**	3	931, 3035, 3107
NsiI	2	3033, 3105	SplI	1	422
NspI	4	931, 3035, 3107, 3448	**SspI**	5	2515, 2668, 5141, 5694, 5809
PacI	1	1592	**StuI**	1	222
PaeR7I	1	33	**StyI**	4	130, 223, 2384, 2940
PflMI	1	2236			
Ppu10I	2	3029, 3101	**VspI**	2	2603, 4509
PpuMI	1	1447	**XbaI**	1	315
PshAI	1	3259	XcmI	1	1003
Psp5II	1	1447	**XhoI**	1	33
PspAI	1	1	**XmaI**	1	1
PvuI	2	4707, 5753	**XmnI**	1	4936
SacI	1	18			

Table 13. Restriction Enzymes That Do Not Cut the pGL2-Control Vector.

AatII	BlpI	**CspI**	**NruI**	**SgfI**
AflII	Bpu1102I	EagI	PinAI	**SnaBI**
AgeI	Bsp120I	Eco52I	PmeI	**SpeI**
ApaI	**BssHII**	Eco72I	PmlI	SrfI
AscI	Bst1107I	FseI	**PstI**	Sse8387I
BalI	**Bst98I**	**I-PpoI**	**PvuII**	SwaI
BbrPI	**BstXI**	**NdeI**	RsrII	**Tth111I**
BclI	**BstZI**	**NotI**	**SacII**	

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

7.F. pGL2-Control Vector Restriction Sites (continued)

Table 14. Restriction Enzymes That Cut the pGL2-Control Vector More Than Six Times.

AciI	Bst71I	**HaeIII**	**MboII**	Sau96I
AluI	**BstOI**	HgaI	MnlI	ScrFI
BanI	BstUI	**HhaI**	MseI	SfaNI
BbvI	**CfoI**	**HinfI**	**MspI**	**TaqI**
BsaOI	**DdeI**	**HpaII**	**NciI**	TfiI
BsaJI	**DpnI**	HphI	**NdeII**	**Tru9I**
BsaMI	DpnII	**Hsp92II**	NlaIII	**XhoII**
BsmI	**DraI**	MaeI	NlaIV	
Bsp1286I	EarI	MaeII	PleI	
BsrI	Fnu4HI	MaeIII	**RsaI**	
BsrSI	**FokI**	**MboI**	**Sau3AI**	

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

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7.H. Related Products

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GLprimer2 (counter clockwise)	2µg	E1661
RVprimer3 (clockwise)	2µg	E4481
RVprimer4 (counter clockwise)	2µg	E4491
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	100 preps	A2495

Luciferase Assay Systems

Product	Size	Cat.#
Luciferase Assay System	100 assays	E1500
Bright-Glo™ Luciferase Assay System	10ml	E2610
Steady-Glo® Luciferase Assay System	10ml	E2510
Dual-Luciferase® Reporter Assay System	100 assays	E1910
Dual-Glo® Luciferase Assay System	10ml	E2920
ONE-Glo™ Luciferase Assay System*	10ml	E6110

Available in additional sizes. *For Laboratory Use.

pGL4 Luciferase Vectors

Product	Size	Cat.#
pGL4.10[*luc2*] Vector	20µg	E6651
pGL4.11[*luc2P*] Vector	20µg	E6661
pGL4.12[*luc2CP*] Vector	20µg	E6671
pGL4.13[*luc2*/SV40] Vector	20µg	E6681
pGL4.14[*luc2*/Hygro] Vector	20µg	E6691
pGL4.17[*luc2*/Neo] Vector	20µg	E6721
pGL4.20[*luc2*/Puro] Vector	20µg	E6751
pGL4.23[*luc2*/minP] Vector	20µg	E8411
pGL4.26[*luc2*/minP/Hygro] Vector	20µg	E8441
pGL4.29[*luc2P*/CRE/Hygro] Vector	20µg	E8471
pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector	20µg	E8481
pGL4.31[*luc2P*/*GAL4*UAS/Hygro] Vector	20µg	C9351
pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector	20µg	E8491

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Revised 2/09

7.H. Related Products (continued)

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Product	Cat.#
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GloMax®-Multi Luminescence Module	E7041
GloMax®-Multi Fluorescence Module	E7051
GloMax®-Multi Absorbance Module	E7061
GloMax® 20/20 Luminometer	E5311
GloMax® 20/20 Luminometer with Single Auto-Injector	E5321
GloMax® 20/20 Luminometer with Dual Auto-Injector	E5331
GloMax® 96 Microplate Luminometer	E6501
GloMax® 96 Microplate Luminometer with Single Reagent Injector	E6511
GloMax® 96 Microplate Luminometer with Dual Reagent Injectors	E6521

*Cat.# E7031 cannot be sold separately and must be purchased with at least one detection module (Cat.# E7041, E7051 or E7061).

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