Technical Manual

pGL3 Luciferase Reporter Vectors

INSTRUCTIONS FOR USE OF PRODUCTS E1741, E1751, E1761 AND E1771.

www.promega.com

PRINTED IN USA.
Revised 9/07

AF9TM033 0907TM033

Part# TM033

pGL3 Luciferase Reporter Vectors

All technical literature is available on the Internet at: www.promega.com/tbs/
Please visit the web site to verify that you are using the most current version of this Technical Bulletin. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com

Promega Corporation · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA
Toll Free in USA 800-356-9526 · Phone 608-274-4330 · Fax 608-277-2516 · **www.promega.com**
Printed in USA. Part# TM033
Revised 9/07

I. Description

The pGL3 Luciferase Reporter Vectors[a,b] provide a basis 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 backbone of the pGL3 Luciferase Reporter Vectors is designed for increased expression, and contains a modified coding region for firefly (*Photinus pyralis*) luciferase that has been optimized for monitoring transcriptional activity in transfected eukaryotic cells. The assay of this genetic reporter is rapid, sensitive and quantitative. In addition, these Luciferase Reporter Vectors contain numerous features aiding in the structural characterization of the putative regulatory sequences under investigation.

II. Product Components and Storage Conditions

Product	Size	Cat.#
pGL3-Control Vector	20µg	E1741
pGL3-Basic Vector	20µg	E1751
pGL3-Promoter Vector	20µg	E1761
pGL3-Enhancer Vector	20µg	E1771

Information on related products, including the Luciferase Assay System, is provided in Sections IV–VI and VIII.K.

Storage Conditions: Store the pGL3 Luciferase Reporter Vectors at –20°C.

III. pGL3 Vector Maps and Sequence Reference Points

The listings of restriction sites for the pGL3 Luciferase Reporter Vectors are provided in Section VIII.G–J.

Note: The specific transcriptional characteristics of the pGL3 Vectors will vary for different cell types. This may be particularly true for COS cells, which contain the SV40 large T antigen. The SV40 large T antigen promotes replication from the SV40 origin, which is found in the promoter of the pGL3-Promoter and pGL3-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 other cell and vector combinations.

III.A. pGL3-Basic Vector

The pGL3-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 *luc*+ gene.

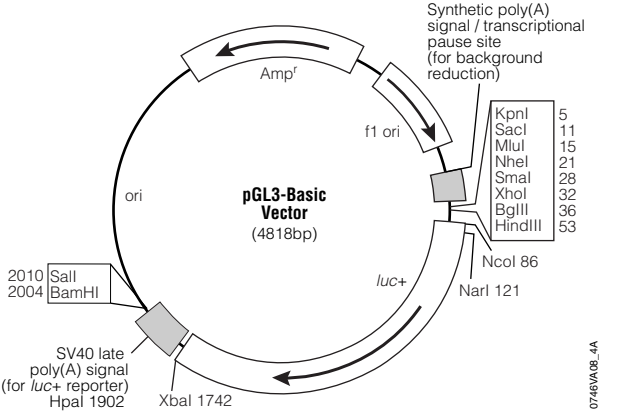

Figure 1. pGL3-Basic Vector circle map. Additional description: *luc*+, cDNA encoding the modified firefly luciferase; Amp^r, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of replication in *E. coli.* Arrows within *luc*+ and the Amp^r gene indicate the direction of transcription; the arrow in the f1 ori indicates the direction of ssDNA strand synthesis.

pGL3-Basic Vector Sequence Reference Points:

Promoter	(none)
Enhancer	(none)
Multiple cloning region	1–58
Luciferase gene (*luc*+)	88–1740
GLprimer2 binding site	89–111
SV40 late poly(A) signal	1772–1993
RVprimer4 binding site	2080–2061
ColE1-derived plasmid replication origin	2318
β-lactamase gene (Amp^r)	3080–3940
f1 origin	4072–4527
upstream poly(A) signal	4658–4811
RVprimer3 binding site	4760–4779

III.B. pGL3-Enhancer Vector

The pGL3-Enhancer Vector contains an SV40 enhancer located downstream of *luc*+ and the poly(A) signal. This aids in the verification of functional promoter elements because the presence of an enhancer will often result in transcription of *luc*+ at higher levels.

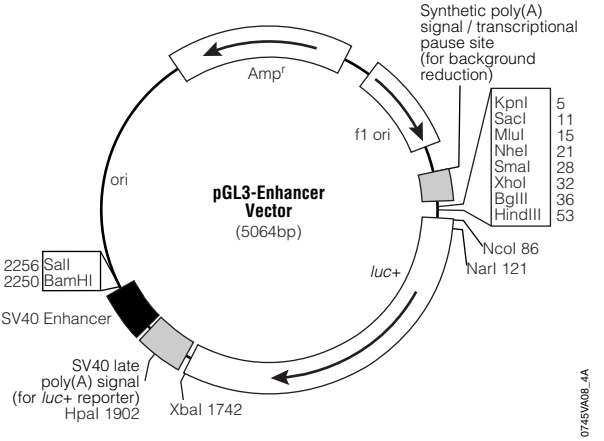

Figure 2. The pGL3-Enhancer Vector circle map. Additional description: *luc*+, cDNA encoding the modified firefly luciferase; Amp^r, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli*. Arrows within *luc*+ and the Amp^r gene indicate the direction of transcription; the arrow in f1 ori indicates the direction of ssDNA strand synthesis.

pGL3-Enhancer Vector Sequence Reference Points:

Promoter	(none)
Multiple cloning region	1–58
Luciferase gene (*luc*+)	88–1740
GLprimer2 binding site	89–111
SV40 late poly(A) signal	1772–1993
Enhancer	2013–2249
RVprimer4 binding site	2307–2326
ColE1-derived plasmid replication origin	2564
β-lactamase gene (Amp^r)	3329–4186
f1 origin	4318–4773
upstream poly(A) signal	4904–5057
RVprimer3 binding site	5006–5025

III.C. pGL3-Promoter Vector

The pGL3-Promoter Vector contains an SV40 promoter upstream of the luciferase gene. DNA fragments containing putative enhancer elements can be inserted either upstream or downstream of the promoter-*luc*+ transcriptional unit.

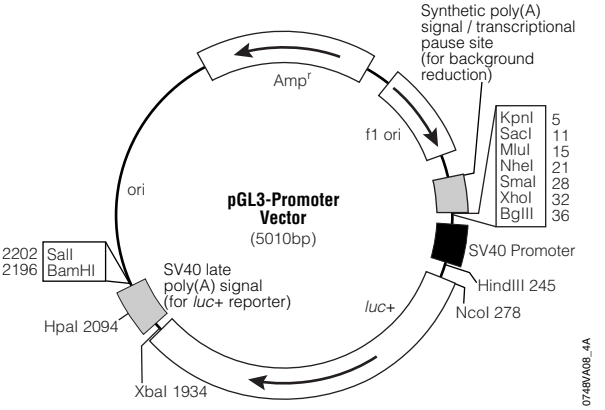

Figure 3. The pGL3-Promoter Vector circle map. Additional description: *luc*+, cDNA encoding the modified firefly luciferase; Amp^r, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli*. Arrows within *luc*+ and the Amp^r gene indicate the direction of transcription; the arrow in f1 ori indicates the direction of ssDNA strand synthesis.

pGL3-Promoter Vector Sequence Reference Points:

Enhancer	(none)
Multiple cloning region	1–41
Promoter	48–250
GLprimer2 binding region	281–303
Luciferase gene (*luc*+)	280–1932
SV40 late poly(A) signal	1964–2185
RVprimer4 binding region	2253–2272
ColE1-derived plasmid replication origin	2510
β-lactamase gene (Amp^r)	3272–4132
f1 origin	4264–4719
Upstream poly(A) signal	4850–5003
RVprimer3 binding region	4952–4971

III.D. pGL3-Control Vector

The pGL3-Control Vector contains SV40 promoter and enhancer sequences, resulting in strong expression of *luc*+ 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 pGL3 recombinants.

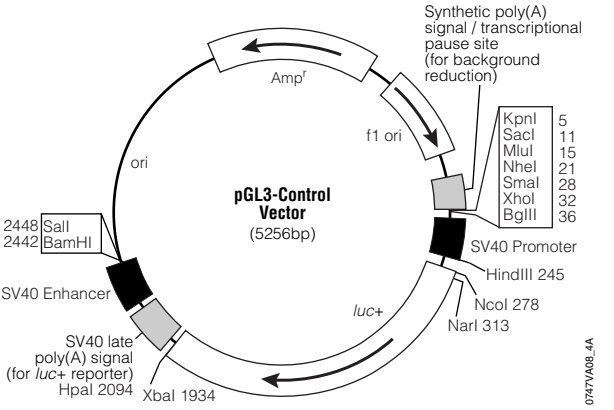

Figure 4. pGL3-Control Vector circle map. Additional description: *luc*+, cDNA encoding the modified firefly luciferase; Amp^r, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli*. Arrows within *luc*+ and the Amp^r gene indicate the direction of transcription; the arrow in f1 ori indicates the direction of ssDNA strand synthesis.

pGL3-Control Vector Sequence Reference Points:

Multiple cloning region	1–41
Promoter	48–250
Luciferase gene (*luc*+)	280–1932
GLprimer2 binding site	281–303
SV40 late poly(A) signal	1964–2185
Enhancer	2205–2441
RVprimer4 binding site	2499–2518
ColE1-derived plasmid replication origin	2756
β-lactamase gene (Amp^r)	3518–4378
f1 origin	4510–4965
upstream poly(A) signal	5096–5249
RVprimer3 binding site	5198–5217

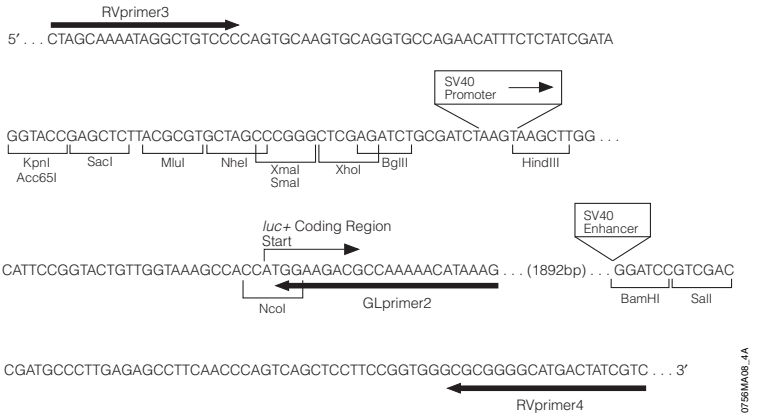

Figure 5. pGL3 Vector multiple cloning regions. Shown are the upstream and downstream cloning sites and the locations of the sequencing primers (GLprimer2, RVprimer3 and RVprimer4). The large primer arrows indicate the direction of sequencing. The positions of the promoter (in the pGL3-Promoter and pGL3-Control Vectors) and the enhancer (in the pGL3-Enhancer and pGL3-Control Vectors) are shown as insertions into the sequence of the pGL3-Basic Vector. (Note that the promoter replaces four bases [AAGT] of the pGL3-Basic Vector.) The sequence shown is of the DNA strand generated from the f1 ori.

IV. Cloning Methods

IV.A. Cloning Strategies

The restriction sites for XhoI and SalI have compatible ends, as do BglII and BamHI. Therefore, cloning into the XhoI or BglII sites upstream of *luc*+, or the downstream SalI or BamHI sites, allows easy interchange of DNA inserts between upstream and downstream positions relative to the luciferase reporter 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 generating nested deletions (see Section VIII.D). Specifically, the KpnI or SacI site is needed to generate a 3′ overhang upstream of the insert.

Please refer to Sections VIII.G–J for additional information on XhoI digestion.

IV.B. Preparation of pGL3 Vectors and Insert DNA for Cloning

The fragment and vector DNA should be digested with restriction enzymes that will generate compatible ends for cloning. In some cases, the ends of the DNA fragment may require modification, either by using synthetic linkers, by a PCR amplification using primers containing sites for appropriate restriction enzymes, or by filling in the restriction site overhangs. It may be advantageous to treat the vector DNA with calf intestinal alkaline phosphatase (CIAP; Cat.# M2825) or TSAP Thermosensitive Alkaline Phosphatase (Cat.# M9910) to remove 5´ phosphate groups, thus preventing reclosure of the vector on itself without an insert. Sufficient DNA should be prepared to perform control reactions for digestion, ligation and transformation steps.

To ensure capture of the correct insert DNA, the desired restriction fragment can be purified by electrophoresis on an acrylamide or agarose gel and then recovered from the gel by one of several methods, such as using the *Wizard® PCR Preps DNA Purification System Technical Bulletin* #TB118. Alternatively, unfractionated restriction fragments can be cloned into the target plasmid, and the desired recombinant then can be identified by gel electrophoresis of plasmid DNA.

Protocols for restriction digestion, alkaline phosphatase treatment, linker ligation and transformation of competent cells can be found in *Molecular Cloning, A Laboratory Manual* (1).

IV.C. Transformation Protocols for pGL3 Vectors

Because the Luciferase Reporter 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 1.0mM thiamine-HCl prior to preparation of competent cells and transformation. This selects for the presence of the F´ episome.

IV.D. Isolation of Plasmid DNA

The Wizard® *Plus* SV Minipreps DNA Purification System (Cat.# A1340, A1470) may be used for small-scale preparation of plasmid DNA for screening clones. DNA suitable for transfection may be purified using the PureYield™ Plasmid Midipreps System (Cat.# A2492, A2495).

V. Transfection of Mammalian Cells

Transfection of DNA into eukaryotic cells may be mediated by cationic lipid compounds (2), calcium phosphate (3,4), DEAE-dextran (3,5), or electroporation (4). Transfection systems based on cationic lipids (TransFast™ Transfection Reagent, Transfectam® Reagent and Tfx™ Reagents) and calcium phosphate (Profection® Mammalian Transfection System) 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 System Technical Manual* (#TM012). All of these documents are available on our web site at: **www.promega.com/tbs/**

VI. Assay 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, we have 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

VI. Assay of Luciferase Activity (continued)

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. All 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 (6). 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 (6).

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, 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 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 maximum	~15 minutes maximum

NH = nonhomogeneous (first create a lysate); H = homogeneous

VII. Sequencing of Luciferase Reporter Vectors

You may desire to sequence the DNA inserted into the Luciferase Reporter Vectors. Two examples of such applications are to determine the exact position of generated deletions and to confirm production of a site-specific mutation. Three primers are available for sequencing the pGL3 Vectors: RVprimer3 (Reporter Vector Primer 3) for sequencing clockwise across the upstream cloning sites, RVprimer4 for sequencing counterclockwise across the BamHI and SalI cloning sites downstream of *luc*+, and GLprimer2 for sequencing counterclockwise upstream of *luc*+.

RVprimer3	5´-CTAGCAAAATAGGCTGTCCC-3´
RVprimer4	5´-GACGATAGTCATGCCCCGCG-3´
GLprimer2	5´-CTTTATGTTTTTGGCGTCTTCCA-3´

RVprimer3 is especially useful for identifying positions of nested deletions.
Note: All three primers can be used for dsDNA sequencing, but only RVprimer4 and GLprimer2 also may be used for ssDNA sequencing.

VIII. Appendix

VIII.A. Common Structural Elements of the pGL3 Luciferase Reporter Vectors

Except for the inclusion of promoters and enhancers, the four pGL3 Luciferase Reporter Vectors are structurally identical. Each plasmid's distinguishing features are summarized in Section III. The pGL3 Vectors each 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.

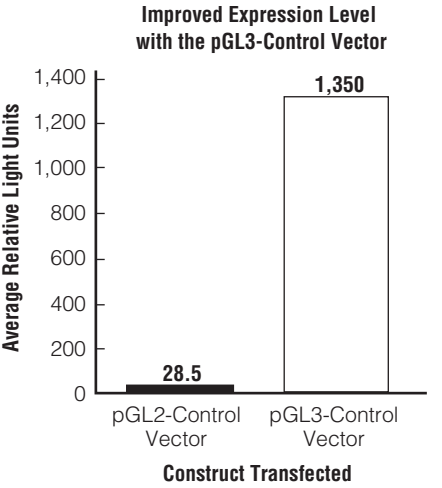

Figure 6. Comparison of luciferase activities expressed in HeLa cells transfected with the pGL2-Control and pGL3-Control Reporter Vectors. The expression level of *luc*+ is dramatically higher with the pGL3-Control Vectors. In repeated experiments with several cell lines, we observed 20- to 100-fold higher luciferase activity from cells transfected with pGL3-Control. Luciferase activity was measured with a Turner Designs luminometer. (Absolute light values and relative expression profiles may vary between different cell types.)

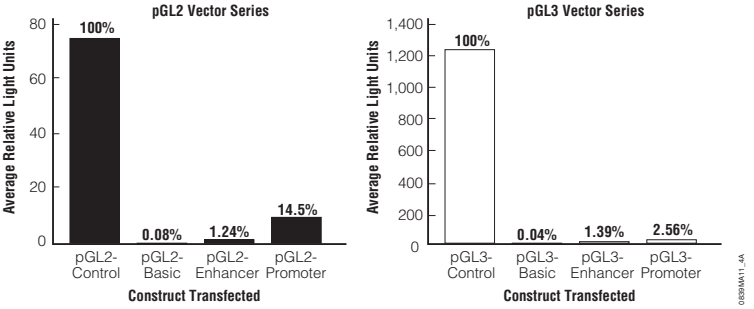

Figure 7. A representative experiment comparing luciferase activities expressed in HeLa cells transfected with the pGL2 and pGL3 Vector series. The increase in luciferase expression observed with these new vectors provides greater sensitivity, while maintaining relatively low background luciferase expression.

VIII.B. Advantages of the pGL3 Vectors

The pGL3 Reporter 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 pGL2 Reporter Vectors (Figure 6), while maintaining relatively low background luciferase expression (Figure 7).

The substantial increase in the expression of luciferase observed with the pGL3 Vectors provides greater sensitivity. It may now be possible to obtain measurable luciferase expression in cell types that are difficult to transfect or when studying weak promoter elements. Users of the pGL2 and pGL3 Vectors should be aware, however, that absolute light unit values and relative expression profiles vary between different cell types (7). Therefore, it is important to include the appropriate control vectors in all experiments.

Further refinements have been made since the pGL3 Vectors became available. Our newest series of luciferase reporter vectors, the pGL4 Luciferase Vectors, provide additional features and benefits as compared to the pGL3 Vectors. For more information, see the *pGL4 Luciferase Reporter Vectors Technical Manual* #TM259 available at: **www.promega.com/tbs/**

VIII.C. The pGL3 Vectors *luc*+ Gene

Modifications that distinguish the *luc*+ gene from the native luciferase gene generally fall into four categories: i) the C-terminal tripeptide has been removed to eliminate peroxisome targeting of the expressed protein; ii) codon usage was improved for expression in plant and animal cells; iii) two potential sites of N-glycosylation were removed; and iv) several DNA sequence changes were made to disrupt extended palindromes, remove internal restriction sites, and eliminate consensus sequences recognized by genetic regulatory binding proteins, thus helping to ensure that the reporter gene itself is unaffected by spurious host transcriptional signals. (For a detailed description of the modifications to the *luc*+ gene, see reference 8.)

Four major modifications were made to the vector backbone: i) the SV40 early poly(A) signal has been replaced with the SV40 late poly(A) signal to increase the efficiency of transcription termination and polyadenylation of the luciferase transcripts (9); ii) a synthetic poly(A) and transcriptional pause site (10,11) have been placed upstream of the multiple cloning site to terminate spurious transcription, which may initiate within the vector backbone; iii) the small T intron has been removed to prevent reduced reporter gene expression due to cryptic RNA splicing (12,13); and iv) a Kozak consensus sequence (14) has been inserted to increase the efficiency of translation initiation of the luciferase gene (7; Table 3).

There is a newer luciferase gene available, *luc2*. The *luc2* gene not only shares the same features as *luc*+, but the sequence was codon-optimized for expression in mammalian cells. For further information about the *luc2* gene present in the pGL4 Luciferase Vectors, see Technical Manual #TM259 available at: **www.promega.com/tbs/**

Table 3. Changes Made to the pGL3 Vectors.

Changes Made	Purpose of Modification	Reference
Modifications made to the luciferase gene (*luc* to *luc*+).	Changes eliminate peroxisome targeting of expressed protein, eliminate consensus binding sequences for various genetic regulatory proteins, improve codon usage for mammalian and plant cells, and provide convenient restriction sites.	(8)
A unique NcoI site created at 5´ end of *luc*+ gene. NcoI sites removed from SV40 enhancer and promoter regions.	Ability to create N-terminal gene fusions with *luc*+ using unique NcoI site.	
Intron from SV40 small T antigen removed.	Intron from SV40 small T antigen can reduce expression when placed 3´ of certain genes due to cryptic splicing.	(12,13)
Poly(A) site for background reduction changed from SV40 early site to a synthetic poly(A) and transcriptional pause site.	Avoids possible recombination between two SV40 poly(A) sequences in the same plasmid.	(9,10)
Poly(A) signal for *luc*+ changed from early to late SV40 poly(A) signal.	Late SV40 poly(A) signal is more efficient than early SV40 poly(A).	(7)
Kozak consensus sequence created immediately 5´ of the *luc*+ gene.	Provides optimal translation efficiency.	(14)
Unique XbaI site created just downstream of the *luc*+ gene.	User convenience; facilitates subcloning of the *luc*+ gene.	
SmaI site moved to internal position in multiple cloning region.	User convenience; blunt-ended inserts can now be cleaved on either side by restriction endonucleases.	

VIII.D. 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 nested deletions following the method of Henikoff (15) and then assaying for changes in biological activity. This method takes advantage of the unique properties of Exonuclease III (Exo III), which will digest 5´ overhangs but not 3´ overhangs or α-phosphorothioate nucleotide filled-in overhangs. Nested deletions of an insert DNA can be made directly in the pGL3 family of Reporter Vectors using this method, eliminating the need for subcloning steps. The multiple cloning region of the pGL3 Vectors contains upstream KpnI and SacI restriction sites, which can be used to generate the 3´ overhangs resistant to Exo III (Figures 1–5). After treatment with Exo III, S1 nuclease is added to remove the resulting ssDNA overhangs, 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 Luciferase Reporter Vectors.

VIII.E. Composition of Buffers and Solutions

M-9 plates (1 liter)

15g	agarose

Add 15g agarose to 750ml water and autoclave. Cool to 50°C. Add:

2.0ml	1M $MgSO_4$
0.1ml	1M $CaCl_2$
10.0ml	20% glucose (filter sterilized)
1.0ml	1M thiamine-HCl
200ml	5X M-9 salts

5X M-9 salts (1 liter)

34g	Na_2HPO_4
15g	KH_2PO_4
2.5g	NaCl
5g	NH_4Cl

Dissolve in deionized water. Divide into 200ml aliquots and autoclave.

VIII.F. References

1. Sambrook, J. *et al.* (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY.
2. Schenborn, E. and Goiffon, V. (1991) Optimization of Transfectam®-mediated transfection using a luciferase reporter system. *Promega Notes* **33**, 8–11.
3. Cullen, B.R. (1987) Use of eukaryotic expression technology in the functional analysis of cloned genes. *Methods Enzymol.* **152**, 684–704.
4. Ausubel, F.M. *et al.* (1988) *Current Protocols in Molecular Biology*, John Wiley and Sons, NY.
5. Rosenthal, N. (1987) Identification of regulatory elements of cloned genes with functional assays. *Methods Enzymol.* **152**, 704–20.
6. Hawkins, E., Butler, B. and Wood, K.V. (2000) Bright-Glo™ and Steady-Glo™ Luciferase Assay Systems: Reagents for academic and industrial applications. *Promega Notes* **75**, 3–6.
7. Groskreutz, D.J. *et al.* (1995) Increased expression and convenience with the new pGL3 Luciferase Reporter Vectors. *Promega Notes* **50**, 2–8.
8. Sherf, B.A. and Wood, K.V. (1994) Firefly luciferase engineered for improved genetic reporting. *Promega Notes* **49**, 14–21.
9. 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.
10. Levitt, N. *et al.* (1989) Definition of an efficient synthetic poly(A) site. *Genes and Dev.* **3**, 1019–25.
11. Enriquez-Harris, P. *et al.* (1991) A pause site for RNA polymerase II is associated with termination of transcription. *EMBO J.* **10**, 1833–42.
12. 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.
13. 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.
14. Kozak, M. (1989) The scanning model for translation: An update. *J. Cell Biol.* **108**, 229–41.
15. Henikoff, S. (1987) Unidirectional digestion with exonuclease III in DNA sequence analysis. *Methods Enzymol.* **155**, 156.

VIII.G. pGL3-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. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number **U47295**) and on the Internet at: **www.promega.com/vectors/**

Table 4. Restriction Enzymes That Cut the pGL3-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AccI	1	2011
AccIII	2	783, 1299
Acc65I	1	1
AcyI	4	95, 121, 1514, 3690
AflIII	3	15, 581, 2260
Alw26I	5	1111, 1343, 1409, 3214, 3990
Alw44I	2	2574, 3820
AlwNI	1	2676
AspHI	5	11, 1553, 2578, 3739, 3824
AvaI	3	26, 32, 1144
AvaII	3	1267, 3291, 3513
BamHI	1	2004
BanII	4	11, 33, 1112, 4231
BbeI	1	124
BbsI	4	98, 1376, 1492, 2089
BbuI	1	751
BclI	1	668
BglI	2	3273, 4541
BglII	1	36
BsaI	1	3214
BsaAI	1	4302
BsaBI	1	2003
BsaHI	4	95, 121, 1514, 3690
BsaMI	3	60, 1823, 1916
BsmI	3	60, 1823, 1916
BspHI	3	671, 2980, 3988
BspMI	3	1477, 1486, 4781
BsrGI	1	578
BssSI	2	2433, 3817
BstZI	3	1755, 1759, 4651
ClaI	3	1997, 4709, 4813
Csp45I	1	257
DraI	4	1963, 3019, 3038, 3730
DraII	1	1267
DraIII	1	4305
DrdI	3	1489, 2368, 4349
DsaI	2	86, 458
EaeI	4	1755, 1759, 3541, 4651
EagI	3	1755,1759, 4651
EclHKI	1	3153
Eco47III	1	2136
Eco52I	3	1755, 1759, 4651
EcoICRI	1	9
EcoNI	3	645, 1045, 1705
EheI	1	122
FseI	1	1761
FspI	2	3375, 4548
HincII	3	1392, 1902, 2012
HindII	3	1392, 1902, 2012
HindIII	1	53
HpaI	1	1902
Hsp92I	4	95, 121, 1514, 3690

Table 4. Restriction Enzymes That Cut the pGL3-Basic Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
KasI	1	120	**SacI**	1	11
KpnI	1	5	**SalI**	1	2010
MluI	1	15	**ScaI**	3	253, 3633, 4716
NaeI	3	1759, 2130, 4199	SgrAI	1	1516
NarI	1	121	**SinI**	3	1267, 3291, 3513
NcoI	1	86	**SmaI**	1	28
NgoMIV	3	1757, 2128, 4197	**SphI**	1	751
NheI	1	21	SrfI	1	28
NotI	1	4651	**SspI**	3	3957, 4510, 4625
NspI	2	751, 2264	**StyI**	1	86
PaeR7I	2	1675, 4266	**VspI**	1	3325
PpuMI	1	1267	**XbaI**	1	1742
PshAI	1	2075	XcmI	1	823
Psp5II	1	1267	**XhoI***	1	32
PspAI	1	26	**XmaI**	1	26
PvuI	2	3523, 4569	**XmnI**	1	3752

*Due to the extent of supercoiling in this vector, the XhoI site has proven difficult to cut to completion under standard restriction digest conditions. For single XhoI digests, we recommend digesting the vector for a minimum of 2 hours using 20 units of enzyme per microgram of DNA at 37°C to ensure complete digestion. If performing a double digest with XhoI and another enzyme, linearize the vector using the companion enzyme prior to carrying out the XhoI digest. Under these conditions, XhoI will cut the vector following standard reactions conditions.

Table 5. Restriction Enzymes That Do Not Cut the pGL3-Basic Vector.

AatII	Bpu1102 I	Eco81I	PmeI	**SpeI**
AccB7I	Bsp120I	**EcoRI**	PmlI	SplI
AflII	**BssHII**	**EcoRV**	Ppu10I	Sse8387I
AgeI	Bst1107I	**I-PpoI**	**PstI**	**StuI**
ApaI	**Bst98I**	**NdeI**	**PvuII**	SwaI
AscI	**BstEII**	**NruI**	RsrII	**Tth111I**
AvrII	**BstXI**	**NsiI**	**SacII**	
BalI	**Bsu36I**	PacI	**SfiI**	
BbrPI	**CspI**	PflMI	**SgfI**	
BlpI	Eco72I	PinAI	**SnaBI**	

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

VIII.G. pGL3-Basic Vector Restriction Sites (continued)

Table 6. Restriction Enzymes That Cut the pGL3-Basic Vector 6 or More Times.

AciI	BstUI	HgaI	MnlI	Sau96I
AluI	**CfoI**	**HhaI**	MseI	ScrFI
BanI	Cfr10I	**HinfI**	**MspI**	SfaNI
BbvI	**DdeI**	**HpaII**	**MspA1I**	**TaqI**
BsaOI	**DpnI**	HphI	**NciI**	TfiI
BsaJI	DpnII	**Hsp92II**	**NdeII**	**Tru9I**
Bsp1286I	EarI	MaeI	NlaIII	**XhoII**
BsrI	Fnu4HI	MaeII	NlaIV	
BsrSI	**FokI**	MaeIII	PleI	
Bst71I	**HaeII**	**MboI**	**RsaI**	
BstOI	**HaeIII**	**MboII**	**Sau3AI**	

VIII.H. pGL3-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. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number **U47297**) and on the Internet at: **www.promega.com/vectors/**

Table 7. Restriction Enzymes that cut the pGL3-Enhancer Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AccI	1	2257	BbeI	1	124
AccIII	2	783,1299	BbsI	4	98, 1376, 1492, 2335
Acc65I	1	1			
AcyI	4	95, 121, 1514, 3936	**BbuI**	3	751, 2108, 2180
			BclI	1	668
AflIII	3	15, 581 ,2506	**BglI**	2	3519, 4787
Alw26I	5	1111, 1343, 1409, 3460, 4236	**BglII**	1	36
			BsaI	1	3460
Alw44I	2	2820, 4066	BsaAI	1	4548
AlwNI	1	2922	BsaBI	1	2003
AspHI	5	11, 1553, 2824, 3985, 4070	BsaHI	4	95, 121, 1514, 3936
AvaI	3	26, 32, 1144	**BsaMI**	3	60, 1823, 1916
AvaII	3	1267, 3537, 3759	BsmI	3	60, 1823, 1916
BamHI	1	2250	BspHI	3	671, 3226, 4234
BanII	4	11, 33, 1112, 4477	BspMI	3	1477, 1486, 5027

Table 7. Restriction Enzymes that cut the pGL3-Enhancer Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location
BsrGI	1	578
BssSI	2	2679, 4063
BstZI	3	1755, 1759, 4897
ClaI	3	1997, 4955, 5059
Csp45I	1	257
DraI	4	1963, 3265, 3284, 3976
DraII	1	1267
DraIII	1	4551
DrdI	3	1489, 2614, 4595
DsaI	2	86, 458
EaeI	4	1755, 1759, 3787, 4897
EagI	3	1755, 1759, 4897
EclHKI	1	3399
Eco47III	1	2382
Eco52I	3	1755, 1759, 4897
EcoICRI	1	9
EcoNI	3	645, 1045, 1705
EheI	1	122
FseI	1	1761
FspI	2	3621, 4794
HincII	3	1392, 1902, 2258
HindII	3	1392, 1902, 2258
HindIII	1	53
HpaI	1	1902
Hsp92I	4	95, 121, 1514, 3936
KasI	1	120
KpnI	1	5
MluI	1	15
NaeI	3	1759, 2376, 4445
NarI	1	121
NcoI	1	86
NgoMIV	3	1757, 2374, 4443
NheI	1	21
NotI	1	4897
NsiI	2	2106, 2178
NspI	4	751, 2108, 2180, 2510
PaeR7I	2	1675, 4512
Ppu10I	2	2102, 2174
PpuMI	1	1267
PshAI	1	2321
Psp5II	1	1267
PspAI	1	26
PvuI	2	3769, 4815
SacI	1	11
SalI	1	2256
ScaI	3	253, 3879, 4962
SgrAI	1	1516
SinI	3	1267, 3537, 3759
SmaI	1	28
SphI	3	751, 2108, 2180
SrfI	1	28
SspI	3	4203, 4756, 4871
StyI	1	86
VspI	1	3571
XbaI	1	1742
XcmI	1	823
XhoI*	1	32
XmaI	1	26
XmnI	1	3998

*Due to the extent of supercoiling in this vector, the XhoI site has proven difficult to cut to completion under standard restriction digest conditions. For single XhoI digests, we recommend digesting the vector for a minimum of 2 hours using 20 units of enzyme per microgram of DNA at 37°C to ensure complete digestion. If performing a double digest with XhoI and another enzyme, linearize the vector using the companion enzyme prior to carrying out the XhoI digest. Under these conditions, XhoI will cut the vector following standard reactions conditions.

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

VIII.H. pGL3-Enhancer Vector Restriction Sites (continued)

Table 8. Restriction Enzymes That Do Not Cut the pGL3-Enhancer Vector.

AatII	BlpI	**CspI**	PflMI	**SgfI**
AccB7I	Bpu1102I	Eco72I	PinAI	**SnaBI**
AflII	Bsp120I	Eco81I	PmeI	**SpeI**
AgeI	**BssHII**	**EcoRI**	PmlI	SplI
ApaI	Bst1107I	**EcoRV**	PstI	Sse8387I
AscI	B**st98I**	**I-PpoI**	**PvuII**	**StuI**
AvrII	**BstEII**	**NdeI**	RsrII	SwaI
BalI	**BstXI**	**NruI**	**SacII**	**Tth111I**
BbrPI	**Bsu36I**	PacI	**SfiI**	

Table 9. Restriction Enzymes That Cut the pGL3-Enhancer Vector 6 or More Times.

AciI	BstUI	HgaI	MnlI	Sau96I
AluI	**CfoI**	**HhaI**	MseI	ScrFI
BanI	Cfr10I	**HinfI**	**MspI**	SfaNI
BbvI	**DdeI**	**HpaII**	**MspA1I**	**TaqI**
BsaOI	**DpnI**	HphI	**NciI**	TfiI
BsaJI	DpnII	**Hsp92II**	**NdeII**	**Tru9I**
Bsp1286I	EarI	MaeI	NlaIII	**XhoII**
BsrI	Fnu4HI	MaeII	NlaIV	
BsrSI	**FokI**	MaeIII	PleI	
Bst71I	**HaeII**	**MboI**	**RsaI**	
BstOI	**HaeIII**	**MboII**	**Sau3AI**	

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

VIII.I. pGL3-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. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number **U47298**) and on the Internet at: **www.promega.com/vectors/**

Table 10. Restriction Enzymes That Cut the pGL3-Promoter Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AccI	1	2203
AccIII	2	975, 1491
Acc65I	1	1
AcyI	4	28, 313, 1706, 3882
AflIII	3	15, 773, 2452
Alw26I	5	1303, 1535, 1601, 3406, 4182
Alw44I	2	2766, 4012
AlwNI	1	2868
AspHI	5	11, 1745, 2770, 3931, 4016
AvaI	3	26, 32, 1336
AvaII	3	1459, 3483, 3705
AvrII	1	229
BamHI	1	2196
BanII	4	11, 33, 1304, 4423
BbeI	1	316
BbsI	4	290, 1568, 1684 2281
BbuI	1	943
BclI	1	860
BglI	3	182, 3465, 4733
BglII	1	36
BsaI	1	3406
BsaAI	1	4494
BsaBI	2	48, 2195
BsaHI	4	287, 313, 1706, 3882
BsaMI	3	252, 2015, 2108
BsmI	3	252, 2015, 2108
BspHI	3	863, 3172, 4180
BspMI	3	1669, 1678, 4973
BsrGI	1	770
BssSI	2	2625, 4009
BstZI	3	1947, 1951, 4843
ClaI	3	2189, 4901, 5005
Csp45I	1	449
DraI	4	2155, 3211, 3230, 3922
DraII	1	1459
DraIII	1	4497
DrdI	3	1681, 2560, 4541
DsaI	2	278, 650
EaeI	4	1947, 1951, 3733, 4843
EagI	3	1947, 1951, 4843
EclHKI	1	3345
Eco47III	1	2328
Eco52I	3	1947, 1951, 4843
EcoICRI	1	9
EcoNI	3	837, 1237, 1897
EheI	1	314
FseI	1	1953
FspI	2	3567, 4740
HincII	3	1584, 2094, 2204
HindII	3	1584, 2094, 2204
HindIII	1	245
HpaI	1	2094
Hsp92I	4	287, 313, 1706, 3882
KasI	1	312

VIII.I. pGL3-Promoter Vector Restriction Sites (continued)

Table 10. Restriction Enzymes That Cut the pGL3-Promoter Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
KpnI	1	5	**ScaI**	3	445, 3825, 4908
MluI	1	15	**SfiI**	1	182
NaeI	3	1951, 2322, 4391	SgrAI	1	1708
NarI	1	313	**SinI**	3	1459, 3483, 3705
NcoI	1	278	**SmaI**	1	28
NgoMIV	3	1949, 2320, 4389	**SphI**	1	943
NheI	1	21	SrfI	1	28
NotI	1	4843	**SspI**	3	4149, 4702, 4817
NspI	2	943, 2456	**StuI**	1	228
PaeR7I	2	1867, 4458	**StyI**	2	229, 278
PpuMI	1	1459	**VspI**	1	3517
PshAI	1	2267	**XbaI**	1	1934
Psp5II	1	1459	XcmI	1	1015
PspAI	1	26	**XhoI***	1	32
PvuI	2	3715, 4761	**XmaI**	1	26
SacI	1	11	**XmnI**	1	3944
SalI	1	2202			

*Due to the extent of supercoiling in this vector, the XhoI site has proven difficult to cut to completion under standard restriction digest conditions. For single XhoI digests, we recommend digesting the vector for a minimum of 2 hours using 20 units of enzyme per microgram of DNA at 37°C to ensure complete digestion. If performing a double digest with XhoI and another enzyme, linearize the vector using the companion enzyme prior to carrying out the XhoI digest. Under these conditions, XhoI will cut the vector following standard reactions conditions.

Table 11. Restriction Enzymes That Do Not Cut the pGL3-Promoter Vector.

AatII	Bpu1102I	Eco72I	PflMI	**SgfI**
AccB7I	Bsp120I	Eco81I	PinAI	**SnaBI**
AflII	**BssHII**	**EcoRI**	PmeI	**SpeI**
AgeI	Bst1107I	**EcoRV**	PmlI	SplI
ApaI	**Bst98I**	**I-PpoI**	Ppu10I	Sse8387I
AscI	**BstEII**	**NdeI**	**PstI**	SwaI
BalI	**BstXI**	**NruI**	**PvuII**	**Tth111I**
BbrPI	**Bsu36I**	**NsiI**	RsrII	
BlpI	**CspI**	PacI	**SacII**	

Table 12. Restriction Enzymes That Cut the pGL3-Promoter Vector 6 or More Times.

AciI	BstUI	HgaI	MnlI	Sau96I
AluI	**CfoI**	**HhaI**	MseI	ScrFI
BanI	Cfr10I	**HinfI**	**MspI**	SfaNI
BbvI	**DdeI**	**HpaII**	**MspA1I**	**TaqI**
BsaOI	**DpnI**	HphI	**NciI**	TfiI
BsaJI	DpnII	**Hsp92II**	**NdeII**	**Tru9I**
Bsp1286I	EarI	MaeI	NlaIII	**XhoII**
BsrI	Fnu4HI	MaeII	NlaIV	
BsrSI	**FokI**	MaeIII	PleI	
Bst71I	**HaeII**	**MboI**	**RsaI**	
BstOI	**HaeIII**	**MboII**	**Sau3AI**	

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

VIII.J. pGL3-Control Vector Restriction Sites

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that this information has not been verified 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. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number **U47296**) and on the Internet at: **www.promega.com/vectors/**

Table 13. Restriction Enzymes That Cut the pGL3-Control Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AccI	1	2449	BspMI	3	1669, 1678, 5219
AccIII	2	975, 1491	BsrGI	1	770
Acc65I	1	1	BssSI	2	2871, 4255
AcyI	4	287, 313, 1706, 4128	**BstZI**	3	1947, 1951, 5089
			ClaI	3	2189, 5147, 5251
AflIII	3	15, 773, 2698	**Csp45I**	1	449
Alw26I	5	1303, 1535, 1601, 3652, 4428	**DraI**	4	2155, 3457, 3476, 4168
Alw44I	2	3012, 4258	DraII	1	1459
AlwNI	1	3114	DraIII	1	4743
AspHI	5	11, 1745, 3016, 4177, 4262	DrdI	3	1681, 2806, 4787
			DsaI	2	278, 650
AvaI	3	26, 32, 1336	EaeI	4	1947, 1951, 3979, 5089
AvaII	3	1459, 3729, 3951			
AvrII	1	229	EagI	3	1947, 1951, 5089
BamHI	1	2442	**EclHKI**	1	3591
BanII	4	11, 33, 1304, 4669	**Eco47III**	1	2574
BbeI	1	316	Eco52I	3	1947, 1951, 5089
BbsI	4	290, 1568, 1684, 2527	**EcoICRI**	1	9
			EcoNI	3	837, 1237, 1897
BbuI	3	943, 2300, 2372	EheI	1	314
BclI	1	860	FseI	1	1953
BglI	3	182, 3711, 4979	FspI	2	3813, 4986
BglII	1	36	**HincII**	3	1584, 2094, 2450
BsaI	1	3652	HindII	3	1584, 2094, 2450
BsaAI	1	4740	**HindIII**	1	245
BsaBI	2	48, 2195	**HpaI**	1	2094
BsaHI	4	287, 313, 1706, 4128	**Hsp92I**	4	287, 313, 1706, 4128
BsaMI	3	252, 2015, 2108	KasI	1	312
BsmI	3	252, 2015, 2108	**KpnI**	1	5
BspHI	3	863, 3418, 4426	**MluI**	1	15

Table 13. Restriction Enzymes That Cut the pGL3-Control Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NaeI	3	1951, 2568, 4637	**SalI**	1	2448
NarI	1	313	**ScaI**	3	445, 4071, 5154
NcoI	1	278	**SfiI**	1	182
NgoMIV	3	1949, 2566, 4635	SgrAI	1	1708
NheI	1	21	**SinI**	3	1459, 3729, 3951
NotI	1	5089	**SmaI**	1	28
NsiI	2	2298, 2370	**SphI**	3	943, 2300, 2372
NspI	4	943, 2300, 2372, 2702	SrfI	1	28
			SspI	3	4395, 4948, 5063
PaeR7I	2	1867, 4704	**StuI**	1	228
Ppu10I	2	2294, 2366	**StyI**	2	229, 278
PpuMI	1	1459	**VspI**	1	3763
PshAI	1	2513	**XbaI**	1	1934
Psp5II	1	1459	XcmI	1	1015
PspAI	1	26	**XhoI***	1	32
PvuI	2	3961, 5007	**XmaI**	1	26
SacI	1	11	**XmnI**	1	4190

*Due to the extent of supercoiling in this vector, the XhoI site has proven difficult to cut to completion under standard restriction digest conditions. For single XhoI digests, we recommend digesting the vector for a minimum of 2 hours using 20 units of enzyme per microgram of DNA at 37°C to ensure complete digestion. If performing a double digest with XhoI and another enzyme, linearize the vector using the companion enzyme prior to carrying out the XhoI digest. Under these conditions, XhoI will cut the vector following standard reactions conditions.

Table 14. Restriction Enzymes That Do Not Cut the pGL3-Control Vector.

AatII	Bpu1102I	Eco72I	PinAI	**SpeI**
AccB7I	Bsp120I	Eco81I	PmeI	SplI
AflII	**BssHII**	**EcoRI**	PmlI	Sse8387I
AgeI	Bst1107I	**EcoRV**	**PstI**	SwaI
ApaI	**Bst98I**	**I-PpoI**	**PvuII**	**Tth111I**
AscI	**BstEII**	**NdeI**	RsrII	
BalI	**BstXI**	**NruI**	**SacII**	
BbrPI	**Bsu36I**	PacI	**SgfI**	
BlpI	**CspI**	PflMI	**SnaBI**	

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

VIII.J. pGL3-Control Vector Restriction Sites (continued)

Table 15. Restriction Enzymes That Cut the pGL3-Control Vector 6 or More Times.

AciI	**BstOI**	**HaeIII**	**MboI**	PleI
AluI	BstUI	HgaI	**MboII**	**RsaI**
BanI	**CfoI**	**HhaI**	MnlI	**Sau3AI**
BbvI	Cfr10I	**HinfI**	MseI	Sau96I
BsaOI	**DdeI**	**HpaII**	**MspI**	ScrFI
BsaJI	**DpnI**	HphI	**MspA1I**	SfaNI
Bsp1286I	EarI	**Hsp92II**	**NciI**	**TaqI**
BsrI	Fnu4HI	MaeI	**NdeII**	TfiI
BsrSI	**FokI**	MaeII	NlaIII	**Tru9I**
Bst71I	**HaeII**	MaeIII	NlaIV	**XhoII**

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

VIII.K. Related Products

Product	Size	Cat.#
GLprimer2 (counter clockwise)	2µg	E1661
RVprimer3 (clockwise)	2µg	E4481
RVprimer4 (counter clockwise)	2µg	E4491
PureYield™ Plasmid Midiprep System	25 preps	A2492
	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.

Luminometers

Product	Size	Cat.#
GloMax® 96 Microplate Luminometer	1 each	E6501
GloMax® 20/20 Luminometer	1 each	E5311

Available with single or dual injectors.

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*/Gal4UAS/Hygro] Vector	20µg	C9351

The complete listing of pGL4 Luciferase Vectors can be found at:
www.promega.com/pgl4/

[a]U.S. Pat. No. 5,670,356 has been issued to Promega Corporation for a modified luciferase technology.

[b]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. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.