pGL3 Luciferase Reporter Vectors

Promega

Technical Manual No. 033

INSTRUCTIONS FOR USE OF PRODUCTS E1741, E1751, E1761, E1771.
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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 pGL2 Luciferase Reporter Vectors[b] was redesigned for the pGL3 Vectors 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, the Luciferase Reporter Vectors contain numerous features aiding in the structural characterization of the putative regulatory sequences under investigation.

II. pGL3 Vector Maps and Sequence Reference Points

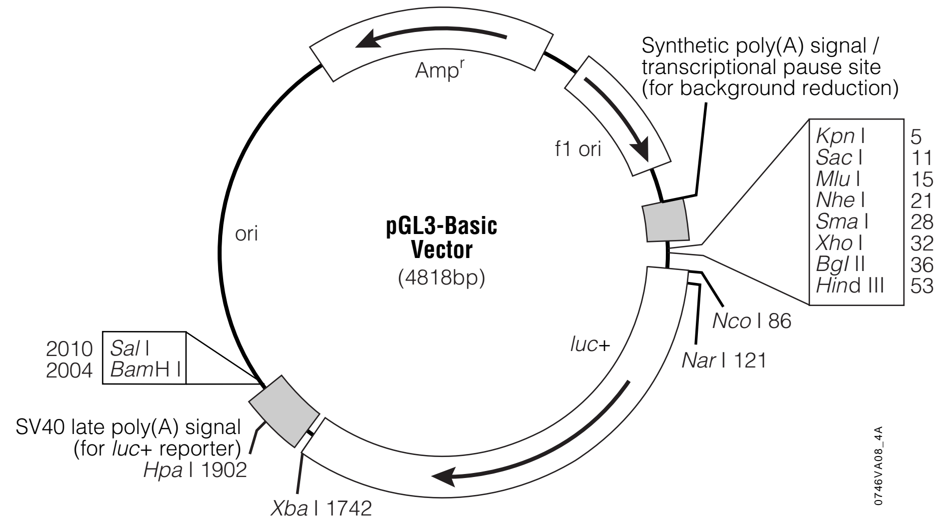

Figure 1. pGL3-Basic Vector circle map. Additional description: *luc*+, cDNA encoding the modified firefly luciferase; Amp^r, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of replication in *E. coli*. Arrows within *luc*+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

SV40 Promoter	(none)
SV40 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
Col E 1-derived plasmid replication origin	2318
β-lactamase gene (Amp^r)	3080–3940
f1 origin	4072–4527
Synthetic poly(A) signal	4658–4811
RVprimer3 binding site	4760–4779

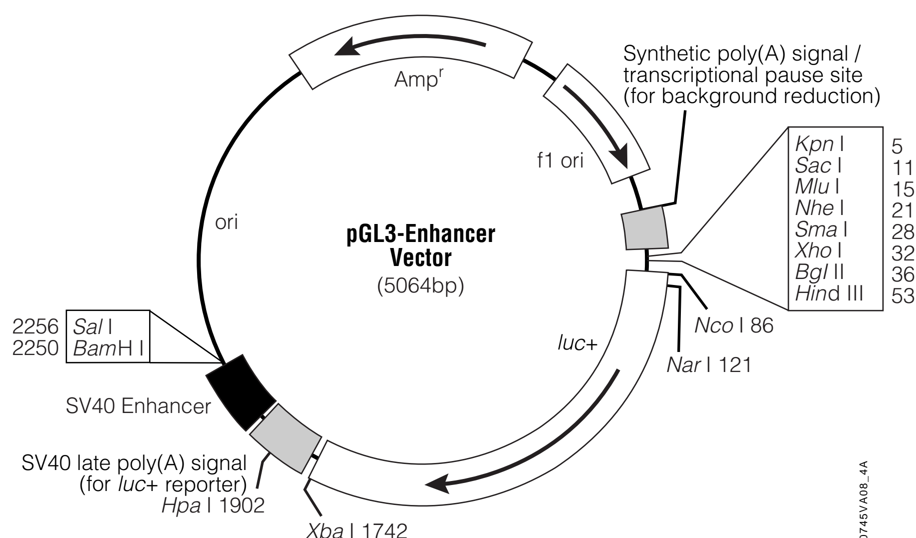

Figure 2. The pGL3-Enhancer Vector circle map. Additional description: *luc*+, cDNA encoding the modified firefly luciferase; Amp^r, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli*. Arrows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

SV40 Promoter	(none)
Multiple cloning region	1–58
Luciferase gene (*luc*+)	88–1740
GLprimer2 binding site	89–111
SV40 late poly(A) signal	1772–1993
SV40 Enhancer	2013–2249
RVprimer4 binding site	2307–2326
Col E 1-derived plasmid replication origin	2564
β-lactamase gene (Amp^r)	3326–4186
f1 origin	4318–4773
Synthetic poly(A) signal	4904–5057
RVprimer3 binding site	5006–5025

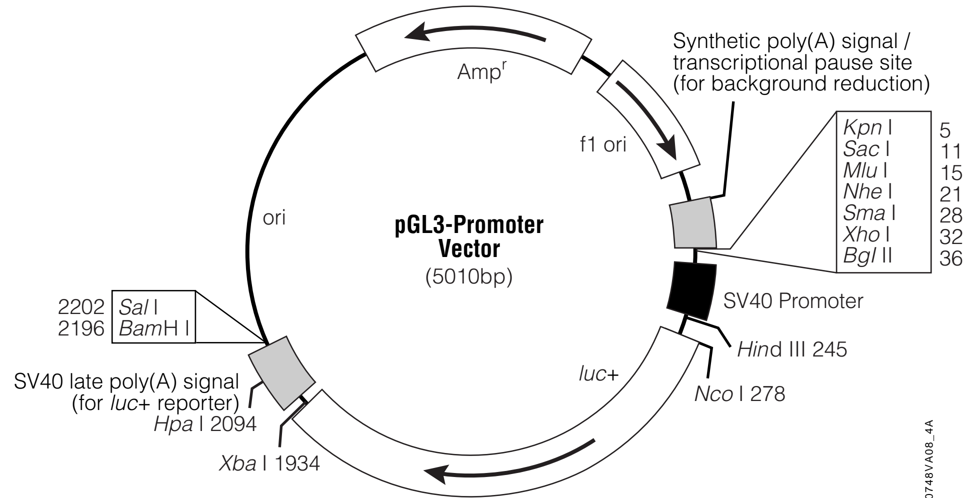

Figure 3. The pGL3-Promoter Vector circle map. Additional description: *luc*+, cDNA encoding the modified firefly luciferase; Amp^r, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of replication in *E. coli.* Arrows within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

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

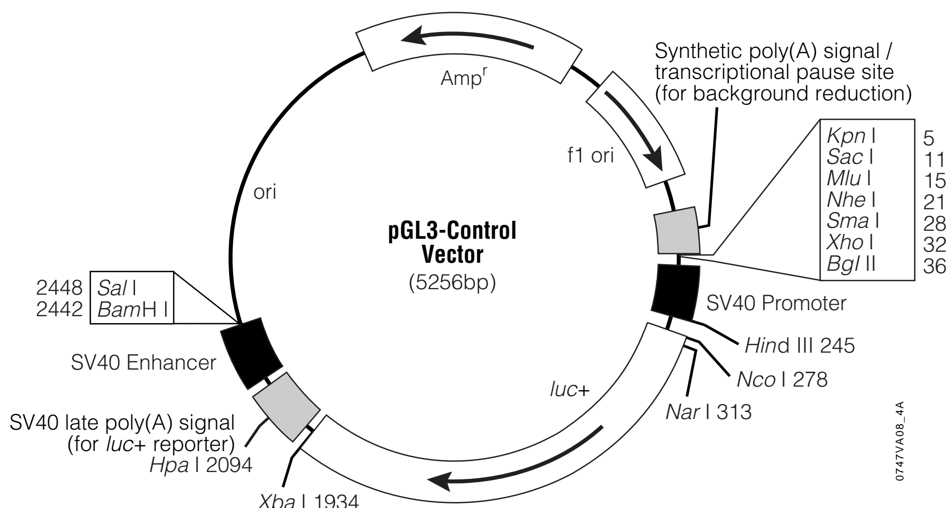

Figure 4. pGL3-Control Vector circle map. Additional description: *luc*+, cDNA encoding the modified firefly luciferase; Ampr, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of replication in *E. coli.* Arrows within *luc*+ and the Ampr 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
SV40 Promoter	48–250
Luciferase gene (*luc*+)	280–1932
GLprimer2 binding site	281–303
SV40 late poly(A) signal	1964–2185
SV40 Enhancer	2205–2441
RVprimer4 binding site	2499–2518
*Col*E 1-derived plasmid replication origin	2756
β-lactamase gene (Ampr)	3518–4378
f1 origin	4510–4965
Synthetic poly(A) signal	5096–5249
RVprimer3 binding site	5198–5217

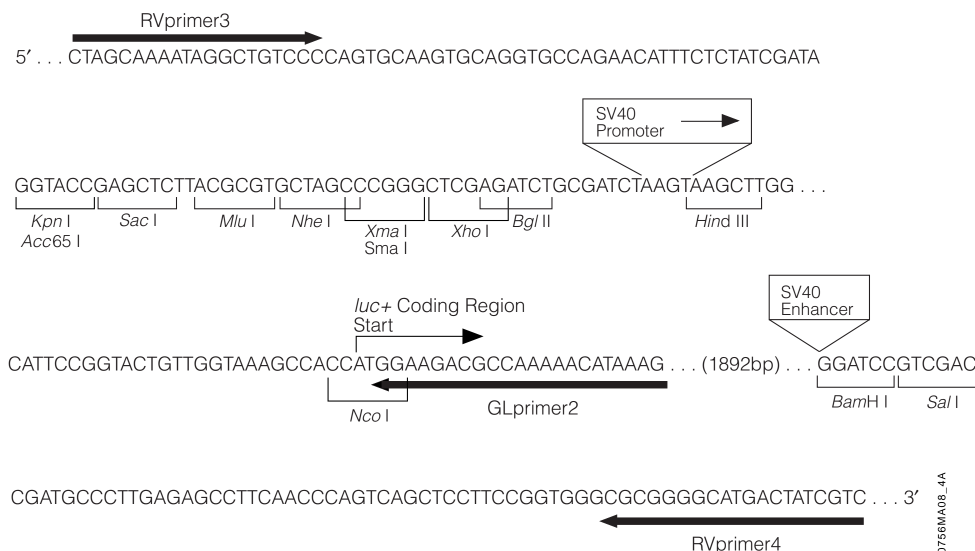

Figure 5. pGL3 Vector multiple cloning regions. The upstream and downstream cloning sites and the location of the sequencing primers, GLprimer2, RVprimer3 and RVprimer4 are shown. The large primer arrows indicate the direction of sequencing. The positions of the promoter (in the pGL3-Promoter and pGL3-Control 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.

III. Product Components

Product	Size	Cat.#
pGL3-Control Vector[a,b]	20µg	E1741
pGL3-Basic Vector[a,b]	20µg	E1751
pGL3-Promoter Vector[a,b]	20µg	E1761
pGL3-Enhancer Vector[a,b]	20µg	E1771

Vectors are supplied with a glycerol stock of bacterial strain JM109 cells. The JM109 cells do not contain the vector and are not competent cells. Information on related products, including the Luciferase Assay System, is provided in Sections III–VIII.

Product	Size	Cat.#
GLprimer2 (counter clockwise)	2µg	E1661
RVprimer3 (clockwise)	2µg	E4481
RVprimer4 (counter clockwise)	2µg	E4491

Storage Conditions: Store the pGL3 Luciferase Reporter Vectors at –20°C and the glycerol stock of JM109 cells at –70°C.

IV. Cloning Methods

A. Cloning Strategies

The restriction sites for *Xh*o I and *Sal* I have compatible ends, as do *Bgl* II and *Bam*H I. Therefore, cloning into the *Xho* I or *Bgl* II sites upstream of *luc*+, or the downstream *Sal* I or *Bam*H I sites, allows for 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 examinable.

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 VII). Specifically, the *Kpn* I or *Sac* I site is needed to generate a 3´-overhang upstream of the insert.

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[c] 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 Shrimp Alkaline Phosphatase (Cat.# M8201) 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*[d] *Technical Bulletin*, #TB118. Alternatively, nonfractionated restriction fragments can be cloned into the target plasmid, and the desired recombinant can then be identified by gel electrophoresis of plasmid DNA.

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Protocols for restriction digestion, alkaline phosphatase treatment, linker ligation and transformation of competent cells can be found in Promega's *Protocols and Applications Guide* (1) or in *Molecular Cloning, A Laboratory Manual* (2).

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 Luciferase Reporter Vectors are supplied with JM109 bacterial cells (*end*A1, *rec*A1, *gyr*A96, *thi*, *hsd* R17, (r_K–,m_K+), *rel*A1, *sup*E44, Δ(*lac–pro*AB), [F´, *tra*D36, *pro*AB, *lac*I^qZΔM15]). 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.

D. Isolation of Plasmid DNA

The *Wizard*® Plus *SV Minipreps DNA Purification System*(e) *Technical Bulletin*, #TB225, or the *Wizard*® Plus *Midipreps DNA Purification System*(f) *Technical Bulletin*, #TB173, may be used for small-scale preparation of plasmid DNA for screening clones. Large-scale DNA preparations can be made for sequencing or restriction digestion using the *Wizard*® Plus *Maxipreps DNA Purification System*(f) *Technical Bulletin*, #TB139, or the *Wizard*® Plus *Megapreps DNA Purification System*(f) *Technical Bulletin*, #TB140. DNA suitable for transfection may be purified using a modification of the Wizard® Maxipreps protocol (3) or by using the *Wizard*® *PureFection Plasmid DNA Purification System*(g) *Technical Bulletin*, #TB259, or by CsCl gradient preparation (4).

Note: An *Experienced User's Protocol* can be found at the end of this Technical Manual.

The following protocol allows for the rapid isolation of large quantities of plasmid DNA without the need for column purification or banding in CsCl gradients (4). The procedure takes advantage of the rapid alkaline denaturation of plasmid and chromosomal DNA and the selective renaturation of plasmid DNA following neutralization of the solution. The polyethylene glycol (PEG) precipitation step is included to help remove contaminants that could interfere with restriction digestions, sequencing procedures or transfection of mammalian cells.

The volume of the culture used may be adjusted depending upon the amount of DNA required for subsequent manipulations. The Luciferase Reporter Vectors are high copy number plasmids (200–300 copies per cell). Using the protocol described below, 150–500µg of DNA can be obtained from a 250ml culture.

Materials to Be Supplied by the User
(Solution compositions are provided in Section X.F.)

- LB medium
- lysis buffer for plasmid preps
- TE-saturated phenol:chloroform: isoamyl alcohol
- TE buffer
- 13% (w/v) polyethylene glycol (M.W. 6,000–8,000) in water
- potassium acetate solution (pH 4.8)

1. Prepare 250ml of culture by incubating overnight in LB medium containing 100µg/ml ampicillin.
2. Centrifuge the cells at 5,000 × *g* for 15 minutes at 4°C. Remove and discard the supernatant.
3. Resuspend the cells in 6ml of freshly prepared ice-cold lysis buffer by careful pipetting with a 10ml pipette. Incubate in ice water for 20 minutes.
4. Add 12ml of 0.1N NaOH, 1% SDS (prepared fresh). Mix carefully and thoroughly by inversion. Do not vortex.
5. Add 7.5ml of potassium acetate solution (pH 4.8). Mix carefully by inversion and incubate in ice water for 10 minutes.
6. Centrifuge at 12,000 × *g* for 15 minutes. Transfer the supernatant to a fresh tube, avoiding the white precipitate. Add 50µl of RNase A (1mg/ml stock) to the supernatant. Incubate for 20 minutes at 37°C.
7. Extract with 1 volume of TE-saturated phenol:chloroform:isoamyl alcohol. Centrifuge at 12,000 × *g* for 10 minutes.
8. Save the upper, aqueous phase and repeat the TE-saturated phenol:chloroform:isoamyl alcohol extraction as described in Step 7 above.
9. Extract with one volume of chloroform:isoamyl alcohol (24:1) by vortexing for 1 minute. Centrifuge at 12,000 × *g* for 10 minutes.
10. Transfer the upper, aqueous phase to a fresh tube and add 2 volumes of 100% ethanol. Centrifuge at 12,000 × *g* for 20 minutes.
11. **Optional:** Dissolve the pellet in 1.6ml of water. Add 0.4ml of 4M NaCl and mix. Add 2ml of 13% (w/v) polyethylene glycol (PEG, M.W. 6,000–8,000) and mix. Incubate in ice water for 60 minutes. PEG is used to separate small nucleotides from plasmid DNA. Centrifuge at 12,000 × *g* for 10 minutes.
12. Remove the supernatant and wash the pellet with 70% ethanol. Centrifuge at 12,000 × *g* for 5 minutes.
13. Dry the pellet under vacuum. Dissolve the pellet in water or TE buffer (100–500µl).

V. Transfection of Mammalian Cells

Transfection of DNA into eukaryotic cells may be mediated by cationic lipid compounds (5), calcium phosphate (6,7), DEAE-dextran (6,8), or electroporation (7). Transfection systems based on cationic lipids (e.g., Transfectam® Reagent[h] [Cat.# E1232], TransFast™ Reagent[i] [Cat.# E2431], or Tfx™ Reagents[j] [Cat.# E1811, E2381, E2391]), calcium phosphate and DEAE-dextran are available from Promega. For information on the Transfectam® protocol, please request the *Transfectam® Reagent Technical Bulletin*, #TB116. For information regarding use of the TransFast™ Transfection Reagent, request Technical Bulletin #TB260. Protocols for the use of Tfx™ Reagents are included in Technical Bulletin #TB216. For transfection procedures using calcium phosphate or DEAE-dextran, please request the *ProFection® Mammalian Transfection System Technical Manual*, #TM012 (Cat.# E1200, E1210).

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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, Promega has developed three luciferase assays with different, but complementary, characteristics: Luciferase Assay System[k] (Cat.# E1500), Bright-Glo™ Luciferase Assay System[k] (Cat.# E2610), and Steady-Glo® Luciferase Assay System[k] (Cat.# E2510). Reagent choice depends on weighing the relative importance of experimental format, assay sensitivity, and luminescence duration.

Table 1. Characteristics of Promega's Luciferase Assay Reagents.

	Bright-Glo™ Reagent	Steady-Glo® Reagent	Luciferase Assay Reagent
Format	NH or H	NH or H	NH
Process	continuous	batch	bench scale
Number of Steps	1	1	4
Sensitivity	highest	lower	higher
Signal Half-Life	~30 minutes	~5 hours	~12 minutes
Precision	High	High	High
Cell Lysis Time	~2 minutes maximum	~5 minutes maximum	NA
Reagent Prep Time	<30 seconds	<30 seconds	Up to 40 minutes

NH = nonhomogeneous; 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™ and Steady-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 very 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 experimental designs in which many microplates are processed as a batch.

In contrast, the Bright-Glo™ Reagent provides high assay sensitivity with lower luminescence duration (<10% decrease per 5 minutes). This reagent is designed for general research applications and for experimental designs 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 Luciferase Assay System, Bright-Glo™ Reagent and Steady-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, or the *Bright-Glo™ Luciferase Assay System Technical Manual*, #TM052.

VII. Generation of Nested Deletions

Unidirectional deletions of any inserted DNA can be made using a procedure developed by Henikoff (10) in which Exonuclease III (Exo III) is used to specifically digest insert DNA from a 5´ protruding or blunt-ended restriction site. In the pGL3 Luciferase Reporter Vectors, these 5´ overhangs are supplied by digesting the plasmid with *Bgl* II, *Mlu* I, *Nhe* I, *Xho* I or *Xma* I. When the plasmids are cut with *Kpn* I or *Sac* I, which yield 3´ overhangs, the Exo III will be unable to digest in the other direction.

The uniform rate of enzyme digestion allows deletions of various lengths to be made simply by removing timed aliquots from the reaction. Given that small deletions (less than 500 bases) are probably desired, we recommend performing the reactions at a lower temperature (between 4–16°C). Samples from the Exo III reaction are removed at timed intervals to tubes containing S1 nuclease, which removes the remaining single-stranded tails. The low pH and the presence of zinc cations in the S1 buffer effectively inhibit further digestion by Exo III. After neutralization and heat inactivation of the S1 nuclease, Klenow DNA polymerase is added to flush the ends, which are then ligated to circularize the deletion-containing vectors. The ligation mixtures are used directly to transform competent cells. Each successive time point yields a collection of subclones containing clustered deletions extending further into the original insert.

For a more detailed protocol, please request the *Erase-a-Base® System Technical Manual*, #TM006.

VIII. Generation of Single-Stranded DNA and Site-Specific Mutations

A. Production of Single-Stranded DNA

To generate single-stranded DNA (ssDNA) from the pGL3 Vectors, bacterial cells containing pGL3 Vectors are infected with an appropriate helper phage. The plasmid then enters the f1 replication mode, and the resulting ssDNA is exported from the cell as an encapsulated phage-like particle. The single-stranded plasmid DNA is purified from the supernatant by simple precipitation and extraction procedures. Promega's *Protocols and Applications Guide* (1) contains protocols for the preparation and analysis of ssDNA suitable for mutagenesis and sequencing (1,11–13).

B. Generation of Site-Specific Mutations

Site-specific mutagenesis, as developed by Hutchinson *et al.* (14), is accomplished by hybridizing to ssDNA a synthetic oligonucleotide that is complementary to the single-stranded template except for a region of mismatch near the center. It is this region that contains the desired nucleotide change or changes. Following hybridization with the single-stranded target DNA, the oligonucleotide is extended with DNA polymerase to create a double-stranded structure. The nick is then sealed with DNA ligase, and the duplex structure is transformed into an *E. coli* host. Theoretically, the yield of mutants using the Hutchinson procedure should be 50% (due to semi-conservative replication). In practice, however, the mutant yield may be much lower, often only a few percent or less. This is assumed to be due to factors such as incomplete in vitro polymerization, primer displacement by the DNA polymerase used in the fill-in reaction, and in vivo host-directed mismatch repair mechanisms, which favor repair of the nonmethylated, newly synthesized DNA strand. Because of the low mutant yield, methods have been developed to increase the mutation frequency.

Promega's Altered Sites® II in vitro Mutagenesis Systems[l] (Cat.# Q6080, Q6090, Q6210) use antibiotic selection to obtain consistently high mutagenesis frequencies (often >90%) using ssDNA or dsDNA templates. These systems provide a simple, one-day procedure for generation and selection of oligonucleotide-directed mutants and include the ability to perform sequential rounds of mutagenesis without subcloning and to express the mutated gene products in vivo or in vitro. For further information, please request the *Altered Sites® II in vitro Mutagenesis System Technical Manual,* #TM001.

IX. Sequencing of Luciferase Reporter Vectors

It may be desirable to sequence the DNA inserted into the Luciferase Reporter Vectors. Two examples of such applications are to determine the exact position of generated deletions (see Section VII) and to confirm production of a site-specific mutation (see Section VIII.B). 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 *Bam*H I and *Sal* I 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 that all three primers can be used for dsDNA sequencing, but only RVprimer4 and GLprimer2 can also be used for ssDNA sequencing.

There are many methods for DNA sequencing; the most appropriate method will depend on the specific application and on your experience. Thermal cycle sequencing takes advantage of the intrinsic properties of the DNA polymerase isolated from *Thermus aquaticus* (*Taq* DNA polymerase). *Thermus aquaticus* is an extremely thermophilic microorganism whose DNA polymerase shows thermal stability to 95°C (15,16). Promega's *fmol*®[m,o] and SILVER SEQUENCE™ DNA Sequencing Systems[m,n,o] include Promega's Sequencing Grade *Taq* DNA Polymerase[o]

(Cat.# M2031, M2035), which is a modified form that gives superior results on dsDNA templates because it lacks 5´→3´ exonuclease activity. Sequencing Grade *Taq* DNA Polymerase produces a uniform band intensity, low background and a high degree of accuracy.

These sequencing systems use a thermocycling apparatus that yields a linear amplification of template DNA, decreasing the amount of template necessary to obtain sequence data. The high temperatures used in this procedure eliminate the need for alkaline denaturation and ethanol precipitation of dsDNA templates. They also increase the stringency of primer hybridization, providing more accurate sequence data, and decrease DNA secondary structure, permitting polymerization through highly structured regions (16). The *fmol*® System (Cat.# Q4100) provides the option of using either ^{32}P or ^{35}S radioactively end-labeled primers or direct incorporation of radioactive label for sequencing reactions. The SILVER SEQUENCE™ System (Cat.# Q4130), by contrast, uses silver staining detection to eliminate the need for radioactivity in sequencing reactions.

For further information, please request the fmol® or *SILVER SEQUENCE™ Sequencing System Technical Manuals,* #TM024 or #TM023, respectively.

X. Appendix

A. Common Structural Elements of the pGL3 Luciferase Reporter Vectors

Except for the promoters and enhancers, the four pGL3 Luciferase Reporter Vectors are structurally identical. Each plasmid's distinguishing features are summarized in Section X.D. 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 (*Xho* I and *Bgl* II) yield cohesive ends compatible with the downstream sites (*Sal* I and *Bam*H I, respectively), allowing the interchange of the DNA insert for rapid analysis of positional effects.

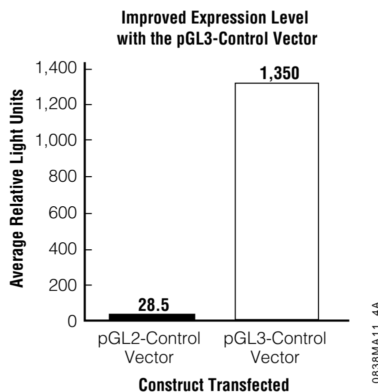

Figure 6. Comparison of luciferase activities expressed in HeLa cells transfected with the pGL2-Control and pGL3-Control Reporter Vectors. The expression level of *luc*+ is dramatically higher with the pGL3-Control Vectors. In repeated experiments with several cell lines, we observed 20- to 100-fold 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.)

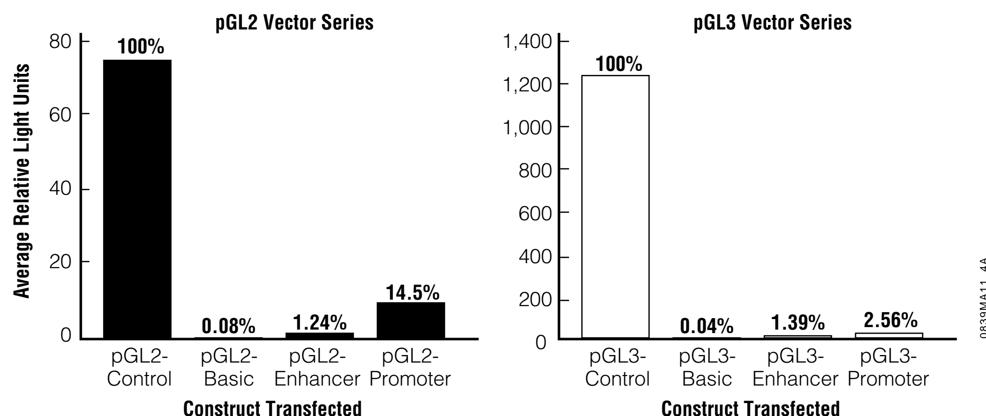

Figure 7. A representative experiment comparing luciferase activities expressed in HeLa cells transfected with the pGL2 and pGL3 Vector series. The increase in luciferase expression observed with these new vectors provides greater sensitivity, while maintaining relatively low background luciferase expression.

B. Advantages of the pGL3 Vectors

The pGL3 Luciferase Reporter Vectors provide significant advances over the pGL2 Reporter Vectors. The pGL3 Reporter Vectors contain a modified firefly luciferase cDNA designated *luc*+ and a redesigned vector backbone. These changes increase luciferase expression, improve in vivo vector stability, and provide greater flexibility in performing genetic manipulations. The modified reporter vectors result 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 these new 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 (17). Therefore, it is important to include the appropriate control vectors in all experiments.

C. Description of the Reporter Vector Changes

Modifications were made to both the luciferase gene (*luc*+) and the vector back-bone. The 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, refer to #TB208 and reference 19.)

Four major modifications were made to the pGL2 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 (19); ii) a synthetic poly(A) and transcriptional pause site (20,21) 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 (22,23); and iv) a Kozak consensus sequence (25) has been inserted to increase the efficiency of translation initiation of the luciferase gene (17; Table 2).

Table 2. Changes Made to the pGL3 Vectors.

Change from pGL2	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.	(18)
A unique *Nco* I site created at 5´ end of *luc*+ gene. *Nco* I sites removed from SV40 enhancer and promoter regions.	Ability to create N-terminal gene fusions with *luc*+ using unique *Nco* I 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.	(23,24)
Poly(A) site for back-ground 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.	(20,21)
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).	(19)
Kozak consensus sequence created immediately 5´ of the *luc*+ gene.	Provides for optimal translation efficiency.	(25)
Unique *Xba* I site created just downstream of the *luc*+ gene.	User convenience; facilitates subcloning of the *luc*+ gene.	
Sma I site moved to internal position in MCS.	User convenience; blunt-ended inserts can now be cleaved on either side by restriction endonucleases.	

D. Distinguishing Features of the pGL3 Luciferase Reporter Vectors

Maps of the pGL3-Basic, pGL3-Promoter, pGL3-Enhancer and pGL3-Control Vectors are shown in Figures 1–4. The DNA sequences and listings of restriction sites for these vectors are provided in Section X.H–K.

pGL3-Basic

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 *Bam*H I or *Sal* I sites downstream of the *luc*+ gene.

pGL3-Enhancer

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.

pGL3-Promoter

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.

pGL3-Control

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 con-venient internal standard for promoter and enhancer activities expressed by pGL3 recombinants.

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.

E. 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 (10) 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 site of the pGL3 Vectors contains

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upstream *Kpn* I and *Sac* I 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.

F. Composition of Buffers and Solutions

lysis buffer for plasmid preps

25mM	Tris-HCl (pH 7.5)
10mM	EDTA
15%	sucrose
2mg/ml	lysozyme

M-9 plates (per liter)

6.0g	Na_2HPO_4
3.0g	KH_2PO_4
0.5g	NaCl
1.0g	NH_4Cl
15g	agar

Add deionized water to approximately 1 liter. Autoclave. Cool to 50°C. Add the following sterilized solutions:

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

TE buffer (pH 8.0)

10mM	Tris-HCl (pH 8.0)
1mM	EDTA

TE-saturated phenol:chloroform:isoamyl alcohol

Mix equal parts of TE buffer and phenol and allow the phases to separate. Then mix 1 part of the lower, phenol phase with 1 part of chloroform: isoamyl alcohol (24:1).

LB medium (per liter)

10g	Bacto®-tryptone
5g	Bacto®-yeast extract
5g	NaCl

potassium acetate (pH 4.8)

Prepare 60ml of 5M potassium acetate. Add 11.5ml of glacial acetic acid and 28.5ml of water. This solution will be 3M with respect to potassium and 5M with respect to acetate. Store at 4°C.

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G. References

1. *Protocols and Applications Guide*, Third Edition, (1996) Promega Corporation.
2. Sambrook, J. *et al.* (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY.
3. Brondyk, B. *et al.* (1994) A comparison of the Wizard™ Maxipreps DNA Purification System and alkaline lysis/cesium chloride method for isolating transfection-quality plasmid DNA. *Promega Notes* **47**, 2–5.
4. Birnboim, H.C. and Doly, J. (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucl. Acids Res.* **7**, 1513–23.
5. Schenborn, E. and Goiffon, V. (1991) Optimization of Transfectam®-mediated transfection using a luciferase reporter system. *Promega Notes* **33**, 8–11.
6. Cullen, B.R. (1987) Use of eukaryotic expression technology in the functional analysis of cloned genes. *Meth. Enzymol.* **152**, 684–704.
7. Ausubel, F.M. *et al.* (1988) *Current Protocols in Molecular Biology*, John Wiley and Sons, NY.
8. Rosenthal, N. (1987) Identification of regulatory elements of cloned genes with functional assays. *Meth. Enzymol.* **152**, 704–20.
9. 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.
10. Henikoff, S. (1987) Unidirectional digestion with exonuclease III in DNA sequence analysis. *Meth. Enzymol.* **155**, 156.
11. Dotto, G.P., Enea, V. and Zinder, N.D. (1981) Functional analysis of bacteriophage f1 intergenic region. *Virology* **114**, 463–73.
12. Dotto, G.P. and Zinder, N.D. (1983) The morphogenetic signal of bacteriophage f1. *Virology* **130**, 252–6.
13. Dotto, G.P., Huriuchi, K. and Zinder, N.D. (1984) The functional origin of bacteriophage f1 DNA replication. Its signals and domains. *J. Mol. Biol.* **172**, 507–21.
14. Hutchison, C.A. *et al.* (1978) Mutagenesis at a specific position in a DNA sequence. *J. Biol. Chem.* **253**, 6551–60.
15. Chien, A., Edgar, D.B. and Trela, J.M. (1976) Deoxyribonucleic acid polymerase from the extreme thermophile *Thermus aquaticus*. *J. Bacteriol.* **127**, 1550–7.
16. Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.T. (1980) Isolation and properties of DNA polymerase from extreme thermophylic bacteria *Thermus aquaticus* YT-1. *Biokhimiya* **45**, 644–51.
17. Innis, M.A. *et al.* (1988) DNA sequencing with *Thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. *Proc. Natl. Acad. Sci. USA* **85**, 9436–40.
18. Groskreutz, D.J. *et al.* (1995) Increased expression and convenience with the new pGL3 Luciferase Reporter Vectors. *Promega Notes* **50**, 2.
19. Sherf, B.A. and Wood, K.V. (1994) Firefly luciferase engineered for improved genetic reporting. *Promega Notes* **49**, 14–21.

20. 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.
21. Levitt, N. *et al.* (1989) Definition of an efficient synthetic poly(A) site. *Genes and Dev.* **3**, 1019–25.
22. Enriquez-Harris, P. *et al.* (1991) A pause site for RNA polymerase II is associated with termination of transcription. *EMBO J.* **10**, 1833–42.
23. 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.
24. 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.
25. Kozak, M. (1989) The scanning model for translation: An update. *J. Cell Biol.* **108**, 229–41.

H. pGL3-Basic Vector Restriction Sites and Sequence

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

Enzyme	# of Sites	Location
Acc I	1	2011
Acc III	2	783, 1299
Acc65 I	1	1
Acy I	4	95, 121, 1514, 3690
Afl III	3	15, 581, 2260
Alw26 I	5	1111, 1343, 1409, 3214, 3990
Alw44 I	2	2574, 3820
AlwN I	1	2676
AspH I	5	11, 1553, 2578, 3739, 3824
Ava I	3	26, 32, 1144
Ava II	3	1267, 3291, 3513
BamH I	1	2004
Ban II	4	11, 33, 1112, 4231
Bbe I	1	124
Bbs I	4	98, 1376, 1492, 2089
Bbu I	1	751
Bcl I	1	668
Bgl I	2	3273, 4541
Bgl II	1	36
Bsa I	1	3214
BsaA I	1	4302
BsaB I	1	2003
BsaH I	4	95, 121, 1514, 3690
BsaM I	3	60, 1823, 1916
Bsm I	3	60, 1823, 1916
BspH I	3	671, 2980, 3988
BspM I	3	1477, 1486, 4781
Bsr BR I	1	2003
BsrG I	1	578
BssS I	2	2433, 3817
BstZ I	3	1755, 1759, 4651
Cla I	3	1997, 4709, 4813
Csp45 I	1	257
Dra I	4	1963, 3019, 3038, 3730
Dra II	1	1267
Dra III	1	4305
Drd I	3	1489, 2368, 4349
Dsa I	2	86, 458
Eae I	4	1755, 1759, 3541, 4651
Eag I	3	1755,1759, 4651
EclHK I	1	3153
Eco47 III	1	2136
Eco52 I	3	1755, 1759, 4651
EcoICR I	1	9
EcoN I	3	645, 1045, 1705
Ehe I	1	122
Fse I	1	1761
Fsp I	2	3375, 4548
Hinc II	3	1392, 1902, 2012
Hind II	3	1392, 1902, 2012
Hind III	1	53
Hpa I	1	1902
Hsp92 I	4	95, 121, 1514, 3690
Kas I	1	120
Kpn I(p)	1	5
Mlu I	1	15
Nae I	3	1759, 2130, 4199
Nar I	1	121
Nco I	1	86
NgoM IV	3	1757, 2128, 4197
Nhe I	1	21
Not I	1	4651
Nsp I	2	751, 2264
PaeR7 I	1	32
PpuM I	1	1267
PshA I	1	2075
Psp5 II	1	1267
PspA I	1	26
Pvu I	2	3523, 4569
Sac I	1	11
Sal I	1	2010
Sca I	3	253, 3633, 4716
SgrA I	1	1516
Sin I	3	1267, 3291, 3513
Sma I	1	28
Sph I	1	751
Srf I	1	28
Ssp I	3	3957, 4510, 4625
Sty I	1	86
Vsp I	1	3325
Xba I	1	1742
Xcm I	1	823
Xho I	1	32
Xma I	1	26
Xmn I	1	3752

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Table 4. Restriction Enzymes That Do Not Cut the pGL3-Basic Vector.

Aat II	_Bbr_P I	**_Bst_X I**	**_Nde_ I**	_Ppu_10 I	**_Spe_ I**
Acc B7 I	_Blp_ I	**_Bsu_36 I**	**_Nru_ I**	**_Pst_ I**	_Spl_ I
Afl II	_Bpu_ 1102 I	**_Csp_ I**	**_Nsi_ I**	**_Pvu_ II**	_Sse_8387 I
Age I	_Bsp_120 I	_Eco_72 I	_Pac_ I	_Rsr_ II	**_Stu_ I**
Apa I	**_Bss_H II**	_Eco_81 I	_Pfl_M I	**_Sac_ II**	_Swa_ I
Asc I	_Bst_1107 I	**_Eco_R I**	_Pin_A I	**_Sfi_ I**	**_Tth_111 I**
Avr II	**_Bst_98 I**	**_Eco_R V**	_Pme_ I	**_Sgf_ I**(q)	
Bal I	**_Bst_E II**	**I-_Ppo_ I**	_Pml_ I	**_Sna_B I**	

Table 5. Restriction Enzymes That Cut the pGL3-Basic Vector 6 or More Times.

Aci I	**_Bst_71 I**	_Fnu_4H I	**_Hsp_92 II**	**_Msp_A1 I**	_Scr_F I
Alu I	**_Bst_O I**	**_Fok_ I**	_Mae_ I	**_Nci_ I**	_Sfa_N I
Ban I	_Bst_U I	**_Hae_ II**	_Mae_ II	**_Nde_ I**	**_Taq_ I**
Bbv I	**_Cfo_ I**	**_Hae_ III**	_Mae_ III	_Nla_ III	_Tfi_ I
_Bsa_0 I	_Cfr_10 I	_Hga_ I	**_Mbo_ I**	_Nla_ IV	**_Tru_9 I**
_Bsa_J I	**_Dde_ I**	**_Hha_ I**	**_Mbo_ II**	_Ple_ I	**_Xho_ II**
_Bsp_1286 I	**_Dpn_ I**	**_Hin_f I**	_Mnl_ I	**_Rsa_ I**	
Bsr I	_Dpn_ II	**_Hpa_ II**	_Mse_ I	**_Sau_3A I**	
Bsr S I	_Ear_ I	_Hph_ I	**_Msp_ I**	**_Sau_96 I**	

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

pGL3-Basic Vector Sequence

The strand shown is the same as the ssDNA strand produced by this vector and also corresponds to the mRNA synthesized from the _luc_+ gene.

```
  1  GGTACCGAGC TCTTACGCGT GCTAGCCCGG GCTCGAGATC TGCGATCTAA
 51  GTAAGCTTGG CATTCCGGTA CTGTTGGTAA AGCCACCATG GAAGACGCCA
101  AAAACATAAA GAAAGGCCCG GCGCCATTCT ATCCGCTGGA AGATGGAACC
151  GCTGGAGAGC AACTGCATAA GGCTATGAAG AGATACGCCC TGGTTCCTGG
201  AACAATTGCT TTTACAGATG CACATATCGA GGTGGACATC ACTTACGCTG
251  AGTACTTCGA AATGTCCGTT CGGTTGGCAG AAGCTATGAA ACGATATGGG
301  CTGAATACAA ATCACAGAAT CGTCGTATGC AGTGAAAACT CTCTTCAATT
351  CTTTATGCCG GTGTTGGGCG CGTTATTTAT CGGAGTTGCA GTTGCGCCCG
401  CGAACGACAT TTATAATGAA CGTGAATTGC TCAACAGTAT GGGCATTTCG
451  CAGCCTACCG TGGTGTTCGT TTCCAAAAAG GGGTTGCAAA AAATTTTGAA
501  CGTGCAAAAA AAGCTCCCAA TCATCCAAAA AATTATTATC ATGGATTCTA
551  AAACGGATTA CCAGGGATTT CAGTCGATGT ACACGTTCGT CACATCTCAT
601  CTACCTCCCG GTTTTAATGA ATACGATTTT GTGCCAGAGT CCTTCGATAG
651  GGACAAGACA ATTGCACTGA TCATGAACTC CTCTGGATCT ACTGGTCTGC
701  CTAAAGGTGT CGCTCTGCCT CATAGAACTG CCTGCGTGAG ATTCTCGCAT
751  GCCAGAGATC CTATTTTTGG CAATCAAATC ATTCCGGATA CTGCGATTTT
```

pGL3-Basic Vector Sequence (continued)

```
 801  AAGTGTTGTT  CCATTCCATC  ACGGTTTTGG  AATGTTTACT  ACACTCGGAT
 851  ATTTGATATG  TGGATTTCGA  GTCGTCTTAA  TGTATAGATT  TGAAGAAGAG
 901  CTGTTTCTGA  GGAGCCTTCA  GGATTACAAG  ATTCAAAGTG  CGCTGCTGGT
 951  GCCAACCCTA  TTCTCCTTCT  TCGCCAAAAG  CACTCTGATT  GACAAATACG
1001  ATTTATCTAA  TTTACACGAA  ATTGCTTCTG  GTGGCGCTCC  CCTCTCTAAG
1051  GAAGTCGGGG  AAGCGGTTGC  CAAGAGGTTC  CATCTGCCAG  GTATCAGGCA
1101  AGGATATGGG  CTCACTGAGA  CTACATCAGC  TATTCTGATT  ACACCCGAGG
1151  GGGATGATAA  ACCGGGCGCG  GTCGGTAAAG  TTGTTCCATT  TTTTGAAGCG
1201  AAGGTTGTGG  ATCTGGATAC  CGGGAAAACG  CTGGGCGTTA  ATCAAAGAGG
1251  CGAACTGTGT  GTGAGAGGTC  CTATGATTAT  GTCCGGTTAT  GTAAACAATC
1301  CGGAAGCGAC  CAACGCCTTG  ATTGACAAGG  ATGGATGGCT  ACATTCTGGA
1351  GACATAGCTT  ACTGGGACGA  AGACGAACAC  TTCTTCATCG  TTGACCGCCT
1401  GAAGTCTCTG  ATTAAGTACA  AAGGCTATCA  GGTGGCTCCC  GCTGAATTGG
1451  AATCCATCTT  GCTCCAACAC  CCCAACATCT  TCGACGCAGG  TGTCGCAGGT
1501  CTTCCCGACG  ATGACGCCGG  TGAACTTCCC  GCCGCCGTTG  TTGTTTTGGA
1551  GCACGGAAAG  ACGATGACGG  AAAAAGAGAT  CGTGGATTAC  GTCGCCAGTC
1601  AAGTAACAAC  CGCGAAAAAG  TTGCGCGGAG  GAGTTGTGTT  TGTGGACGAA
1651  GTACCGAAAG  GTCTTACCGG  AAAACTCGAC  GCAAGAAAAA  TCAGAGAGAT
1701  CCTCATAAAG  GCCAAGAAGG  GCGGAAAGAT  CGCCGTGTAA  TTCTAGAGTC
1751  GGGGCGGCCG  GCCGCTTCGA  GCAGACATGA  TAAGATACAT  TGATGAGTTT
1801  GGACAAACCA  CAACTAGAAT  GCAGTGAAAA  AAATGCTTTA  TTTGTGAAAT
1851  TTGTGATGCT  ATTGCTTTAT  TTGTAACCAT  TATAAGCTGC  AATAAACAAG
1901  TTAACAACAA  CAATTGCATT  CATTTTATGT  TTCAGGTTCA  GGGGGAGGTG
1951  TGGGAGGTTT  TTTAAAGCAA  GTAAAACCTC  TACAAATGTG  GTAAAATCGA
2001  TAAGGATCCG  TCGACCGATG  CCCTTGAGAG  CCTTCAACCC  AGTCAGCTCC
2051  TTCCGGTGGG  CGCGGGGCAT  GACTATCGTC  GCCGCACTTA  TGACTGTCTT
2101  CTTTATCATG  CAACTCGTAG  GACAGGTGCC  GGCAGCGCTC  TTCCGCTTCC
2151  TCGCTCACTG  ACTCGCTGCG  CTCGGTCGTT  CGGCTGCGGC  GAGCGGTATC
2201  AGCTCACTCA  AAGGCGGTAA  TACGGTTATC  CACAGAATCA  GGGGATAACG
2251  CAGGAAAGAA  CATGTGAGCA  AAAGGCCAGC  AAAAGGCCAG  GAACCGTAAA
2301  AAGGCCGCGT  TGCTGGCGTT  TTTCCATAGG  CTCCGCCCCC  CTGACGAGCA
2351  TCACAAAAAT  CGACGCTCAA  GTCAGAGGTG  GCGAAACCCG  ACAGGACTAT
2401  AAAGATACCA  GGCGTTTCCC  CCTGGAAGCT  CCCTCGTGCG  CTCTCCTGTT
2451  CCGACCCTGC  CGCTTACCGG  ATACCTGTCC  GCCTTTCTCC  CTTCGGGAAG
2501  CGTGGCGCTT  TCTCATAGCT  CACGCTGTAG  GTATCTCAGT  TCGGTGTAGG
2551  TCGTTCGCTC  CAAGCTGGGC  TGTGTGCACG  AACCCCCCGT  TCAGCCCGAC
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pGL3-Basic Vector Sequence (continued)

```
2601  CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA
2651  CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA
2701  GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC
2751  TACACTAGAA GAACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC
2801  CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG
2851  GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA
2901  GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG
2951  GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA
3001  TCTTCACCTA GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA
3051  AGTATATATG AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA
3101  GGCACCTATC TCAGCGATCT GTCTATTTCG TTCATCCATA GTTGCCTGAC
3151  TCCCCGTCGT GTAGATAACT ACGATACGGG AGGGCTTACC ATCTGGCCCC
3201  AGTGCTGCAA TGATACCGCG AGACCCACGC TCACCGGCTC CAGATTTATC
3251  AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCCTGCAA
3301  CTTTATCCGC CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA
3351  AGTAGTTCGC CAGTTAATAG TTTGCGCAAC GTTGTTGCCA TTGCTACAGG
3401  CATCGTGGTG TCACGCTCGT CGTTTGGTAT GGCTTCATTC AGCTCCGGTT
3451  CCCAACGATC AAGGCGAGTT ACATGATCCC CCATGTTGTG CAAAAAAGCG
3501  GTTAGCTCCT TCGGTCCTCC GATCGTTGTC AGAAGTAAGT TGGCCGCAGT
3551  GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC
3601  CATCCGTAAG ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC
3651  TGAGAATAGT GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG
3701  GGATAATACC GCGCCACATA GCAGAACTTT AAAAGTGCTC ATCATTGGAA
3751  AACGTTCTTC GGGGCGAAAA CTCTCAAGGA TCTTACCGCT GTTGAGATCC
3801  AGTTCGATGT AACCCACTCG TGCACCCAAC TGATCTTCAG CATCTTTTAC
3851  TTTCACCAGC GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA
3901  AAAAGGGAAT AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT
3951  TTTCAATATT ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA
4001  CATATTTGAA TGTATTTAGA AAAATAAACA AATAGGGGTT CCGCGCACAT
4051  TTCCCCGAAA AGTGCCACCT GACGCGCCCT GTAGCGGCGC ATTAAGCGCG
4101  GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG CCAGCGCCCT
4151  AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC CTTTCTCGCC ACGTTCGCCG
4201  GCTTTCCCCG TCAAGCTCTA AATCGGGGGC TCCCTTTAGG GTTCCGATTT
4251  AGTGCTTTAC GGCACCTCGA CCCCAAAAAA CTTGATTAGG GTGATGGTTC
4301  ACGTAGTGGG CCATCGCCCT GATAGACGGT TTTTCGCCCT TTGACGTTGG
4351  AGTCCACGTT CTTTAATAGT GGACTCTTGT TCCAAACTGG AACAACACTC
4401  AACCCTATCT CGGTCTATTC TTTTGATTTA TAAGGATTT TGCCGATTTC
```

pGL3-Basic Vector Sequence (continued)

```
4451  GGCCTATTGG  TTAAAAAATG  AGCTGATTTA  ACAAAAATTT  AACGCGAATT
4501  TTAACAAAAT  ATTAACGCTT  ACAATTTGCC  ATTCGCCATT  CAGGCTGCGC
4551  AACTGTTGGG  AAGGGCGATC  GGTGCGGGCC  TCTTCGCTAT  TACGCCAGCC
4601  CAAGCTACCA  TGATAAGTAA  GTAATATTAA  GGTACGGGAG  GTACTTGGAG
4651  CGGCCGCAAT  AAAATATCTT  TATTTTCATT  ACATCTGTGT  GTTGGTTTTT
4701  TGTGTGAATC  GATAGTACTA  ACATACGCTC  TCCATCAAAA  CAAAACGAAA
4751  CAAAACAAAC  TAGCAAAATA  GGCTGTCCCC  AGTGCAAGTG  CAGGTGCCAG
4801  AACATTTCTC  TATCGATA
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I. pGL3-Enhancer Vector Restriction Sites and Sequence

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

Enzyme	# of Sites	Location
***Acc* I**	1	2257
***Acc* III**	2	783,1299
***Acc*65 I**	1	1
***Acy* I**	4	95, 121, 1514, 3936
Afl III	3	15, 581 ,2506
***Alw*26 I**	5	1111, 1343, 1409, 3460, 4236
***Alw*44 I**	2	2820, 4066
*Alw*N I	1	2922
*Asp*H I	5	11, 1553, 2824, 3985, 4070
***Ava* I**	3	26, 32, 1144
***Ava* II**	3	1267, 3537, 3759
***Bam*H I**	1	2250
***Ban* II**	4	11, 33, 1112, 4477
Bbe I	1	124
Bbs I	4	98, 1376, 1492, 2335
***Bbu* I**	3	751, 2108, 2180
***Bcl* I**	1	668
***Bgl* I**	2	3519, 4787
***Bgl* II**	1	36
Bsa I	1	3460
*Bsa*A I	1	4548
*Bsa*B I	1	2003
*Bsa*H I	4	95, 121, 1514, 3936
***Bsa*M I**	3	60, 1823, 1916
Bsm I	3	60, 1823, 1916
*Bsp*H I	3	671, 3226, 4234
*Bsp*M I	3	1477, 1486, 5027
***Bsr* BR I**	1	2003
*Bsr*G I	1	578
*Bss*S I	2	2679, 4063
***Bst*Z I**	3	1755, 1759, 4897
***Cla* I**	3	1997, 4955, 5059
***Csp*45 I**	1	257
***Dra* I**	4	1963, 3265, 3284, 3976
Dra II	1	1267
Dra III	1	4551
Drd I	3	1489, 2614, 4595
Dsa I	2	86, 458
Eae I	4	1755, 1759, 3787, 4897
Eag I	3	1755, 1759, 4897
***Ecl*HK I**	1	3399
***Eco*47 III**	1	2382
***Eco*52 I**	3	1755, 1759, 4897
***Eco*ICR I**	1	9
*Eco*N I	3	645, 1045, 1705
Ehe I	1	122
Fse I	1	1761
Fsp I	2	3621, 4794
***Hinc* II**	3	1392, 1902, 2258
*Hin*d II	3	1392, 1902, 2258
***Hin*d III**	1	53
***Hpa* I**	1	1902
***Hsp*92 I**	4	95, 121, 1514, 3936
Kas I	1	120
***Kpn* I**	1	5
***Mlu* I**	1	15
***Nae* I**	3	1759, 2376, 4445
***Nar* I**	1	121
***Nco* I**	1	86
***Ngo*M IV**	3	1757, 2374, 4443
***Nhe* I**	1	21
***Not* I**	1	4897
***Nsi* I**	2	2106, 2178
Nsp I	4	751, 2108, 2180, 2510
*Pae*R7 I	1	32
*Ppu*10 I	2	2102, 2174
*Ppu*M I	1	1267
*Psh*A I	1	2321
*Psp*5 II	1	1267
*Psp*A I	1	26
***Pvu* I**	2	3769, 4815
***Sac* I**	1	11
***Sal* I**	1	2256
***Sca* I**	3	253, 3879, 4962
*Sgr*A I	1	1516
***Sin* I**	3	1267, 3537, 3759
***Sma* I**	1	28
***Sph* I**	3	751, 2108, 2180

Table 6. Restriction Enzymes That Cut the pGL3-Enhancer Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location
Srf I	1	28
***Ssp* I**	3	4203, 4756, 4871
***Sty* I**	1	86
***Vsp* I**	1	3571
***Xba* I**	1	1742
Xcm I	1	823
***Xho* I**	1	32
***Xma* I**	1	26
***Xmn* I**	1	3998

Table 7. Restriction Enzymes That Do Not Cut the pGL3-Enhancer Vector.

***Aat* II**	*Bbr*P I	***Bst*X I**	***Nde* I**	***Pvu* II**	*Sse*8387 I
***Acc* B7 I**	*Blp* I	***Bsu*36 I**	***Nru* I**	*Rsr* II	***Stu* I**
Afl II	*Bpu* 1102I	***Csp* I**	*Pac* I	***Sac* II**	*Swa* I
***Age* I**	*Bsp*120 I	*Eco*72 I	*Pfl*M I	***Sfi* I**	***Tth*III I**
***Apa* I**	***Bss*H II**	*Eco*81 I	*Pin*A I	***Sgf* I**	
Asc I	*Bst*1107 I	***Eco*R I**	*Pme* I	***Sna*B I**	
Avr II	***Bst*98 I**	***Eco*R V**	*Pml* I	***Spe* I**	
***Bal* I**	***Bst*E II**	**I-*Ppo* I**	***Pst* I**	*Spl* I	

Table 8. Restriction Enzymes That Cut the pGL3-Enhancer Vector 6 or More Times.

Aci I	***Bst*71 I**	*Fnu*4H I	***Hsp*92 II**	***Msp*A1 I**	*Scr*F I
***Alu* I**	***Bst*O I**	***Fok* I**	*Mae* I	***Nci* I**	*Sfa*N I
***Ban* I**	*Bst*U I	***Hae* II**	*Mae* II	***Nde* II**	***Taq* I**
Bbv I	***Cfo* I**	***Hae* III**	*Mae* III	*Nla* III	*Tfi* I
***Bsa*0 I**	*Cfr*10 I	*Hga* I	***Mbo* I**	*Nla* IV	***Tru*9 I**
*Bsa*J I	***Dde* I**	***Hha* I**	***Mbo* II**	*Ple* I	***Xho* II**
***Bsp*1286 I**	***Dpn* I**	***Hinf* I**	*Mnl* I	***Rsa* I**	
Bsr I	*Dpn* II	***Hpa* II**	*Mse* I	***Sau*3A I**	
***Bsr*S I**	*Ear* I	*Hph* I	***Msp* I**	***Sau*96 I**	

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

pGL3-Enhancer Vector Sequence

The strand shown is the same as the ssDNA strand produced by this vector and also corresponds to the mRNA synthesized from the *luc*+ gene.

```
  1 GGTACCGAGC TCTTACGCGT GCTAGCCCGG GCTCGAGATC TGCGATCTAA
 51 GTAAGCTTGG CATTCCGGTA CTGTTGGTAA AGCCACCATG GAAGACGCCA
101 AAAACATAAA GAAAGGCCCG GCGCCATTCT ATCCGCTGGA AGATGGAACC
151 GCTGGAGAGC AACTGCATAA GGCTATGAAG AGATACGCCC TGGTTCCTGG
201 AACAATTGCT TTTACAGATG CACATATCGA GGTGGACATC ACTTACGCTG
251 AGTACTTCGA AATGTCCGTT CGGTTGGCAG AAGCTATGAA ACGATATGGG
301 CTGAATACAA ATCACAGAAT CGTCGTATGC AGTGAAAACT CTCTTCAATT
351 CTTTATGCCG GTGTTGGGCG CGTTATTTAT CGGAGTTGCA GTTGCGCCCG
401 CGAACGACAT TTATAATGAA CGTGAATTGC TCAACAGTAT GGGCATTTCG
451 CAGCCTACCG TGGTGTTCGT TTCCAAAAAG GGGTTGCAAA AAATTTTGAA
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pGL3-Enhancer Vector Sequence (continued)

```
 501  CGTGCAAAAA AAGCTCCCAA TCATCCAAAA AATTATTATC ATGGATTCTA
 551  AAACGGATTA CCAGGGATTT CAGTCGATGT ACACGTTCGT CACATCTCAT
 601  CTACCTCCCG GTTTTAATGA ATACGATTTT GTGCCAGAGT CCTTCGATAG
 651  GGACAAGACA ATTGCACTGA TCATGAACTC CTCTGGATCT ACTGGTCTGC
 701  CTAAAGGTGT CGCTCTGCCT CATAGAACTG CCTGCGTGAG ATTCTCGCAT
 751  GCCAGAGATC CTATTTTTGG CAATCAAATC ATTCCGGATA CTGCGATTTT
 801  AAGTGTTGTT CCATTCCATC ACGGTTTTGG AATGTTTACT ACACTCGGAT
 851  ATTTGATATG TGGATTTCGA GTCGTCTTAA TGTATAGATT TGAAGAAGAG
 901  CTGTTTCTGA GGAGCCTTCA GGATTACAAG ATTCAAAGTG CGCTGCTGGT
 951  GCCAACCCTA TTCTCCTTCT TCGCCAAAAG CACTCTGATT GACAAATACG
1001  ATTTATCTAA TTTACACGAA ATTGCTTCTG GTGGCGCTCC CCTCTCTAAG
1051  GAAGTCGGGG AAGCGGTTGC CAAGAGGTTC CATCTGCCAG GTATCAGGCA
1101  AGGATATGGG CTCACTGAGA CTACATCAGC TATTCTGATT ACACCCGAGG
1151  GGGATGATAA ACCGGGCGCG GTCGGTAAAG TTGTTCCATT TTTTGAAGCG
1201  AAGGTTGTGG ATCTGGATAC CGGGAAAACG CTGGGCGTTA ATCAAAGAGG
1251  CGAACTGTGT GTGAGAGGTC CTATGATTAT GTCCGGTTAT GTAAACAATC
1301  CGGAAGCGAC CAACGCCTTG ATTGACAAGG ATGGATGGCT ACATTCTGGA
1351  GACATAGCTT ACTGGGACGA AGACGAACAC TTCTTCATCG TTGACCGCCT
1401  GAAGTCTCTG ATTAAGTACA AAGGCTATCA GGTGGCTCCC GCTGAATTGG
1451  AATCCATCTT GCTCCAACAC CCCAACATCT TCGACGCAGG TGTCGCAGGT
1501  CTTCCCGACG ATGACGCCGG TGAACTTCCC GCCGCCGTTG TTGTTTTGGA
1551  GCACGGAAAG ACGATGACGG AAAAAGAGAT CGTGGATTAC GTCGCCAGTC
1601  AAGTAACAAC CGCGAAAAAG TTGCGCGGAG GAGTTGTGTT TGTGGACGAA
1651  GTACCGAAAG GTCTTACCGG AAAACTCGAC GCAAGAAAAA TCAGAGAGAT
1701  CCTCATAAAG GCCAAGAAGG GCGGAAAGAT CGCCGTGTAA TTCTAGAGTC
1751  GGGGCGGCCG GCCGCTTCGA GCAGACATGA TAAGATACAT TGATGAGTTT
1801  GGACAAACCA CAACTAGAAT GCAGTGAAAA AAATGCTTTA TTTGTGAAAT
1851  TTGTGATGCT ATTGCTTTAT TTGTAACCAT TATAAGCTGC AATAAACAAG
1901  TTAACAACAA CAATTGCATT CATTTTATGT TTCAGGTTCA GGGGGAGGTG
1951  TGGGAGGTTT TTTAAAGCAA GTAAAACCTC TACAAATGTG GTAAAATCGA
2001  TAAGGATCTG AACGATGGAG CGGAGAATGG GCGGAACTGG GCGGAGTTAG
2051  GGGCGGGATG GGCGGAGTTA GGGGCGGGAC TATGGTTGCT GACTAATTGA
2101  GATGCATGCT TTGCATACTT CTGCCTGCTG GGGAGCCTGG GGACTTTCCA
2151  CACCTGGTTG CTGACTAATT GAGATGCATG CTTTGCATAC TTCTGCCTGC
2201  TGGGGAGCCT GGGGACTTTC CACACCCTAA CTGACACACA TTCCACAGCG
2251  GATCCGTCGA CCGATGCCCT TGAGAGCCTT CAACCCAGTC AGCTCCTTCC
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pGL3-Enhancer Vector Sequence (continued)

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2301  GGTGGGCGCG GGGCATGACT ATCGTCGCCG CACTTATGAC TGTCTTCTTT
2351  ATCATGCAAC TCGTAGGACA GGTGCCGGCA GCGCTCTTCC GCTTCCTCGC
2401  TCACTGACTC GCTGCGCTCG GTCGTTCGGC TGCGGCGAGC GGTATCAGCT
2451  CACTCAAAGG CGGTAATACG GTTATCCACA GAATCAGGGG ATAACGCAGG
2501  AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG
2551  CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC
2601  AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG
2651  ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA
2701  CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG
2751  GCGCTTTCTC ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT
2801  TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT
2851  GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC
2901  TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA
2951  TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA
3001  CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTTC
3051  GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG
3101  CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA AAAAAAGGAT
3151  CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC
3201  GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT
3251  CACCTAGATC CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA
3301  TATATGAGTA AACTTGGTCT GACAGTTACC AATGCTTAAT CAGTGAGGCA
3351  CCTATCTCAG CGATCTGTCT ATTTCGTTCA TCCATAGTTG CCTGACTCCC
3401  CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT GGCCCCAGTG
3451  CTGCAATGAT ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA
3501  ATAAACCAGC CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC CTGCAACTTT
3551  ATCCGCCTCC ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA
3601  GTTCGCCAGT TAATAGTTTG CGCAACGTTG TTGCCATTGC TACAGGCATC
3651  GTGGTGTCAC GCTCGTCGTT TGGTATGGCT TCATTCAGCT CCGGTTCCCA
3701  ACGATCAAGG CGAGTTACAT GATCCCCCAT GTTGTGCAAA AAAGCGGTTA
3751  GCTCCTTCGG TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA
3801  TCACTCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG TCATGCCATC
3851  CGTAAGATGC TTTTCTGTGA CTGGTGAGTA CTCAACCAAG TCATTCTGAG
3901  AATAGTGTAT GCGGCGACCG AGTTGCTCTT GCCCGGCGTC AATACGGGAT
3951  AATACCGCGC CACATAGCAG AACTTTAAAA GTGCTCATCA TTGGAAAACG
4001  TTCTTCGGGG CGAAAACTCT CAAGGATCTT ACCGCTGTTG AGATCCAGTT
4051  CGATGTAACC CACTCGTGCA CCCAACTGAT CTTCAGCATC TTTTACTTTC
4101  ACCAGCGTTT CTGGGTGAGC AAAAACAGGA AGGCAAAATG CCGCAAAAAA
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pGL3-Enhancer Vector Sequence (continued)

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4151  GGGAATAAGG  GCGACACGGA  AATGTTGAAT  ACTCATACTC  TTCCTTTTTC
4201  AATATTATTG  AAGCATTTAT  CAGGGTTATT  GTCTCATGAG  CGGATACATA
4251  TTTGAATGTA  TTTAGAAAAA  TAAACAAATA  GGGGTTCCGC  GCACATTTCC
4301  CCGAAAAGTG  CCACCTGACG  CGCCCTGTAG  CGGCGCATTA  AGCGCGGCGG
4351  GTGTGGTGGT  TACGCGCAGC  GTGACCGCTA  CACTTGCCAG  CGCCCTAGCG
4401  CCCGCTCCTT  TCGCTTTCTT  CCCTTCCTTT  CTCGCCACGT  TCGCCGGCTT
4451  TCCCCGTCAA  GCTCTAAATC  GGGGGCTCCC  TTTAGGGTTC  CGATTTAGTG
4501  CTTTACGGCA  CCTCGACCCC  AAAAAACTTG  ATTAGGGTGA  TGGTTCACGT
4551  AGTGGGCCAT  CGCCCTGATA  GACGGTTTTT  CGCCCTTTGA  CGTTGGAGTC
4601  CACGTTCTTT  AATAGTGGAC  TCTTGTTCCA  AACTGGAACA  ACACTCAACC
4651  CTATCTCGGT  CTATTCTTTT  GATTTATAAG  GGATTTTGCC  GATTTCGGCC
4701  TATTGGTTAA  AAAATGAGCT  GATTTAACAA  AAATTTAACG  CGAATTTTAA
4751  CAAAATATTA  ACGCTTACAA  TTTGCCATTC  GCCATTCAGG  CTGCGCAACT
4801  GTTGGGAAGG  GCGATCGGTG  CGGGCCTCTT  CGCTATTACG  CCAGCCCAAG
4851  CTACCATGAT  AAGTAAGTAA  TATTAAGGTA  CGGGAGGTAC  TTGGAGCGGC
4901  CGCAATAAAA  TATCTTTATT  TTCATTACAT  CTGTGTGTTG  GTTTTTTGTG
4951  TGAATCGATA  GTACTAACAT  ACGCTCTCCA  TCAAAACAAA  ACGAAACAAA
5001  ACAAACTAGC  AAAATAGGCT  GTCCCCAGTG  CAAGTGCAGG  TGCCAGAACA
5051  TTTCTCTATC  GATA
```

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J. pGL3-Promoter Vector Restriction Sites and Sequence

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

Enzyme	# of Sites	Location
***Acc* I**	1	2203
***Acc* III**	2	975, 1491
***Acc*65 I**	1	1
***Acy* I**	4	28, 313, 1706, 3882
Afl III	3	15, 773, 2452
***Alw*26 I**	5	1303, 1535, 1601, 3406, 4182
***Alw*44 I**	2	2766, 4012
*Alw*N I	1	2868
*Asp*H I	5	11, 1745, 2770, 3931, 4016
***Ava* I**	3	26, 32, 1336
***Ava* II**	3	1459, 3483, 3705
Avr II	1	229
***Bam*H I**	1	2196
***Ban* II**	4	11, 33, 1304, 4423
Bbe I	1	316
Bbs I	4	290, 1568, 1684 2281
***Bbu* I**	1	943
***Bcl* I**	1	860
***Bgl* I**	3	182, 3465, 4733
***Bgl* II**	1	36
Bsa I	1	3406
*Bsa*A I	1	4494
*Bsa*B I	2	48, 2195
*Bsa*H I	4	287, 313, 1706, 3882
***Bsa*M I**	3	252, 2015, 2108
Bsm I	3	252, 2015, 2108
*Bsp*H I	3	863, 3172, 4180
*Bsp*M I	3	1669, 1678, 4973
***Bsr* BR I**	2	48, 2195
*Bsr*G I	1	770
*Bss*S I	2	2625, 4009
***BstZ* I**	3	1947, 1951, 4843
***Cla* I**	3	2189, 4901, 5005
***Csp*45 I**	1	449
***Dra* I**	4	2155, 3211, 3230, 3922
Dra II	1	1459
Dra III	1	4497
Drd I	3	1681, 2560, 4541
Dsa I	2	278, 650
Eae I	4	1947, 1951, 3733, 4843
Eag I	3	1947, 1951, 4843
***Ecl*HK I**	1	3345
***Eco*47 III**	1	2328
***Eco*52 I**	3	1947, 1951, 4843
***Eco*ICR I**	1	9
*Eco*N I	3	837, 1237, 1897
Ehe I	1	314
Fse I	1	1953
Fsp I	2	3567, 4740
***Hinc* II**	3	1584, 2094, 2204
Hind II	3	1584, 2094, 2204
***Hind* III**	1	245
***Hpa* I**	1	2094
***Hsp*92 I**	4	287, 313, 1706, 3882
Kas I	1	312
***Kpn* I**	1	5
***Mlu* I**	1	15
***Nae* I**	3	1951, 2322, 4391
***Nar* I**	1	313
***Nco* I**	1	278
***Ngo*M IV**	3	1949, 2320, 4389
***Nhe* I**	1	21
***Not* I**	1	4843
Nsp I	2	943, 2456
*Pae*R7 I	1	32
*Ppu*M I	1	1459
*Psh*A I	1	2267
*Psp*5 II	1	1459
*Psp*A I	1	26
***Pvu* I**	2	3715, 4761
***Sac* I**	1	11
***Sal* I**	1	2202
***Sca* I**	3	445, 3825, 4908
***Sfi* I**	1	182
*Sgr*A I	1	1708
***Sin* I**	3	1459, 3483, 3705
***Sma* I**	1	28
***Sph* I**	1	943
Srf I	1	28
***Ssp* I**	3	4149, 4702, 4817
***Stu* I**	1	228

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Table 9. Restriction Enzymes That Cut the pGL3-Promoter Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location
***Sty* I**	2	229, 278
***Vsp* I**	1	3517
***Xba* I**	1	1934
Xcm I	1	1015
***Xho* I**	1	32
***Xma* I**	1	26
***Xmn* I**	1	3944

Table 10. Restriction Enzymes That Do Not Cut the pGL3-Promoter Vector.

***Aat* II**	*Blp* I	***Bsu*36 I**	***Nru* I**	***Pst* I**	*Sse*8387 I
***Acc* B7 I**	*Bpu* 1102I	***Csp* I**	***Nsi* I**	***Pvu* II**	*Swa* I
Afl II	*Bsp*120 I	*Eco*72 I	*Pac* I	*Rsr* II	***Tth*111 I**
***Age* I**	***Bss*H II**	*Eco*81 I	*Pfl*M I	***Sac* II**	
***Apa* I**	*Bst*1107 I	***Eco*R I**	*Pin*A I	***Sgf* I**	
Asc I	***Bst*98 I**	***Eco*R V**	*Pme* I	***Sna*B I**	
***Bal* I**	***Bst*E II**	***I-Ppo* I**	*Pml* I	***Spe* I**	
*Bbr*P I	***Bst*X I**	***Nde* I**	*Ppu*10 I	*Spl* I	

Table 11. Restriction Enzymes That Cut the pGL3-Promoter Vector 6 or More Times.

Aci I	***Bst*71 I**	*Fnu*4H I	***Hsp*92 II**	***Msp*A1 I**	*Scr*F I
***Alu* I**	***Bst*O I**	***Fok* I**	*Mae* I	***Nci* I**	*Sfa*N I
***Ban* I**	*Bst*U I	***Hae* II**	*Mae* II	***Nde* II**	***Taq* I**
Bbv I	**Cfo I**	***Hae* III**	*Mae* III	*Nla* III	*Tfi* I
***Bsa*0 I**	*Cfr*10 I	*Hga* I	***Mbo* I**	*Nla* IV	***Tru*9 I**
*Bsa*J I	***Dde* I**	***Hha* I**	***Mbo* II**	*Ple* I	***Xho* II**
***Bsp*1286 I**	***Dpn* I**	***Hin*f I**	*Mnl* I	***Rsa* I**	
Bsr I	*Dpn* II	***Hpa* II**	*Mse* I	***Sau*3A I**	
***Bsr* S I**	*Ear* I	*Hph* I	***Msp* I**	***Sau*96 I**	

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

pGL3-Promoter Vector Sequence

The strand shown is the same as the ssDNA strand produced by this vector and corresponds to the mRNA synthesized from the *luc+* gene.

```
  1 GGTACCGAGC TCTTACGCGT GCTAGCCCGG GCTCGAGATC TGCGATCTGC
 51 ATCTCAATTA GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG
101 CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATC GCTGACTAAT
151 TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC
201 AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTT
251 GGCATTCCGG TACTGTTGGT AAAGCCACCA TGGAAGACGC CAAAAACATA
301 AAGAAAGGCC CGGCGCCATT CTATCCGCTG GAAGATGGAA CCGCTGGAGA
351 GCAACTGCAT AAGGCTATGA AGAGATACGC CCTGGTTCCT GGAACAATTG
401 CTTTTACAGA TGCACATATC GAGGTGGACA TCACTTACGC TGAGTACTTC
451 GAAATGTCCG TTCGGTTGGC AGAAGCTATG AAACGATATG GGCTGAATAC
```

pGL3-Promoter Vector Sequence (continued)

```
 501  AAATCACAGA ATCGTCGTAT GCAGTGAAAA CTCTCTTCAA TTCTTTATGC
 551  CGGTGTTGGG CGCGTTATTT ATCGGAGTTG CAGTTGCGCC CGCGAACGAC
 601  ATTTATAATG AACGTGAATT GCTCAACAGT ATGGGCATTT CGCAGCCTAC
 651  CGTGGTGTTC GTTTCCAAAA AGGGGTTGCA AAAAATTTTG AACGTGCAAA
 701  AAAAGCTCCC AATCATCCAA AAAATTATTA TCATGGATTC TAAAACGGAT
 751  TACCAGGGAT TTCAGTCGAT GTACACGTTC GTCACATCTC ATCTACCTCC
 801  CGGTTTTAAT GAATACGATT TTGTGCCAGA GTCCTTCGAT AGGGACAAGA
 851  CAATTGCACT GATCATGAAC TCCTCTGGAT CTACTGGTCT GCCTAAAGGT
 901  GTCGCTCTGC CTCATAGAAC TGCCTGCGTG AGATTCTCGC ATGCCAGAGA
 951  TCCTATTTTT GGCAATCAAA TCATTCCGGA TACTGCGATT TTAAGTGTTG
1001  TTCCATTCCA TCACGGTTTT GGAATGTTTA CTACACTCGG ATATTTGATA
1051  TGTGGATTTC GAGTCGTCTT AATGTATAGA TTTGAAGAAG AGCTGTTTCT
1101  GAGGAGCCTT CAGGATTACA AGATTCAAAG TGCGCTGCTG GTGCCAACCC
1151  TATTCTCCTT CTTCGCCAAA AGCACTCTGA TTGACAAATA CGATTTATCT
1201  AATTTACACG AAATTGCTTC TGGTGGCGCT CCCCTCTCTA AGGAAGTCGG
1251  GGAAGCGGTT GCCAAGAGGT TCCATCTGCC AGGTATCAGG CAAGGATATG
1301  GGCTCACTGA GACTACATCA GCTATTCTGA TTACACCCGA GGGGGATGAT
1351  AAACCGGGCG CGGTCGGTAA AGTTGTTCCA TTTTTTGAAG CGAAGGTTGT
1401  GGATCTGGAT ACCGGGAAAA CGCTGGGCGT TAATCAAAGA GGCGAACTGT
1451  GTGTGAGAGG TCCTATGATT ATGTCCGGTT ATGTAAACAA TCCGGAAGCG
1501  ACCAACGCCT TGATTGACAA GGATGGATGG CTACATTCTG GAGACATAGC
1551  TTACTGGGAC GAAGACGAAC ACTTCTTCAT CGTTGACCGC CTGAAGTCTC
1601  TGATTAAGTA CAAAGGCTAT CAGGTGGCTC CCGCTGAATT GGAATCCATC
1651  TTGCTCCAAC ACCCCAACAT CTTCGACGCA GGTGTCGCAG GTCTTCCCGA
1701  CGATGACGCC GGTGAACTTC CCGCCGCCGT TGTTGTTTTG GAGCACGGAA
1751  AGACGATGAC GGAAAAAGAG ATCGTGGATT ACGTCGCCAG TCAAGTAACA
1801  ACCGCGAAAA AGTTGCGCGG AGGAGTTGTG TTTGTGGACG AAGTACCGAA
1851  AGGTCTTACC GGAAAACTCG ACGCAAGAAA AATCAGAGAG ATCCTCATAA
1901  AGGCCAAGAA GGGCGGAAAG ATCGCCGTGT AATTCTAGAG TCGGGGCGGC
1951  CGGCCGCTTC GAGCAGACAT GATAAGATAC ATTGATGAGT TTGGACAAAC
2001  CACAACTAGA ATGCAGTGAA AAAAATGCTT TATTTGTGAA ATTTGTGATG
2051  CTATTGCTTT ATTTGTAACC ATTATAAGCT GCAATAAACA AGTTAACAAC
2101  AACAATTGCA TTCATTTTAT GTTTCAGGTT CAGGGGGAGG TGTGGGAGGT
2151  TTTTTAAAGC AAGTAAAACC TCTACAAATG TGGTAAAATC GATAAGGATC
2201  CGTCGACCGA TGCCCTTGAG AGCCTTCAAC CCAGTCAGCT CCTTCCGGTG
2251  GGCGCGGGGC ATGACTATCG TCGCCGCACT TATGACTGTC TTCTTTATCA
2301  TGCAACTCGT AGGACAGGTG CCGGCAGCGC TCTTCCGCTT CCTCGCTCAC
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pGL3-Promoter Vector Sequence (continued)

```
2351  TGACTCGCTG  CGCTCGGTCG  TTCGGCTGCG  GCGAGCGGTA  TCAGCTCACT
2401  CAAAGGCGGT  AATACGGTTA  TCCACAGAAT  CAGGGGATAA  CGCAGGAAAG
2451  AACATGTGAG  CAAAAGGCCA  GCAAAAGGCC  AGGAACCGTA  AAAAGGCCGC
2501  GTTGCTGGCG  TTTTTCCATA  GGCTCCGCCC  CCCTGACGAG  CATCACAAAA
2551  ATCGACGCTC  AAGTCAGAGG  TGGCGAAACC  CGACAGGACT  ATAAAGATAC
2601  CAGGCGTTTC  CCCCTGGAAG  CTCCCTCGTG  CGCTCTCCTG  TTCCGACCCT
2651  GCCGCTTACC  GGATACCTGT  CCGCCTTTCT  CCCTTCGGGA  AGCGTGGCGC
2701  TTTCTCATAG  CTCACGCTGT  AGGTATCTCA  GTTCGGTGTA  GGTCGTTCGC
2751  TCCAAGCTGG  GCTGTGTGCA  CGAACCCCCC  GTTCAGCCCG  ACCGCTGCGC
2801  CTTATCCGGT  AACTATCGTC  TTGAGTCCAA  CCCGGTAAGA  CACGACTTAT
2851  CGCCACTGGC  AGCAGCCACT  GGTAACAGGA  TTAGCAGAGC  GAGGTATGTA
2901  GGCGGTGCTA  CAGAGTTCTT  GAAGTGGTGG  CCTAACTACG  GCTACACTAG
2951  AAGAACAGTA  TTTGGTATCT  GCGCTCTGCT  GAAGCCAGTT  ACCTTCGGAA
3001  AAAGAGTTGG  TAGCTCTTGA  TCCGGCAAAC  AAACCACCGC  TGGTAGCGGT
3051  GGTTTTTTTG  TTTGCAAGCA  GCAGATTACG  CGCAGAAAAA  AAGGATCTCA
3101  AGAAGATCCT  TTGATCTTTT  CTACGGGGTC  TGACGCTCAG  TGGAACGAAA
3151  ACTCACGTTA  AGGGATTTTG  GTCATGAGAT  TATCAAAAAG  GATCTTCACC
3201  TAGATCCTTT  TAAATTAAAA  ATGAAGTTTT  AAATCAATCT  AAAGTATATA
3251  TGAGTAAACT  TGGTCTGACA  GTTACCAATG  CTTAATCAGT  GAGGCACCTA
3301  TCTCAGCGAT  CTGTCTATTT  CGTTCATCCA  TAGTTGCCTG  ACTCCCCGTC
3351  GTGTAGATAA  CTACGATACG  GGAGGGCTTA  CCATCTGGCC  CCAGTGCTGC
3401  AATGATACCG  CGAGACCCAC  GCTCACCGGC  TCCAGATTTA  TCAGCAATAA
3451  ACCAGCCAGC  CGGAAGGGCC  GAGCGCAGAA  GTGGTCCTGC  AACTTTATCC
3501  GCCTCCATCC  AGTCTATTAA  TTGTTGCCGG  GAAGCTAGAG  TAAGTAGTTC
3551  GCCAGTTAAT  AGTTTGCGCA  ACGTTGTTGC  CATTGCTACA  GGCATCGTGG
3601  TGTCACGCTC  GTCGTTTGGT  ATGGCTTCAT  TCAGCTCCGG  TTCCCAACGA
3651  TCAAGGCGAG  TTACATGATC  CCCCATGTTG  TGCAAAAAAG  CGGTTAGCTC
3701  CTTCGGTCCT  CCGATCGTTG  TCAGAAGTAA  GTTGGCCGCA  GTGTTATCAC
3751  TCATGGTTAT  GGCAGCACTG  CATAATTCTC  TTACTGTCAT  GCCATCCGTA
3801  AGATGCTTTT  CTGTGACTGG  TGAGTACTCA  ACCAAGTCAT  TCTGAGAATA
3851  GTGTATGCGG  CGACCGAGTT  GCTCTTGCCC  GGCGTCAATA  CGGGATAATA
3901  CCGCGCCACA  TAGCAGAACT  TTAAAAGTGC  TCATCATTGG  AAAACGTTCT
3951  TCGGGGCGAA  AACTCTCAAG  GATCTTACCG  CTGTTGAGAT  CCAGTTCGAT
4001  GTAACCCACT  CGTGCACCCA  ACTGATCTTC  AGCATCTTTT  ACTTTCACCA
4051  GCGTTTCTGG  GTGAGCAAAA  ACAGGAAGGC  AAAATGCCGC  AAAAAAGGGA
4101  ATAAGGGCGA  CACGGAAATG  TTGAATACTC  ATACTCTTCC  TTTTTCAATA
4151  TTATTGAAGC  ATTTATCAGG  GTTATTGTCT  CATGAGCGGA  TACATATTTG
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pGL3-Promoter Vector Sequence (continued)

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4201  AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA
4251  AAAGTGCCAC CTGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT
4301  GGTGGTTACG CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG
4351  CTCCTTTCGC TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCTTTCCC
4401  CGTCAAGCTC TAAATCGGGG GCTCCCTTTA GGGTTCCGAT TTAGTGCTTT
4451  ACGGCACCTC GACCCCAAAA AACTTGATTA GGGTGATGGT TCACGTAGTG
4501  GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG
4551  TTCTTTAATA GTGGACTCTT GTTCCAAACT GGAACAACAC TCAACCCTAT
4601  CTCGGTCTAT TCTTTTGATT TATAAGGGAT TTTGCCGATT TCGGCCTATT
4651  GGTTAAAAAA TGAGCTGATT TAACAAAAAT TTAACGCGAA TTTTAACAAA
4701  ATATTAACGC TTACAATTTG CCATTCGCCA TTCAGGCTGC GCAACTGTTG
4751  GGAAGGGCGA TCGGTGCGGG CCTCTTCGCT ATTACGCCAG CCCAAGCTAC
4801  CATGATAAGT AAGTAATATT AAGGTACGGG AGGTACTTGG AGCGGCCGCA
4851  ATAAAATATC TTTATTTTCA TTACATCTGT GTGTTGGTTT TTTGTGTGAA
4901  TCGATAGTAC TAACATACGC TCTCCATCAA AACAAAACGA AACAAAACAA
4951  ACTAGCAAAA TAGGCTGTCC CCAGTGCAAG TGCAGGTGCC AGAACATTTC
5001  TCTATCGATA
```

K. pGL3-Control Vector Restriction Sites and Sequence

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

Enzyme	# of Sites	Location
Acc I	1	2449
Acc III	2	975, 1491
Acc65 I	1	1
Acy I	4	287, 313, 1706, 4128
Afl III	3	15, 773, 2698
Alw26 I	5	1303, 1535, 1601, 3652, 4428
Alw44 I	2	3012, 4258
*Alw*N I	1	3114
*Asp*H I	5	11, 1745, 3016, 4177, 4262
Ava I	3	26, 32, 1336
Ava II	3	1459, 3729, 3951
Avr II	1	229
BamH I	1	2442
Ban II	4	11, 33, 1304, 4669
Bbe I	1	316
Bbs I	4	290, 1568, 1684, 2527
Bbu I	3	943, 2300, 2372
Bcl I	1	860
Bgl I	3	182, 3711, 4979
Bgl II	1	36
Bsa I	1	3652
*Bsa*A I	1	4740
*Bsa*B I	2	48, 2195
*Bsa*H I	4	287, 313, 1706,4128
BsaM I	3	252, 2015, 2108
Bsm I	3	252, 2015, 2108
*Bsp*H I	3	863, 3418, 4426
*Bsp*M I	3	1669, 1678, 5219
Bsr BR I	2	48, 2195
*Bsr*G I	1	770
*Bss*S I	2	2871, 4255
BstZ I	3	1947, 1951, 5089
Cla I	3	2189, 5147, 5251
Csp45 I	1	449
Dra I	4	2155, 3457, 3476, 4168
Dra II	1	1459
Dra III	1	4743
Drd I	3	1681, 2806, 4787
Dsa I	2	278, 650
Eag I	3	1947, 1951, 5089
EclH K I	1	3591
Eco47 III	1	2574
Eae I	4	1947, 1951, 3979,
Eco52 I	3	1947, 1951, 5089
EcoICR I	1	9
*Eco*N I	3	837, 1237, 1897
Ehe I	1	314
Fse I	1	1953
Fsp I	2	3813, 4986
Hinc II	3	1584, 2094, 2450
*Hin*d II	3	1584, 2094, 2450
Hind III	1	245
Hpa I	1	2094
Hsp92 I	4	287, 313, 1706, 4128
Kas I	1	312
Kpn I	1	5
Mlu I	1	15
Nae I	3	1951, 2568, 4637
Nar I	1	313
Nco I	1	278
NgoM IV	3	1949, 2566, 4635
Nhe I	1	21
Not I	1	5089
Nsi I	2	2298, 2370
Nsp I	4	943, 2300, 2372, 2702
*Pae*R7 I	1	32
*Ppu*10 I	2	2294, 2366
*Ppu*M I	1	1459
*Psh*A I	1	2513
*Psp*5 II	1	1459
*Psp*A I	1	26
Pvu I	2	3961, 5007
Sac I	1	11
Sal I	1	2448
Sca I	3	445, 4071, 5154
Sfi I	1	182
*Sgr*A I	1	1708
Sin I	3	1459, 3729, 3951
Sma I	1	28
Sph I	3	943, 2300, 2372
Srf I	1	28
Ssp I	3	4395, 4948, 5063
Stu I	1	228
Sty I	2	229, 278
Vsp I	1	3763
Xba I	1	1934
Xcm I	1	1015
Xho I	1	32
Xma I	1	26
Xmn I	1	4190

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Table 13. Restriction Enzymes That Do Not Cut the pGL3-Control Vector.

***Aat* II**	*Bbr*P I	***Bst*E II**	***Eco*R V**	*Pme* I	***Sna*B I**
***Acc* B7 I**	*Blp* I	***Bst*X I**	**I-*Ppo* I**	*Pml* I	***Spe* I**
Afl II	*Bpu* 1102I	***Bsu*3 6 I**	***Nde* I**	***Pst* I**	*Spl* I
***Age* I**	*Bsp*120 I	***Csp* I**	***Nru* I**	***Pvu* II**	*Sse*8387 I
***Apa* I**	***Bss*H II**	*Eco*72 I	*Pac* I	*Rsr* II	*Swa* I
Asc I	*Bst*1 107 I	*Eco*81 I	*Pfl*M I	***Sac* II**	***Tth*111 I**
***Bal* I**	***Bst*9 8 I**	***Eco*R I**	*Pin*A I	***Sgf* I**	

Table 14. Restriction Enzymes That Cut the pGL3-Control Vector 6 or More Times.

Aci I	***Bst*71 I**	***Fok* I**	*Mae* I	***Nci* I**	*Sfa*N I
***Alu* I**	***Bst*O I**	***Hae* II**	*Mae* II	***Nde* II**	***Taq* I**
***Ban* I**	*Bst*U I	***Hae* III**	*Mae* III	*Nla* III	*Tfi* I
Bbv I	***Cfo* I**	*Hga* I	***Mbo* I**	*Nla* IV	***Tru*9 I**
***Bsa*0 I**	*Cfr*1 0 I	***Hha* I**	***Mbo* II**	*Ple* I	***Xho* II**
*Bsa*J I	***Dde* I**	***Hin*f I**	*Mnl* I	***Rsa* I**	
***Bsp*1286 I**	***Dpn* I**	***Hpa* II**	*Mse* I	***Sau*3 A I**	
Bsr I	*Ear* I	*Hph* I	***Msp***	***Sau*9 6 I**	
***Bsr* S I**	*Fnu*4H I	***Hsp*9 2 II**	***Msp*A1 I**	*Scr*F I	

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

pGL3-Control Vector Sequence

The strand shown is the same as the ssDNA strand produced by this vector and also corresponds to the mRNA synthesized from the *luc+* gene.

```
  1 GGTACCGAGC TCTTACGCGT GCTAGCCCGG GCTCGAGATC TGCGATCTGC
 51 ATCTCAATTA GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG
101 CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATC GCTGACTAAT
151 TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC
201 AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTT
251 GGCATTCCGG TACTGTTGGT AAAGCCACCA TGGAAGACGC CAAAAACATA
301 AAGAAAGGCC CGGCGCCATT CTATCCGCTG GAAGATGGAA CCGCTGGAGA
351 GCAACTGCAT AAGGCTATGA AGAGATACGC CCTGGTTCCT GGAACAATTG
401 CTTTTACAGA TGCACATATC GAGGTGGACA TCACTTACGC TGAGTACTTC
451 GAAATGTCCG TTCGGTTGGC AGAAGCTATG AAACGATATG GGCTGAATAC
501 AAATCACAGA ATCGTCGTAT GCAGTGAAAA CTCTCTTCAA TTCTTTATGC
551 CGGTGTTGGG CGCGTTATTT ATCGGAGTTG CAGTTGCGCC CGCGAACGAC
601 ATTTATAATG AACGTGAATT GCTCAACAGT ATGGGCATTT CGCAGCCTAC
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pGL3-Control Vector Sequence (continued)

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 651  CGTGGTGTTC GTTTCCAAAA AGGGGTTGCA AAAAATTTTG AACGTGCAAA
 701  AAAAGCTCCC AATCATCCAA AAAATTATTA TCATGGATTC TAAAACGGAT
 751  TACCAGGGAT TTCAGTCGAT GTACACGTTC GTCACATCTC ATCTACCTCC
 801  CGGTTTTAAT GAATACGATT TTGTGCCAGA GTCCTTCGAT AGGGACAAGA
 851  CAATTGCACT GATCATGAAC TCCTCTGGAT CTACTGGTCT GCCTAAAGGT
 901  GTCGCTCTGC CTCATAGAAC TGCCTGCGTG AGATTCTCGC ATGCCAGAGA
 951  TCCTATTTTT GGCAATCAAA TCATTCCGGA TACTGCGATT TTAAGTGTTG
1001  TTCCATTCCA TCACGGTTTT GGAATGTTTA CTACACTCGG ATATTTGATA
1051  TGTGGATTTC GAGTCGTCTT AATGTATAGA TTTGAAGAAG AGCTGTTTCT
1101  GAGGAGCCTT CAGGATTACA AGATTCAAAG TGCGCTGCTG GTGCCAACCC
1151  TATTCTCCTT CTTCGCCAAA AGCACTCTGA TTGACAAATA CGATTTATCT
1201  AATTTACACG AAATTGCTTC TGGTGGCGCT CCCCTCTCTA AGGAAGTCGG
1251  GGAAGCGGTT GCCAAGAGGT TCCATCTGCC AGGTATCAGG CAAGGATATG
1301  GGCTCACTGA GACTACATCA GCTATTCTGA TTACACCCGA GGGGGATGAT
1351  AAACCGGGCG CGGTCGGTAA AGTTGTTCCA TTTTTTGAAG CGAAGGTTGT
1401  GGATCTGGAT ACCGGGAAAA CGCTGGGCGT TAATCAAAGA GGCGAACTGT
1451  GTGTGAGAGG TCCTATGATT ATGTCCGGTT ATGTAAACAA TCCGGAAGCG
1501  ACCAACGCCT TGATTGACAA GGATGGATGG CTACATTCTG GAGACATAGC
1551  TTACTGGGAC GAAGACGAAC ACTTCTTCAT CGTTGACCGC CTGAAGTCTC
1601  TGATTAAGTA CAAAGGCTAT CAGGTGGCTC CCGCTGAATT GGAATCCATC
1651  TTGCTCCAAC ACCCCAACAT CTTCGACGCA GGTGTCGCAG GTCTTCCCGA
1701  CGATGACGCC GGTGAACTTC CCGCCGCCGT TGTTGTTTTG GAGCACGGAA
1751  AGACGATGAC GGAAAAAGAG ATCGTGGATT ACGTCGCCAG TCAAGTAACA
1801  ACCGCGAAAA AGTTGCGCGG AGGAGTTGTG TTTGTGGACG AAGTACCGAA
1851  AGGTCTTACC GGAAAACTCG ACGCAAGAAA AATCAGAGAG ATCCTCATAA
1901  AGGCCAAGAA GGGCGGAAAG ATCGCCGTGT AATTCTAGAG TCGGGGCGGC
1951  CGGCCGCTTC GAGCAGACAT GATAAGATAC ATTGATGAGT TTGGACAAAC
2001  CACAACTAGA ATGCAGTGAA AAAAATGCTT TATTTGTGAA ATTTGTGATG
2051  CTATTGCTTT ATTTGTAACC ATTATAAGCT GCAATAAACA AGTTAACAAC
2101  AACAATTGCA TTCATTTTAT GTTTCAGGTT CAGGGGGAGG TGTGGGAGGT
2151  TTTTTAAAGC AAGTAAAACC TCTACAAATG TGGTAAAATC GATAAGGATC
2201  TGAACGATGG AGCGGAGAAT GGGCGGAACT GGGCGGAGTT AGGGGCGGGA
2251  TGGGCGGAGT TAGGGGCGGG ACTATGGTTG CTGACTAATT GAGATGCATG
2301  CTTTGCATAC TTCTGCCTGC TGGGGAGCCT GGGGACTTTC CACACCTGGT
2351  TGCTGACTAA TTGAGATGCA TGCTTTGCAT ACTTCTGCCT GCTGGGGAGC
2401  CTGGGGACTT TCCACACCCT AACTGACACA CATTCCACAG CGGATCCGTC
2451  GACCGATGCC CTTGAGAGCC TTCAACCCAG TCAGCTCCTT CCGGTGGGCG
2501  CGGGGCATGA CTATCGTCGC CGCACTTATG ACTGTCTTCT TTATCATGCA
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pGL3-Control Vector Sequence (continued)

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2551  ACTCGTAGGA  CAGGTGCCGG  CAGCGCTCTT  CCGCTTCCTC  GCTCACTGAC
2601  TCGCTGCGCT  CGGTCGTTCG  GCTGCGGCGA  GCGGTATCAG  CTCACTCAAA
2651  GGCGGTAATA  CGGTTATCCA  CAGAATCAGG  GGATAACGCA  GGAAAGAACA
2701  TGTGAGCAAA  AGGCCAGCAA  AAGGCCAGGA  ACCGTAAAAA  GGCCGCGTTG
2751  CTGGCGTTTT  TCCATAGGCT  CCGCCCCCCT  GACGAGCATC  ACAAAAATCG
2801  ACGCTCAAGT  CAGAGGTGGC  GAAACCCGAC  AGGACTATAA  AGATACCAGG
2851  CGTTTCCCCC  TGGAAGCTCC  CTCGTGCGCT  CTCCTGTTCC  GACCCTGCCG
2901  CTTACCGGAT  ACCTGTCCGC  CTTTCTCCCT  TCGGGAAGCG  TGGCGCTTTC
2951  TCATAGCTCA  CGCTGTAGGT  ATCTCAGTTC  GGTGTAGGTC  GTTCGCTCCA
3001  AGCTGGGCTG  TGTGCACGAA  CCCCCCGTTC  AGCCCGACCG  CTGCGCCTTA
3051  TCCGGTAACT  ATCGTCTTGA  GTCCAACCCG  GTAAGACACG  ACTTATCGCC
3101  ACTGGCAGCA  GCCACTGGTA  ACAGGATTAG  CAGAGCGAGG  TATGTAGGCG
3151  GTGCTACAGA  GTTCTTGAAG  TGGTGGCCTA  ACTACGGCTA  CACTAGAAGA
3201  ACAGTATTTG  GTATCTGCGC  TCTGCTGAAG  CCAGTTACCT  TCGGAAAAAG
3251  AGTTGGTAGC  TCTTGATCCG  GCAAACAAAC  CACCGCTGGT  AGCGGTGGTT
3301  TTTTTGTTTG  CAAGCAGCAG  ATTACGCGCA  GAAAAAAAGG  ATCTCAAGAA
3351  GATCCTTTGA  TCTTTTCTAC  GGGGTCTGAC  GCTCAGTGGA  ACGAAAACTC
3401  ACGTTAAGGG  ATTTTGGTCA  TGAGATTATC  AAAAAGGATC  TTCACCTAGA
3451  TCCTTTTAAA  TTAAAAATGA  AGTTTTAAAT  CAATCTAAAG  TATATATGAG
3501  TAAACTTGGT  CTGACAGTTA  CCAATGCTTA  ATCAGTGAGG  CACCTATCTC
3551  AGCGATCTGT  CTATTTCGTT  CATCCATAGT  TGCCTGACTC  CCCGTCGTGT
3601  AGATAACTAC  GATACGGGAG  GGCTTACCAT  CTGGCCCCAG  TGCTGCAATG
3651  ATACCGCGAG  ACCCACGCTC  ACCGGCTCCA  GATTTATCAG  CAATAAACCA
3701  GCCAGCCGGA  AGGGCCGAGC  GCAGAAGTGG  TCCTGCAACT  TTATCCGCCT
3751  CCATCCAGTC  TATTAATTGT  TGCCGGGAAG  CTAGAGTAAG  TAGTTCGCCA
3801  GTTAATAGTT  TGCGCAACGT  TGTTGCCATT  GCTACAGGCA  TCGTGGTGTC
3851  ACGCTCGTCG  TTTGGTATGG  CTTCATTCAG  CTCCGGTTCC  CAACGATCAA
3901  GGCGAGTTAC  ATGATCCCCC  ATGTTGTGCA  AAAAAGCGGT  TAGCTCCTTC
3951  GGTCCTCCGA  TCGTTGTCAG  AAGTAAGTTG  GCCGCAGTGT  TATCACTCAT
4001  GGTTATGGCA  GCACTGCATA  ATTCTCTTAC  TGTCATGCCA  TCCGTAAGAT
4051  GCTTTTCTGT  GACTGGTGAG  TACTCAACCA  AGTCATTCTG  AGAATAGTGT
4101  ATGCGGCGAC  CGAGTTGCTC  TTGCCCGGCG  TCAATACGGG  ATAATACCGC
4151  GCCACATAGC  AGAACTTTAA  AAGTGCTCAT  CATTGGAAAA  CGTTCTTCGG
4201  GGCGAAAACT  CTCAAGGATC  TTACCGCTGT  TGAGATCCAG  TTCGATGTAA
4251  CCCACTCGTG  CACCCAACTG  ATCTTCAGCA  TCTTTTACTT  TCACCAGCGT
4301  TTCTGGGTGA  GCAAAAACAG  GAAGGCAAAA  TGCCGCAAAA  AAGGGAATAA
4351  GGGCGACACG  GAAATGTTGA  ATACTCATAC  TCTTCCTTTT  TCAATATTAT
4401  TGAAGCATTT  ATCAGGGTTA  TTGTCTCATG  AGCGGATACA  TATTTGAATG
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4451  TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATTT CCCCGAAAAG
4501  TGCCACCTGA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG
4551  GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC
4601  TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC TTTCCCCGTC
4651  AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG
4701  CACCTCGACC CCAAAAAACT TGATTAGGGT GATGGTTCAC GTAGTGGGCC
4751  ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT
4801  TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG
4851  GTCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT
4901  AAAAAATGAG CTGATTTAAC AAAAATTTAA CGCGAATTTT AACAAAATAT
4951  TAACGCTTAC AATTTGCCAT TCGCCATTCA GGCTGCGCAA CTGTTGGGAA
5001  GGGCGATCGG TGCGGGCCTC TTCGCTATTA CGCCAGCCCA AGCTACCATG
5051  ATAAGTAAGT AATATTAAGG TACGGGAGGT ACTTGGAGCG GCCGCAATAA
5101  AATATCTTTA TTTTCATTAC ATCTGTGTGT TGGTTTTTTG TGTGAATCGA
5151  TAGTACTAAC ATACGCTCTC CATCAAAACA AAACGAAACA AAACAAACTA
5201  GCAAAATAGG CTGTCCCCAG TGCAAGTGCA GGTGCCAGAA CATTTCTCTA
5251  TCGATA
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[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.

[c]The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

[d]Licensed under U.S. Pat. No. 5,075,430.

[e]U.S. Pat. No. 5,981,235 and Australian Pat. No. 729932 have been issued to Promega Corporation for methods for isolating nucleic acids using alkaline protease. Other patents are pending. Australian Pat. No. 730718 has been issued to Promega Corporation for an improved filtration system and method. Other patents are pending.

[f]U.S. Pat. Nos. 5,658,548 and 5,808,041 and Australian Pat. No. 689815 have been issued to Promega Corporation for nucleic acid purification on silica gel and glass mixtures. Other patents are pending.

[g]U.S. Pat. No. 6,027,945 and Australian Pat. No. 732756 have been issued to Promega Corporation for methods of isolating biological target materials using silica magnetic particles. Other patents are pending. U.S. Pat. No. 6,194,562 has been issued to Promega Corporation for endotoxin reduction in nucleic acid purification. Other patents are pending.

[h]Transfectam is a registered trademark of Promega Corporation, which sells the Transfectam® product for research purposes only under agreement with CNRS-Paris, owner by assignment of U. S. Pat. No. 5,171,678. The Transfectam® product was developed by J.P. Behr and J.P. Loeffler and is covered by the aforementioned patent.

[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]U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289, 5,814,471, Australian Pat. No. 649289 and European Pat. No. 0 553 234 have been issued to Promega Corporation for a firefly luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Other patents are pending. Certain applications of this product may require licenses from others.

[l]U.S. Pat. No. 5,955,363 has been issued to Promega Corporation for a vector for in vitro mutagenesis and use thereof.

[m]The 7-deaza-dGTP component is licensed from Boehringer Mannheim GmbH under U.S. Pat. Nos. 4,804,748 and 5,480,980.

[n]U.S. Pat. Nos. 5,523,206 and 5,654,149, Australian Pat. No. 671820 and European Pat. No. 0 610 615 B1 have been issued to Promega Corporation for non-radioactive DNA sequencing.

[o]U.S. Pat. No. 5,108,892 has been issued to Promega Corporation for the use of a modified *Taq* DNA polymerase to determine DNA sequence and amplify DNA sequence.

[p]Notice to Purchaser: Limited Use License
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[q]U.S. Pat. No. 5,391,487 has been issued to Promega Corporation for Restriction Endonuclease *Sgf* I.

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pGL3 Luciferase Reporter Vectors: *Experienced User's Protocol*

This quick protocol is intended as an easy-to-follow reminder for experienced users. Please follow the complete protocol (Sections IV.A–D) the first time you use the pGL3 Luciferase Reporter Vectors.

Step	Protocol
Isolation of Plasmid DNA (Section IV.D)	1. Prepare an overnight culture in 250ml of LB medium containing 100µg/ml ampicillin. 2. Collect cells by centrifuging at 5,000 × *g* for 15 minutes at 4°C. Discard the supernatant. 3. Resuspend cells in 6ml of freshly prepared ice-cold lysis buffer. Mix carefully using a 10ml pipet. Incubate the tube containing the cells and lysis buffer in ice water for 20 minutes. 4. Add 12ml of 0.1N NaOH, 1% SDS (prepared fresh). Mix carefully and thoroughly by inversion. Do not vortex. 5. Add 7.5ml of potassium acetate solution (pH 4.8). Mix carefully by inversion. Incubate tube in ice water for 10 minutes. 6. Centrifuge at 12,000 × *g* for 15 minutes. Transfer the supernatant to a new tube, avoiding the white precipitate. Add 50µl of RNase A (1mg/ml stock) to the supernatant. Incubate for 20 minutes at 37°C. 7. Extract with one volume of TE-saturated phenol:chloroform:isoamyl alcohol. Centrifuge at 12,000 × *g* for 10 minutes. 8. Save the upper, aqueous phase and repeat the TE-saturated phenol:chloroform:isoamyl alcohol extraction described in Step 7. 9. Extract with one volume of chloroform:isoamyl alcohol (24:1) by vortexing for 1 minute. Centrifuge at 12,000 × *g* for 10 minutes. 10. Transfer the upper aqueous phase to a new tube and add 2 volumes of 100% ethanol. Centrifuge at 12,000 × *g* for 20 minutes. 11. **Optional:** Dissolve the pellet in 1.6ml of water. Add 0.4ml of 4M NaCl and mix. Add 2ml of 13% (w/v) polyethylene glycol and mix. Incubate the tube in ice water for 60 minutes. Centrifuge at 12,000 × *g* for 10 minutes. 12. Remove the supernatant and wash the pellet with 70% ethanol. Centrifuge at 12,000 × *g* for 5 minutes. 13. Dry the pellet under vacuum. Dissolve the pellet in water or TE buffer (100–500µl).