Technical Manual

pGL4 Luciferase Reporter Vectors

INSTRUCTIONS FOR USE OF PRODUCTS E6651, E6661, E6671, E6681, E6691, E6701, E6711, E6721, E6731, E6741, E6751, E6761, E6771, E6881, E6891, E6901, E6911, E6921, E6931, E6941, E6951, E6961, E6971, E6981, E6991, E7501, E7511, E7521, E8411, E8421, E8431, E8441, E8451, E8461, E8471, E8481 AND E8491.

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1. Description

The pGL4 Luciferase Reporter Vectors[a,b,c,d] are the next generation of reporter gene vectors optimized for expression in mammalian cells. Numerous configurations of pGL4 Vectors are available, including those with the synthetic firefly *luc2* (*Photinus pyralis*) and *Renilla hRluc* (*Renilla reniformis*) genes, which have been codon optimized for more efficient expression in mammalian cells. Furthermore, both the reporter genes and the vector backbone, including the *bla* (β-lactamase or Amp^r) and mammalian selectable marker genes for hygromycin (Hygro or Hyg^r), neomycin (Neo or Neo^r) and puromycin (Puro or Puro^r), have been engineered to reduce the number of consensus transcription factor binding sites, reducing background and the risk of anomalous transcription.

The pGL4 Vector backbone, provided with a choice of *luc2* or *hRluc* genes, is also supplied with two Rapid Response™ Luciferase Reporter genes for each luciferase gene. The proteins encoded by these Rapid Response™ Luciferase genes respond more quickly and with greater magnitude to changes in transcriptional activity than their more stable counterparts.

The pGL4 Vector family includes:

- Basic vectors with no promoter that contain a multiple cloning region for cloning a promoter of choice
- Vectors containing a minimal promoter
- Vectors containing response elements and a minimal promoter
- Promoter-containing vectors that can be used as expression controls or as co-reporter vectors

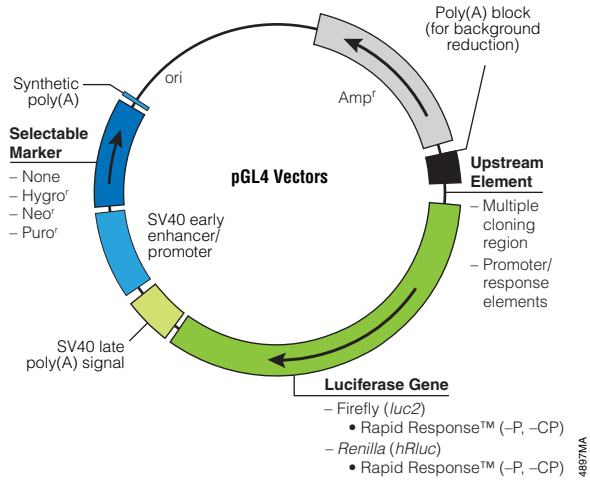


Figure 1. Generic pGL4 Vector map showing the variety of features.

Advantages of the pGL4 Vectors include:

Improved sensitivity and biological relevance due to:

- **Increased reporter gene expression:** Codon optimization of synthetic genes for mammalian expression
- **Reduced background and risk of expression artifacts:** Removal of cryptic DNA regulatory elements and transcription factor binding sites
- **Improved temporal response:** Rapid Response™ technology available using destabilized luciferase genes

Enhanced usability and convenience:

- **Flexible detection options:** Choice of either synthetic *luc2* (*Photinus pyralis*) or *hRluc* (*Renilla reniformis*) reporter genes
- **Easy transition from transient to stable cells:** Choice of mammalian selectable markers
- **Easy transfer from one vector to another:** Common multiple cloning site and a unique *Sfi* I transfer scheme

2. Product Components and Storage Conditions

Product	Size	Cat.#
pGL4.10[*luc2*] Vector[a,b]	20µg	E6651
pGL4.11[*luc2P*] Vector[a,b]	20µg	E6661
pGL4.12[*luc2CP*] Vector[a,b]	20µg	E6671
pGL4.13[*luc2*/SV40] Vector[a,b]	20µg	E6681
pGL4.14[*luc2*/Hygro] Vector[a,b]	20µg	E6691
pGL4.15[*luc2P*/Hygro] Vector[a,b]	20µg	E6701
pGL4.16[*luc2CP*/Hygro] Vector[a,b]	20µg	E6711
pGL4.17[*luc2*/Neo] Vector[a,b]	20µg	E6721
pGL4.18[*luc2P*/Neo] Vector[a,b]	20µg	E6731
pGL4.19[*luc2CP*/Neo] Vector[a,b]	20µg	E6741
pGL4.20[*luc2*/Puro] Vector[a,b]	20µg	E6751
pGL4.21[*luc2P*/Puro] Vector[a,b]	20µg	E6761
pGL4.22[*luc2CP*/Puro] Vector[a,b]	20µg	E6771
pGL4.23[*luc2*/minP] Vector[a,b]	20µg	E8411
pGL4.24[*luc2P*/minP] Vector[a,b]	20µg	E8421
pGL4.25[*luc2CP*/minP] Vector[a,b]	20µg	E8431
pGL4.26[*luc2*/minP/Hygro] Vector[a,b]	20µg	E8441
pGL4.27[*luc2P*/minP/Hygro] Vector[a,b]	20µg	E8451
pGL4.28[*luc2CP*/minP/Hygro] Vector[a,b]	20µg	E8461
pGL4.29[*luc2P*/CRE/Hygro] Vector[a,b]	20µg	E8471
pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector[a,b,c]	20µg	E8481
pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector[a,b,d]	20µg	E8491
pGL4.70[*hRluc*] Vector[a,e]	20µg	E6881
pGL4.71[*hRlucP*] Vector[a,e]	20µg	E6891
pGL4.72[*hRlucCP*] Vector[a,e]	20µg	E6901
pGL4.73[*hRluc*/SV40] Vector[a,e]	20µg	E6911
pGL4.74[*hRluc*/TK] Vector[a,e]	20µg	E6921
pGL4.75[*hRluc*/CM*V*] Vector[a,e,f]	20µg	E6931
pGL4.76[*hRluc*/Hygro] Vector[a,e]	20µg	E6941
pGL4.77[*hRlucP*/Hygro] Vector[a,e]	20µg	E6951
pGL4.78[*hRlucCP*/Hygro] Vector[a,e]	20µg	E6961
pGL4.79[*hRluc*/Neo] Vector[a,e]	20µg	E6971
pGL4.80[*hRlucP*/Neo] Vector[a,e]	20µg	E6981
pGL4.81[*hRlucCP*/Neo] Vector[a,e]	20µg	E6991
pGL4.82[*hRluc*/Puro] Vector[a,e]	20µg	E7501
pGL4.83[*hRlucP*/Puro] Vector[a,e]	20µg	E7511
pGL4.84[*hRlucCP*/Puro] Vector[a,e]	20µg	E7521

2. Product Components and Storage Conditions (continued)

Available Separately:

Product	Size	Cat.#
pGL4.31[*luc2P*/*GAL4*UAS/Hygro] Vector[a,b]	20µg	C9351

The *CheckMate™/Flexi® Vector Mammalian Two-Hybrid System Technical Manual*, #TM283, is shipped with the pGL4.31[*luc2P*/*GAL4*UAS/Hygro] Vector and contains protocol information for use of this vector.

Storage Conditions: Store the pGL4 Luciferase Reporter Vectors at –20°C.

3. General Considerations

3.A. The pGL4 Vector Backbone

To reduce the risk of anomalous expression and increase the reliability of reporter gene expression, the pGL3 Vector region upstream of the reporter gene was re-engineered to create the pGL4 Vectors. The pGL3 Vector region from the start of the reporter gene (the *Nco* I restriction site) to the bacterial origin of replication sequence was also redesigned. The modifications to this region include: a greatly reduced number of consensus transcription factor binding sites (Figure 2); a redesign of the multiple cloning region; removal of the f1 origin of replication; deletion of an intronic sequence; and a reduction in the number of promoter modules. (Promoter modules are composite regulatory elements consisting of at least two transcription factor binding sites separated by a spacer. Promoter modules can have synergistic or antagonistic functions (1).)

Note: The synthetic Amp^r in the pGL4 Vectors expresses a protein sequence identical to that expressed by the Amp^r gene in the pGL3 Vectors.

The only regions of the pGL4 Vector backbone not optimized for reduced anomalous expression are the SV40 late poly(A) signal downstream of the reporter gene and the bacterial origin of replication.

Modifications to the pGL4 Vectors have resulted in an improved signal-to-background ratio. Some of these improved ratios are presented in Table 1, where several pGL4 Vectors are compared to their controls (the corresponding promoterless vectors), and the fold increase in the signal-to-background ratio is compared for the pGL4 Vectors and previous generations of reporter vectors (e.g., pGL3 and phRL Vectors).

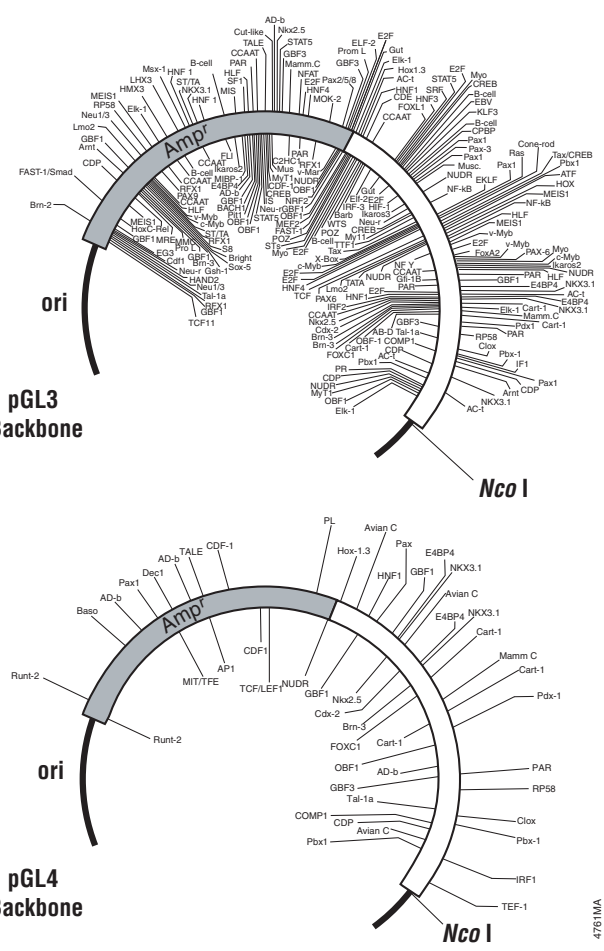


Figure 2. Reduced number of consensus transcription factor binding sites for the pGL4 Vectors. The number of consensus transcription factor binding sites identified in the pGL3 Vector backbone has been greatly reduced in the pGL4 Vector backbone.

Note: The SV40, HSV-TK and CMV promoters contain several consensus transcription factor binding sites. Due to the complex nature of the promoters these sites were not altered. However, the number and type of consensus transcription factor binding sites vary depending on the promoter. For maximum reduction of anomalous expression in your assay system, we recommend that you evaluate the promoters to determine the one that results in the lowest level of anomalous expression with your system.

3.A. The pGL4 Vector Backbone (continued)

Synthetic Poly(A) Signal/Transcriptional Pause Site for Background Reduction
All pGL4 Vectors contain a synthetic poly(A) signal/transcriptional pause site (2) located upstream of either the multiple cloning region (in promoterless vectors) or the SV40, CMV or HSV-TK promoter (in promoter-containing vectors). The synthetic poly(A) signal/transcriptional pause site is present to reduce the effects of spurious transcription on luciferase reporter gene expression.

Table 1. Signal-to-Background Comparison for Several of the pGL4, pGL3 and phRL Vectors.[1]

Vectors	Signal-to-Background Ratio[1]	Fold Increase for pGL4 Vectors[2]
pGL4.13[*luc2*/SV40] vs. pGL4.10[*luc2*]	3,200 ± 340	3.7
pGL3-Control vs. pGL3-Basic	670± 57	
pGL4.73[*hRluc*/SV40] vs. pGL4.70[*hRluc*]	630 ± 78	32
phRL-SV40 vs. phRL-null	19 ± 2	
pGL4.74[*hRluc*/TK] vs. pGL4.70[*hRluc*]	79 ± 8	33
phRL-TK vs. phRL-null	2.3 ± 0.9	
pGL4.75[*hRluc*/CMV] vs. pGL4.70[*hRluc*]	1,100 ± 120	64
phRL-CMV vs. phRL-null	17 ± 2	

[1]To generate signal-to-background ratios the luciferase-containing vectors were transfected into CHO cells. At 24 hours post-transfection the cells were lysed, luminescent signals were generated using the Dual-Luciferase® Assay System and the relative light units were corrected for transfection efficiency, yielding "normalized signals". Signal-to-background ratio = signal from promoter-containing vector/signal from corresponding promoterless vector.
[2]Fold increase for pGL4 Vectors = (signal-to-background ratio from pGL4 Vector/signal-to-background ratio from corresponding pGL3 or phRL Vector) – 1.
The experiment was repeated in CHO and other cell lines (HeLa, NIH/3T3 and HEK 293 cells) generating similar results.

3.B. The pGL4 Reporter Genes

To increase expression and reliability of the firefly luciferase reporter, a synthetic firefly luciferase gene, *luc2*, has been engineered. The gene was synthetically redesigned by changing the codons to those most frequently used in mammalian cells while simultaneously removing most of the consensus sequences for transcription factor binding sites (Figure 3). Additionally, the number of predicted promoter modules present within the *luc*+ reporter gene (and thought to cause anomalous expression) has been reduced to a single module in the *luc2* gene. To maintain the integrity of the firefly luciferase protein sequence from the *luc*+ gene to the *luc2* gene, some of the consensus transcription factor binding sites and promoter modules were not removed. In the transfection experiment shown in Figure 4, the synthetic firefly *luc2* luciferase gene displayed an increase in expression compared to *luc*+.

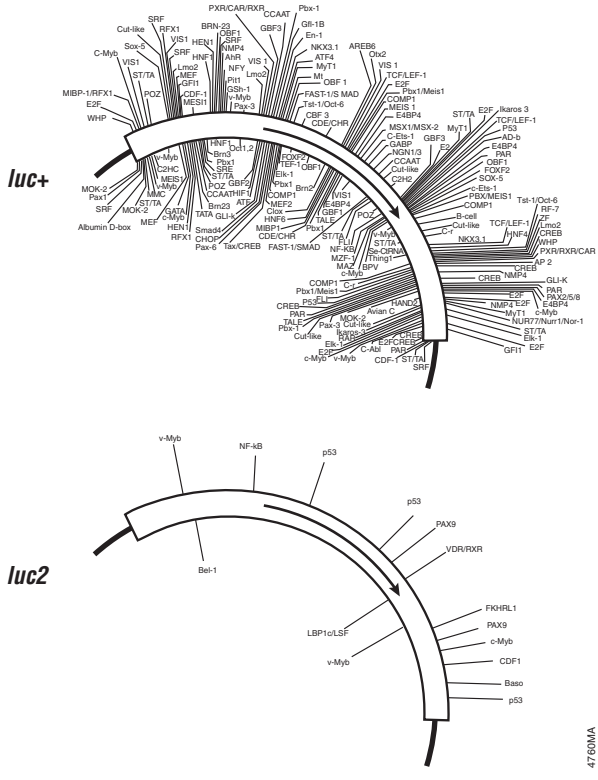


Figure 3. Reduced number of consensus transcription factor binding sites for the *luc2* gene. The number of consensus transcription factor binding sites identified in the *luc*+ gene (top) have been greatly reduced in the *luc*2 gene (below), while conserving the protein sequence.

To ensure that the synthetic construction affected only expression, both the *luc*+ and *luc*2 genes were cloned into the pGL3-Control Vector. The vectors were co-transfected with an internal control for assessing transfection efficiency, and after 24 hours the luminescence (in relative light units) was measured and normalized to the control. When compared to *luc*+, the *luc*2 gene demonstrated a 4.1- to 11.8-fold increase in expression for the four mammalian cell lines tested.

For more information on the *hRluc* gene, please refer to the *Synthetic* Renilla *Luciferase Reporter Vectors Technical Manual,* #TM237. See Table 2 for a list of the features in each pGL4 Vector.

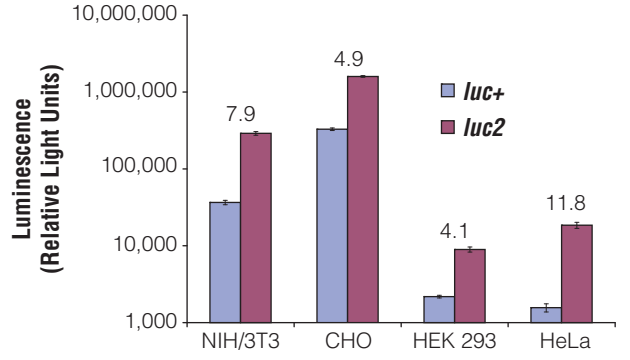


Figure 4. The firefly *luc2* gene displays higher expression than *luc+*. The *luc2* gene was cloned into the pGL3-Control Vector (Cat.# E1741), replacing the *luc+* gene. Thus both firefly luciferase genes were in the same pGL3-Control Vector backbone. The two vectors containing either of the firefly luciferase genes were co-transfected into NIH/3T3, CHO, HEK 293 and HeLa cells using the phRL-TK Vector (Cat.# E6241) for a transfection control. Twenty-four hours post-transfection the cells were lysed with Passive Lysis Buffer (Cat.# E1941) and luminescence was measured using the Dual-Luciferase® Assay System (Cat.# E1910). Luminescence (relative light units) was normalized to the *Renilla* luciferase expression from the phRL-TK Vector transfection control. The fold increase in expression values is listed above each pair of bars. A repeat of this experiment yielded similar results.

Table 2. Features of the pGL4 Vectors.

Vector	Multiple Cloning Region	Reporter Gene	Protein Degradation Sequence	Reporter Gene Promoter/ Response Element	Mammalian Selectable Marker
pGL4.10[*luc2*]	Yes	*luc2*[1]	No	No	No
pGL4.11[*luc2P*]	Yes	"	hPEST	No	No
pGL4.12[*luc2CP*]	Yes	"	hCL1-hPEST	No	No
pGL4.13[*luc2*/SV40]	No	"	No	SV40	No
pGL4.14[*luc2*/Hygro]	Yes	"	No	No	Hygro
pGL4.15[*luc2P*/Hygro]	Yes	"	hPEST	No	Hygro
pGL4.16[*luc2CP*/Hygro]	Yes	"	hCL1-hPEST	No	Hygro
pGL4.17[*luc2*/Neo]	Yes	"	No	No	Neo
pGL4.18[*luc2P*/Neo]	Yes	"	hPEST	No	Neo
pGL4.19[*luc2CP*/Neo]	Yes	"	hCL1-hPEST	No	Neo
pGL4.20[*luc2*/Puro]	Yes	"	No	No	Puro
pGL4.21[*luc2P*/Puro]	Yes	"	hPEST	No	Puro
pGL4.22[*luc2CP*/Puro]	Yes	"	hCL1-hPEST	No	Puro
pGL4.23[*luc2*/minP]	Yes	"	No	minP	No
pGL4.24[*luc2P*/minP]	Yes	"	hPEST	"	No
pGL4.25[*luc2CP*/minP]	Yes	"	hCL1-hPEST	"	No
pGL4.26[*luc2*/minP/Hygro]	Yes	"	No	"	Hygro
pGL4.27[*luc2P*/minP/Hygro]	Yes	"	hPEST	"	Hygro
pGL4.28[*luc2CP*/minP/Hygro]	Yes	"	hCL1-hPEST	"	Hygro
pGL4.29[*luc2P*/CRE/Hygro]	No	"	hPEST	CRE	Hygro
pGL4.30[*luc2P*/NFAT-RE/Hygro]	No	"	hPEST	NFAT-RE	Hygro
pGL4.31[*luc2P*/*GAL4*UAS/Hygro]	No	"	hPEST	*GAL4*UAS	Hygro
pGL4.32[*luc2P*/NF-κB-RE/Hygro]	No	"	hPEST	NF-κB-RE	Hygro

[1]*luc2* is the synthetic firefly luciferase gene.

(continued)

Table 2. Features of the pGL4 Vectors (continued).

Vector	Multiple Cloning Region	Reporter Gene	Protein Degradation Sequence	Reporter Gene Promoter/ Response Element	Mammalian Selectable Marker
pGL4.70[*hRluc*]	Yes	*hRluc*[2]	No	No	No
pGL4.71[*hRlucP*]	Yes	"	hPEST	No	No
pGL4.72[*hRlucCP*]	Yes	"	hCL1-hPEST	No	No
pGL4.73[*hRluc*/SV40]	No	"	No	SV40	No
pGL4.74[*hRluc*/TK]	No	"	No	HSV-TK	No
pGL4.75[*hRluc*/CMV]	No	"	No	CMV	No
pGL4.76[*hRluc*/Hygro]	Yes	"	No	No	Hygro
pGL4.77[*hRlucP*/Hygro]	Yes	"	hPEST	No	Hygro
pGL4.78[*hRlucCP*/Hygro]	Yes	"	hCL1-hPEST	No	Hygro
pGL4.79[*hRluc*/Neo]	Yes	"	No	No	Neo
pGL4.80[*hRlucP*/Neo]	Yes	"	hPEST	No	Neo
pGL4.81[*hRlucCP*/Neo]	Yes	"	hCL1-hPEST	No	Neo
pGL4.82[*hRluc*/Puro]	Yes	"	No	No	Puro
pGL4.83[*hRlucP*/Puro]	Yes	"	hPEST	No	Puro
pGL4.84[*hRlucCP*/Puro]	Yes	"	hCL1-hPEST	No	Puro

[2]*hRluc* is the synthetic *Renilla* gene.

3.C. Distinguishing Features of the pGL4 Vectors

The following features are available for the pGL4 Vectors.

Reporter Genes:

- Firefly luciferase (*luc2*) or *Renilla* luciferase (*hRluc*)
- Regular or Rapid Response™ genes

Upstream Elements:

- Promoterless basic vectors
- Vectors with minimal promoters
- Vectors containing response elements and minimal promoters
- Vectors with constitutive promoters such as SV40, HSV-TK and CMV

Selectable Markers:

- Hygro
- Neo
- Puro

The Rapid Response™ pGL4 Vectors

Computational models predict that genetic reporters with reduced intracellular stability will yield faster response to changes in transcriptional rate and an increase in the relative magnitude of the response (3). Destabilized reporter proteins (i.e., those with faster protein degradation rates) are therefore expected to be more responsive and better suited to monitor rapid processes (such as promoter activation and repression) than those with slower degradation rates.

To generate reporter proteins that have increased protein degradation rates (i.e., destabilized reporters) one or both of two different protein degradation sequences have been incorporated into the synthetic firefly *luc2* and *Renilla hRluc* luciferase genes. The first degradation sequence, PEST, is a forty-amino acid sequence isolated from the C-terminal region of mouse ornithine decarboxylase (4). The second

degradation sequence includes CL1 and PEST. CL1, originally isolated from yeast, has also been shown to increase protein degradation (5). The codons within the PEST and CL1-PEST sequences have been optimized for expression in mammalian cells, and the number of consensus transcription factor binding sites has been reduced. To reflect the changes in these two protein degradation sequences they have been designated hPEST and hCL1-hPEST. The Rapid Response™ pGL4 Reporter gene design is shown in Figure 5.

As the result of inclusion of the protein degradation sequences in the Rapid Response™ pGL4 Vectors, the rate of reporter response to cellular stimuli has increased. In Figure 6 and Table 3 increased rates of reporter response in the CRE (cAMP response element) model system are demonstrated. For the firefly luciferase-based Rapid Response™ pGL4 Reporter Vectors (pGL4.11[*luc2P*] and pGL4.12[*luc2CP*]), the rate of reporter response for the first two hours post-induction increased one- and twofold, respectively, over the *luc2* control (pGL4.10[*luc2*]). Similar results for the same time frame were detected for the *Renilla*-based Rapid Response™ pGL4 Reporter Vectors (pGL4.71[*hRlucP*] and pGL4.72[*hRlucCP*]). The rate of response for the *hRlucP*- and *hRlucCP*-destabilized *Renilla* reporters increased one- and threefold, respectively, when compared to the nondestabilized *hRluc* reporter.

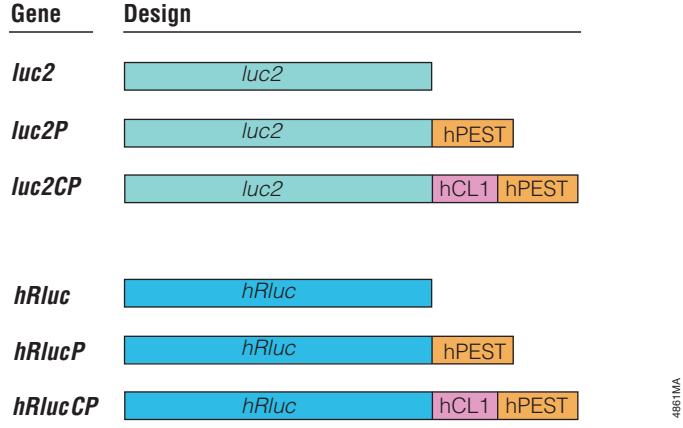


Figure 5. Gene design of the Rapid Response™ pGL4 Reporter Vectors.

A consequence of inclusion of the degradation sequences is that the destabilized luciferase proteins do not accumulate in the cell to the same extent as the nondestabilized luciferase-containing controls. As a result, destabilized reporter proteins typically generate lower signal intensities (Figure 7). The *luc2P* and *luc2CP* proteins displayed 18.7% and 3.9%, respectively, of the relative light units obtained using the firefly luciferase gene *luc2*. Similar reductions in relative light intensities were displayed by the *Renilla* luciferase-based Rapid Response™ pGL4 Reporter Vectors. The *Renilla* reporter proteins *hRlucP* and *hRlucCP* displayed 40% and 5.8%, respectively, of the relative light units of the *hRluc* control. Luminescence obtained using the Rapid Response™ pGL4 Reporter Vectors can vary depending on the mammalian cell line and experimental conditions used, thus optimization is recommended.

Table 3. Increase in Reporter Response for the Rapid Response™ pGL4 Reporter Vectors (results calculated from the data shown in Figure 6).

Vector	Induction at 2 Hours[1]	Fold Increase in Reporter Response Rate[2]
pGL4.10[*luc2*]	6.6	–
pGL4.11[*luc2P*]	13.8	1
pGL4.12[*luc2CP*]	21.6	2
pGL4.70[*hRluc*]	2.7	–
pGL4.71[*hRlucP*]	6.3	1
pGL4.72[*hRlucCP*]	12.2	3

[1]Induction = signal from induced samples/signal from noninduced samples.
[2]Reporter Response Rate = (induction of Rapid Response™ Reporter/induction of a regular reporter) – 1.

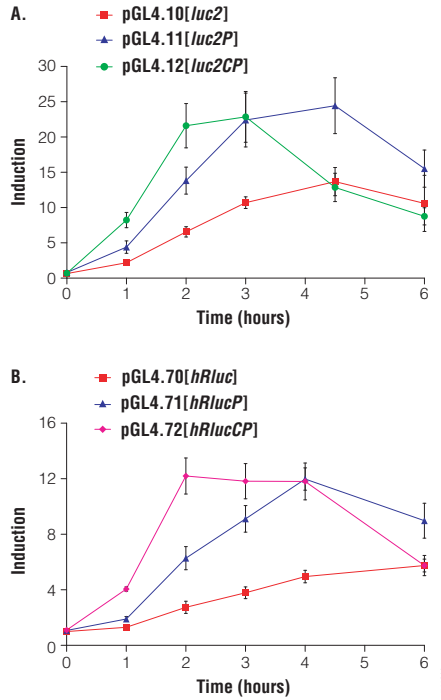


Figure 6. Increase in reporter response rate. To determine the increase in reporter response rate for the Rapid Response™ pGL4 Reporter Vectors, a DNA segment containing multiple CREs (cAMP response element) and a minimal HSV-TK promoter were cloned into each of the four Rapid Response™ pGL4 Reporter Vectors (pGL4.11[*luc2P*], pGL4.12[*luc2CP*], pGL4.71[*hRlucP*] and pGL4.72[*hRlucCP*]) and the two control vectors (pGL4.10[*luc2*] and pGL4.70[*hRluc*]). The CRE-containing vectors were then transiently transfected into HEK 293 cells. Twenty-four hours later 100µM of RO (RO-20-1724) and 1µM of ISO (isoproterenol hydrochloride) were added to the transiently transfected cells to induce reporter gene expression. RO alone (100µM) was added to a subset of the wells to serve as a noninduced control. Cells were harvested, lysed and assayed with either the Luciferase Assay System (Cat.# E1500; Panel A) or the *Renilla* Luciferase Assay System (Cat.# E2810; Panel B). Induction was calculated by dividing the relative light units obtained from the induced wells by the relative light units obtained from noninduced wells. Two repeats of this experiment yielded similar results.

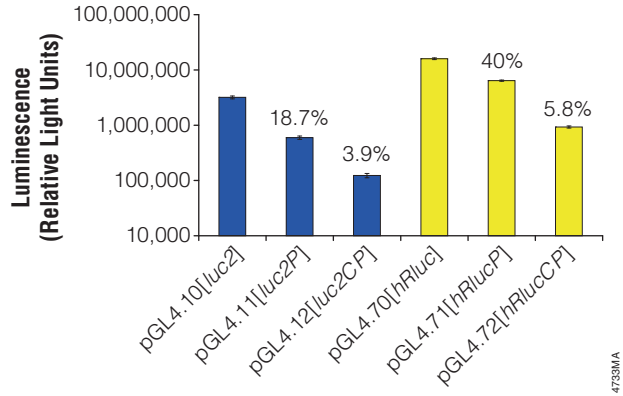


Figure 7. Relative expression of Rapid Response™ pGL4 luciferase genes. To determine the relative expression of the Rapid Response™ pGL4 luciferase genes, a DNA segment containing multiple CREs (cAMP response element) and a minimal HSV-TK promoter was cloned into each of the four Rapid Response™ pGL4 Reporter Vectors (pGL4.11[*luc2P*], pGL4.12[*luc2CP*], pGL4.71[*hRlucP*], and pGL4.72[*hRlucCP*]) and the two control vectors (pGL4.10[*luc2*] and pGL4.70[*hRluc*]), which do not contain degradation sequences. The resulting vectors were co-transfected with a second reporter (transfection control) into HEK 293 cells. The transfection controls for the firefly *luc2*-containing vectors were the phRL-TK Vector and, for the *Renilla hRluc* vectors, the pGL3-Control Vector. Twenty-four hours later the cells were lysed with Passive Lysis Buffer and luminescence (relative light units) was measured using the Dual-Luciferase® Assay System. (The firefly *luc2* data is shown by three bars on the left, while the *hRluc* data is contained in the three bars on the right.) Luminescence (relative light units) was normalized to the transfection control. The effects of the protein degradation sequences on the accumulation of luciferase enzyme are shown as percent expression of the control.

The Promoterless pGL4 Vectors

The promoterless pGL4 Vectors contain no enhancer or promoter elements. These vectors, which contain a multiple cloning region immediately upstream of the luciferase reporter gene, can be used to clone in a desired regulatory element(s) to drive expression of the reporter gene.

The Minimal Promoter pGL4 Vectors

The minimal promoter (minP) vectors contain a TATA-box promoter element immediately upstream of the luciferase reporter gene and immediately downstream of the multiple cloning region. These vectors can be used to clone in a desired promoterless-response element to drive expression of the reporter gene. The minimal TATA promoter has low basal activity and allows for sensitive response element activity measurements.

The CRE, NFAT-RE and NF-kB-RE Reporter Vectors

The pGL4.29[*luc2P*/CRE/Hygro], pGL4.30[*luc2P*/NFAT-RE/Hygro] and pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vectors are designed for use in both transient transfection assays and in stable cell line generation to monitor the cAMP, NFAT and NF-κB signaling pathways, respectively (Figure 8 shows a transient transfection). These vectors feature the Rapid Response™ Luciferase *luc2P* reporter gene and contain a hygromycin selection cassette to facilitate stable cell line expression. pGL4.29[*luc2P*/CRE/Hygro] contains three tandem CRE sequences upstream of the minimal promoter sequence to drive *luc2P* expression.

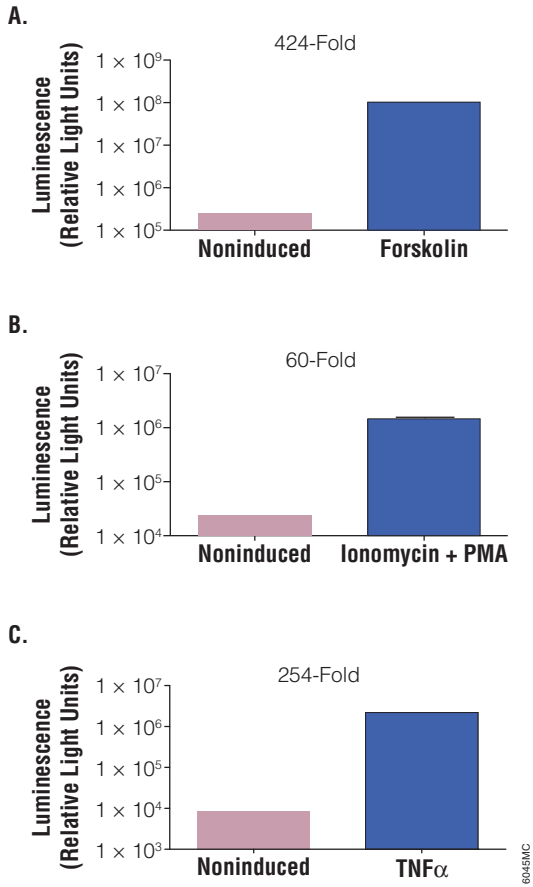


Figure 8. High levels of reporter induction for pGL4.29[*luc2P*/CRE/Hygro], pGL4.30[*luc2P*/NFAT-RE/Hygro] and pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vectors in transient transfection of HEK 293 cells. pGL4.29 (Panel A), pGL4.30 (Panel B) and pGL4.32 (Panel C) Reporter Vectors were transiently transfected into HEK 293 cells. After twenty-four hours, reporter gene expression was induced by adding: 100µM forskolin to the pGL4.29-transfected cells for 5 hours; 1µM ionomycin plus 10ng/ml PMA to the pGL4.30-transfected cells for 17 hours; and 20ng/µl of TNFα to the pGL4.32-transfected cells for 5 hours. Noninduced controls were treated with an equivalent amount of DMSO vehicle for both pGL4.29- and pGL4.30-transfected cells, and with an equivalent amount of standard media for the pGL4.32-transfected cells. Luciferase activity was measured using the Bright-Glo™ Luciferase Assay System (Cat.# E2610) for Panels A and B, and using the ONE-Glo™ Luciferase Assay System (Cat.# E6110) for Panel C. The numbers shown above the graph bars represent fold induction for the respective vectors, calculated by dividing the relative light units obtained for the induced wells by the relative light units obtained from noninduced wells (n = 15; standard error bars are shown). Results will vary depending on the cell line and the transfection protocol used.

The CRE, NFAT-RE and NF-κB-RE Reporter Vectors (continued)

pGL4.30[*luc2P*/NFAT-RE/Hygro] contains three tandem NFAT-RE sequences upstream of the minimal promoter sequence to drive expression of the *luc2P* reporter. pGL4.32[*luc2P*/NF-κB-RE/Hygro] contains 5 tandem repeats of a prototypical NF-κB binding site.

The *GAL4*UAS Reporter Vector

Another response element vector, the pGL4.31[*luc2P/GAL4*UAS/Hygro] Vector is part of the CheckMate™/Flexi® Vector Mammalian Two-Hybrid System. This reporter vector contains five consensus binding sequences, or Upstream Activating Sequences (UAS), for the GAL4 DNA-binding domain (*GAL4*UAS) upstream of a minimal adenoviral promoter and is designed for transcriptional activation of the firefly luciferase by association of the GAL4 DNA-binding and VP16 activation domains bound upstream of the luciferase gene.

The Internal Control pGL4 Vectors

The pGL4.13[*luc2*/SV40] and pGL4.73[*hRluc*/SV40] Vectors are intended for use as internal control reporters and may be used with most experimental reporter vectors to co-transfect mammalian cells. These vectors contain the SV40 early enhancer/promoter region, which provides strong, constitutive expression of luciferase in a variety of cell types, as well as the SV40 origin of replication, which results in transient, episomal replication in cells expressing the SV40 large T antigen such as COS-1 or COS-7 cells (6).

These promoter-containing vectors may not be appropriate for use in examining the function of a response element of interest. Minimal promoter-containing vectors are better suited for examining a response element of interest.

The pGL4.74[*hRluc*/TK] Vector is intended for use as an internal control reporter and may be used with most experimental reporter vectors to co-transfect mammalian cells. The pGL4.74[*hRluc*/TK] Vector contains the herpes simplex virus thymidine kinase (HSV-TK) promoter upstream of *hRluc* to provide low to moderate levels of *Renilla* luciferase expression in co-transfected mammalian cells of both embryonal and mature mammalian tissues (6,7).

The pGL4.75[*hRluc*/CMV] Vector is intended for use as an internal control reporter and may be used with most experimental reporter vectors to co-transfect mammalian cells. The pGL4.75[*hRluc*/CMV] Vector contains the CMV immediate-early enhancer/promoter region, which provides strong, constitutive expression of *Renilla* luciferase in a variety of cell types. The promiscuous nature of the CMV immediate-early enhancer/promoter has been demonstrated in transgenic mice, where its transcriptional activity was observed in 24 of the 28 murine tissues examined (8).

Selectable Markers

Hygromycin B is an aminoglycosidic antibiotic that inhibits protein synthesis by disrupting translocation and promoting mistranslation at the 80S ribosome. Hygromycin B phosphotransferase (Hygro or Hyg^r) inactivates hygromycin B by phosphorylation. For the selection of stable cell lines, an expression cassette for the gene encoding hygromycin B phosphotransferase is available in the pGL4 Vectors listed as “Hygro” in Table 2, under “Mammalian Selectable Markers”.

G418 is an aminoglycosidic antibiotic that inhibits protein synthesis. Neomycin or aminoglycoside 3´ phosphotransferase (Neo or Neo^r) confers resistance to G418. For the selection of stable cell lines, an expression cassette for the gene encoding neomycin phosphotransferase is available in the pGL4 Vectors listed as “Neo” in

Table 2, under "Mammalian Selectable Markers".

Puromycin dihydrochloride is a nucleoside antibiotic that inhibits protein synthesis. Puromycin-N-acetyltransferase (Puro or Puro^r) confers resistance to puromycin. For the selection of stable cell lines, an expression cassette for the gene encoding puromycin-N-acetyltransferase is available in the pGL4 Vectors listed as "Puro" in Table 2, under "Mammalian Selectable Markers".

The genes for hygromycin, neomycin and puromycin resistance have also been designed for reduced anomalous expression and codon optimized by the same process used on the firefly and *Renilla* luciferase reporter genes (earning all nine genes the designation "synthetic"). The expression cassette contains the SV40 early enhancer/promoter to express the Hygro, Neo or Puro resistance gene and a synthetic poly(A) signal at the 3´ end for transcriptional termination (separate from the synthetic poly(A)/translational pause site located upstream of the reporter gene).

3.D. Common Features of the pGL4 Vectors

SV40 Late Poly(A) Signal

In addition to the features previously listed, each of the pGL4 Vectors contains a SV40 late poly(A) signal downstream of the reporter gene. Polyadenylation signals cause the termination of transcription by RNA polymerase II and signal the addition of approximately 200–250 adenosine residues to the 3´-end of the RNA transcript (9). Polyadenylation has been shown to enhance RNA stability and translation efficiency (10,11). The SV40 late poly(A) signal is extremely efficient and has been shown to increase the steady-state level of RNA approximately fivefold more than the SV40 early poly(A) signal (12,13). The SV40 late poly(A) signal has been positioned 3´ to the reporter gene in the pGL4 Vectors to increase the level of luciferase expression.

Redesigned Multiple Cloning Region

The multiple cloning region of the pGL4 Vectors (Figure 9) has been synthetically constructed and is based on the multiple cloning region of the pGL3 Vectors. However, differences between the two multiple cloning regions exist. The pGL4 Vector multiple cloning region includes the following restriction sites: *Bgl* I, *Sfi* I, *Acc*65 I, *Kpn* I, *Eco*ICR I, *Sac* I, *Nhe* I, *Xho* I, *Eco*R V, *Bgl* II, and *Hin*d III. The *Bgl* I, *Sfi* I and *Eco*R V restriction sites have been added to the pGL4 Vector multiple cloning region and are not found in the pGL3 Vector multiple cloning region. The *Mlu* I and *Sma* I restriction sites found in the pGL3 multiple cloning region have not been included in the pGL4 multiple cloning region. The purpose of two *Bgl* I/*Sfi* I restriction sites is to be able to move DNA of interest (i.e., response elements, enhancers, promoters, etc.) among the pGL4 Vectors. Additionally, due to the unique DNA recognition properties of *Bgl* I and *Sfi* I, the two restriction sites in the pGL4 Vectors offer directionality. Thus transfer between pGL4 Vectors by using either the *Bgl* I or *Sfi* I restriction enzymes retains the desired directionality of your DNA fragment of interest.

Note: Additional *Bgl* I and *Eco*R V restriction sites are present in the *hRluc* gene. Therefore, these two enzymes should not be used for cloning into *Renilla*-based pGL4 Vectors.

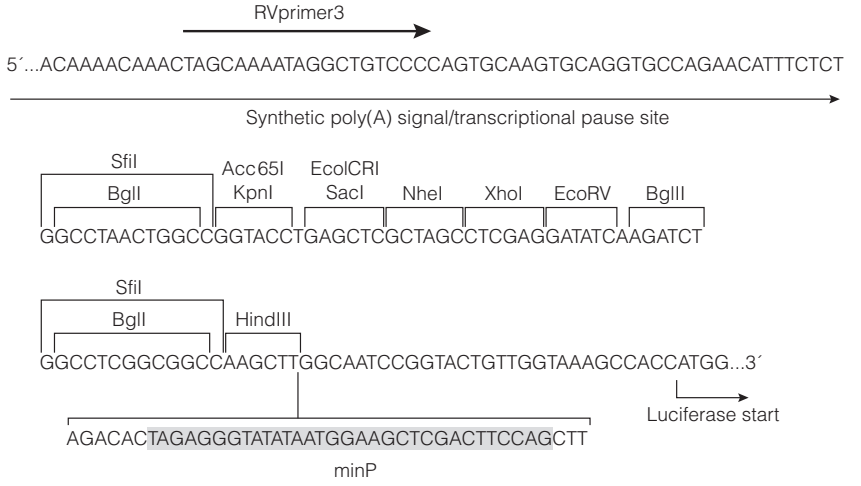


Figure 9. The multiple cloning region of the pGL4 Vectors. This map represents the promoterless pGL4 Vectors but also demonstrates the position and sequence of the minimal promoter, minP, found in the pGL4.23–pGL4.28 Vectors. The minP sequence is shaded in gray. **Note:** The BglI and EcoRV sites are also present in the *hRluc* gene and should not be used for cloning into the *hRluc*- (*Renilla* luciferase-) containing pGL4 Vectors.

4. pGL4 Vector Sequences, Restriction Enzyme Tables and Maps

4.A. Vector Sequences, Restriction Enzyme Tables and GenBank® Numbers

Sequence information and restriction enzyme tables for the pGL4 Vectors are available online at: **www.promega.com/vectors/** The GenBank®/EMBL accession numbers are listed below in Table 4.

Note: Due to the use of updated software for generating vector maps and restriction enzyme tables, the manner in which we report restriction enzyme cut sites has changed. The location is now reported at the 5´-end of the cut DNA (the base to the right of the cut site). The maps and restriction enzyme tables associated with the pGL4 Vectors have been updated accordingly.

Table 4. GenBank®/EMBL Accession Numbers for the pGL4 Vectors.

Vector	GenBank®/EMBL Accession Number
pGL4.10[*luc2*] Vector	AY738222
pGL4.11[*luc2P*] Vector	AY738223
pGL4.12[*luc2CP*] Vector	AY738224
pGL4.13[*luc2*/SV40] Vector	AY738225
pGL4.14[*luc2*/Hygro] Vector	AY864928
pGL4.15[*luc2P*/Hygro] Vector	AY864929
pGL4.16[*luc2CP*/Hygro] Vector	AY864930
pGL4.17[*luc2*/Neo] Vector	DQ188837
pGL4.18[*luc2P*/Neo] Vector	DQ188838

Table 4. GenBank®/EMBL Accession Numbers (continued)

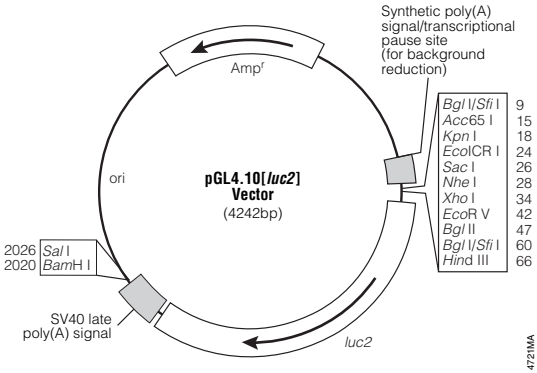
Vector	GenBank®/EMBL Accession Number
pGL4.19[*luc2CP*/Neo] Vector	DQ188839
pGL4.20[*luc2*/Puro] Vector	DQ188840
pGL4.21[*luc2P*/Puro] Vector	DQ188841
pGL4.22[*luc2CP*/Puro] Vector	DQ188842
pGL4.23[*luc2*/minP] Vector	DQ904455
pGL4.24[*luc2P*/minP] Vector	DQ904456
pGL4.25[*luc2CP*/minP] Vector	DQ904457
pGL4.26[*luc2*/minP/Hygro] Vector	DQ904458
pGL4.27[*luc2P*/minP/Hygro] Vector	DQ904459
pGL4.28[*luc2CP*/minP/Hygro] Vector	DQ904460
pGL4.29[*luc2P*/CRE/Hygro] Vector	DQ904461
pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector	DQ904462
pGL4.31[*luc2P*/*GAL4*UAS/Hygro] Vector	DQ487213
pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector	EU581860
pGL4.70[*hRluc*] Vector	AY738226
pGL4.71[*hRlucP*] Vector	AY738227
pGL4.72[*hRlucCP*] Vector	AY738228
pGL4.73[*hRluc*/SV40] Vector	AY738229
pGL4.74[*hRluc*/TK] Vector	AY738230
pGL4.75[*hRluc*/CMV] Vector	AY738231
pGL4.76[*hRluc*/Hygro] Vector	AY864931
pGL4.77[*hRlucP*/Hygro] Vector	AY864932
pGL4.78[*hRlucCP*/Hygro] Vector	AY864933
pGL4.79[*hRluc*/Neo] Vector	DQ188843
pGL4.80[*hRlucP*/Neo] Vector	DQ188844
pGL4.81[*hRlucCP*/Neo] Vector	DQ188845
pGL4.82[*hRluc*/Puro] Vector	DQ188846
pGL4.83[*hRlucP*/Puro] Vector	DQ188847
pGL4.84[*hRlucCP*/Puro] Vector	DQ188848

4.B. pGL4 Vector Maps

The following section contains circular vector maps and sequence reference points for the pGL4 Vectors. For each map a selected set of restriction enzyme sites is also shown, including those in the multiple cloning regions, EcoRI, which denotes the Rapid Response™ Reporter and BamHI and SalI, which denote the selectable marker region. Information for pGL4.31[*luc2P*/*GAL4*UAS/Hygro] Vector can be found in the *CheckMate™/Flexi® Vector Mammalian Two-Hybrid System Technical Manual*, #TM283 and online at: **www.promega.com/vectors/**

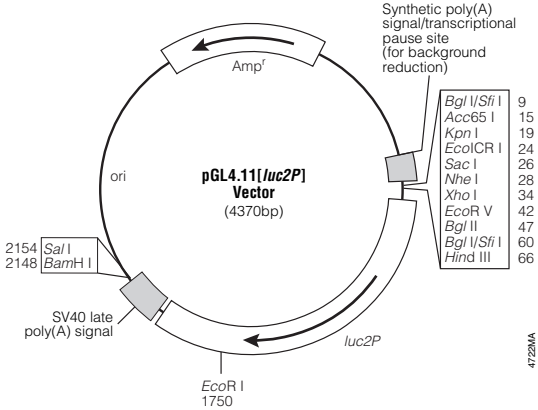
Note: The restriction enzyme sites shown on these vector maps were derived from sequence analysis software and have not been verified by restriction enzyme digestion with each enzyme listed. The location given specifies the 5′ end of the cut DNA (the base to the right of the cut site).

pGL4 *luc2* Vector Maps



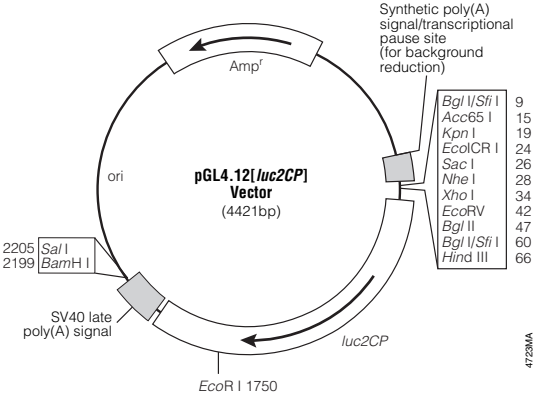
Multiple cloning region	1–70
luc2 reporter gene	100–1752
SV40 late poly(A) signal	1787–2008
Reporter Vector primer 4 binding region	2076–2095
*Col*EI-derived plasmid replication origin	2333
Synthetic β-lactamase (Amp^r) coding region	3124–3984
Synthetic poly(A) signal/transcriptional pause site	4089–4242
Reporter Vector primer 3 binding region	4191–4210

Figure 10. pGL4.10[*luc2*] Vector circle map and sequence reference points.



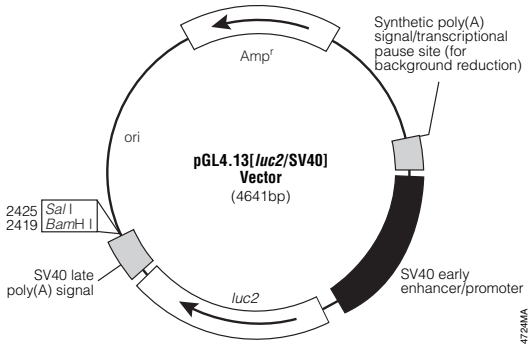
Multiple cloning region	1–70
luc2P reporter gene	100–1875
SV40 late poly(A) signal	1915–2136
Reporter Vector primer 4 binding region	2204–2223
*Col*EI-derived plasmid replication origin	2461
Synthetic β-lactamase (Amp^r) coding region	3252–4112
Synthetic poly(A) signal/transcriptional pause site	4217–4370
Reporter Vector primer 3 binding region	4319–4338

Figure 11. pGL4.11[*luc2P*] Vector circle map and sequence reference points.



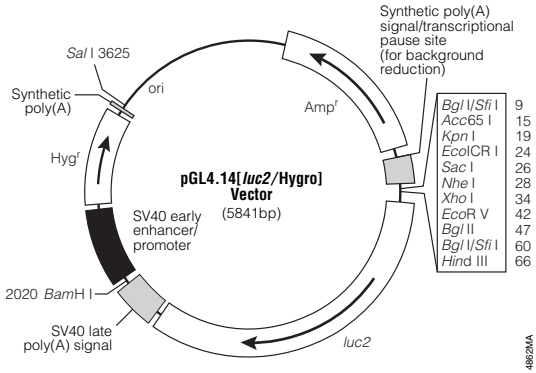
Multiple cloning region	1–70
luc2CP reporter gene	100–1929
SV40 late poly(A) signal	1966–2187
Reporter Vector primer 4 binding region	2255–2274
*Col*EI-derived plasmid replication origin	2512
Synthetic β-lactamase (Amp^r) coding region	3303–4163
Synthetic poly(A) signal/transcriptional pause site	4268–4421
Reporter Vector primer 3 binding region	4370–4389

Figure 12. pGL4.12[*luc2CP*] Vector circle map and sequence reference points.



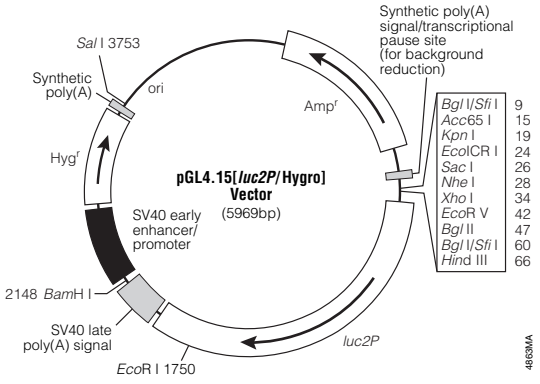
SV40 early enhancer/promoter	51–469
luc2 reporter gene	499–2151
SV40 late poly(A) signal	2186–2407
Reporter Vector primer 4 binding region	2475–2494
*Col*EI-derived plasmid replication origin	2732
Synthetic β-lactamase (Amp^r) coding region	3523–4383
Synthetic poly(A) signal/transcriptional pause site	4488–4641
Reporter Vector primer 3 binding region	4590–4609

Figure 13. pGL4.13[*luc2*/SV40] Vector circle map and sequence reference points.



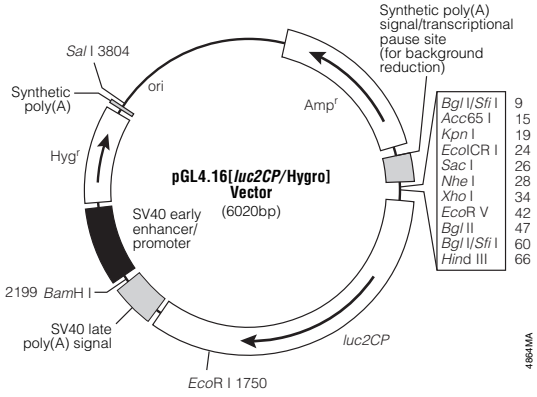
Multiple cloning region	1–70
luc2 reporter gene	100–1752
SV40 late poly(A) signal	1787–2008
SV40 early enhancer/promoter	2056–2474
Synthetic hygromycin (Hyg^r) coding region	2499–3536
Synthetic poly(A) signal	3560–3608
Reporter Vector primer 4 binding region	3675–3694
*Col*EI-derived plasmid replication origin	3932
Synthetic β-lactamase (Amp^r) coding region	4723–5583
Synthetic poly(A) signal/transcriptional pause site	5688–5841
Reporter Vector primer 3 binding region	5790–5809

Figure 14. pGL4.14[*luc2*/Hygro] Vector circle map and sequence reference points.



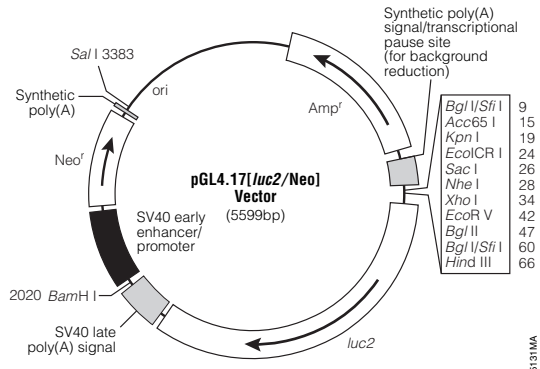
Multiple cloning region	1–70
luc2P reporter gene	100–1875
SV40 late poly(A) signal	1915–2136
SV40 early enhancer/promoter	2184–2602
Synthetic hygromycin (Hyg^r) coding region	2627–3664
Synthetic poly(A) signal	3688–3736
Reporter Vector primer 4 binding region	3803–3822
*Col*EI-derived plasmid replication origin	4060
Synthetic β-lactamase (Amp^r) coding region	4851–5711
Synthetic poly(A) signal/transcriptional pause site	5816–5969
Reporter Vector primer 3 binding region	5918–5937

Figure 15. pGL4.15[*luc2P*/Hygro] Vector circle map and sequence reference points.



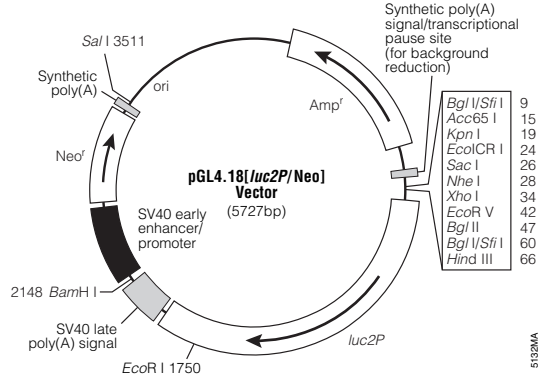
Multiple cloning region	1–70
luc2CP reporter gene	100–1929
SV40 late poly(A) signal	1966–2187
SV40 early enhancer/promoter	2235–2653
Synthetic hygromycin (Hyg^r) coding region	2678–3715
Synthetic poly(A) signal	3739-3787
Reporter Vector primer 4 binding region	3854–3873
*Col*EI-derived plasmid replication origin	4111
Synthetic β-lactamase (Amp^r) coding region	4902–5762
Synthetic poly(A) signal/transcriptional pause site	5867–6020
Reporter Vector primer 3 binding region	5969–5988

Figure 16. pGL4.16[*luc2CP*/Hygro] Vector circle map and sequence reference points.



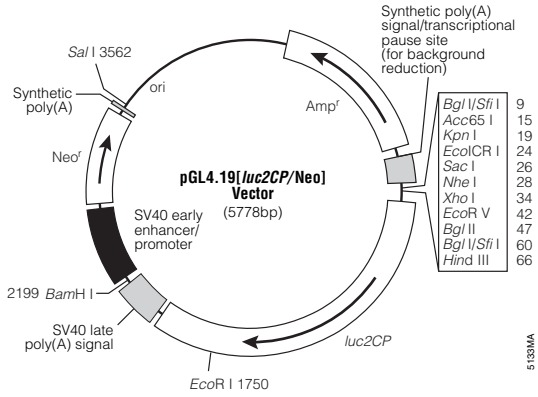
Multiple cloning region	1–70
luc2 reporter gene	100–1752
SV40 late poly(A) signal	1787–2008
SV40 early enhancer/promoter	2056–2474
Synthetic neomycin phosphotransferase (Neo^r) coding region	2499–3293
Synthetic poly(A) signal	3318–3366
Reporter Vector primer 4 (RVprimer4) binding region	3433–3452
*Col*EI-derived plasmid replication origin	3690
Synthetic β-lactamase (Amp^r) coding region	4481–5341
Synthetic poly(A) signal/transcriptional pause site	5446–5599
Reporter Vector primer 3 binding region	5548–5567

Figure 17. pGL4.17[*luc2*/Neo] Vector circle map and sequence reference points.



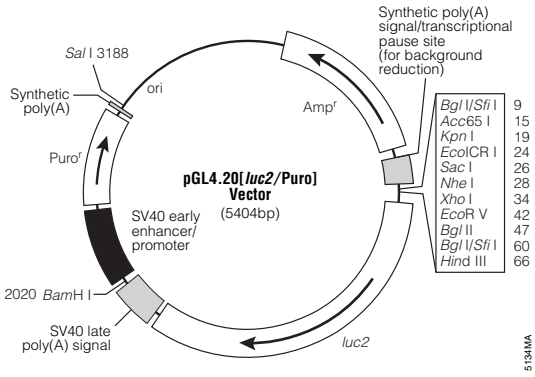
Multiple cloning region	1–70
luc2P reporter gene	100–1875
SV40 late poly(A) signal	1915–2136
SV40 early enhancer/promoter	2184–2602
Synthetic neomycin phosphotransferase (Neo^r) coding region	2627–3421
Synthetic poly(A) signal	3446–3494
Reporter Vector primer 4 binding region	3561–3580
*Col*EI-derived plasmid replication origin	3818
Synthetic β-lactamase (Amp^r) coding region	4609–5469
Synthetic poly(A) signal/transcriptional pause site	5574–5727
Reporter Vector primer 3 binding region	5676–5695

Figure 18. pGL4.18[*luc2P*/Neo] Vector circle map and sequence reference points.



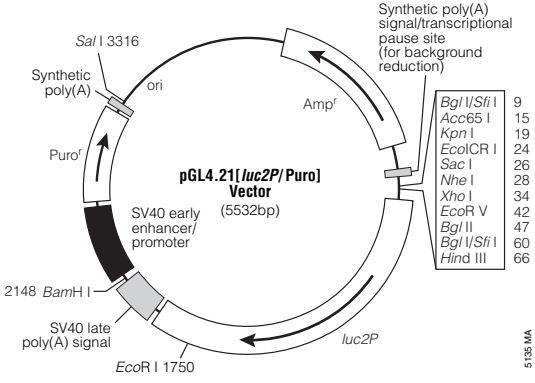
Multiple cloning region	1–70
luc2CP reporter gene	100–1929
SV40 late poly(A) signal	1966–2187
SV40 early enhancer/promoter	2235–2653
Synthetic neomycin phosphotransferase (Neo^r) coding region	2678–3472
Synthetic poly(A) signal	3497–3545
Reporter Vector primer 4 binding region	3612–3631
*Col*EI-derived plasmid replication origin	3869
Synthetic β-lactamase (Amp^r) coding region	4660–5520
Synthetic poly(A) signal/transcriptional pause site	5625–5778
Reporter Vector primer 3 binding region	5727–5746

Figure 19. pGL4.19[*luc2CP*/Neo] Vector circle map and sequence reference points.



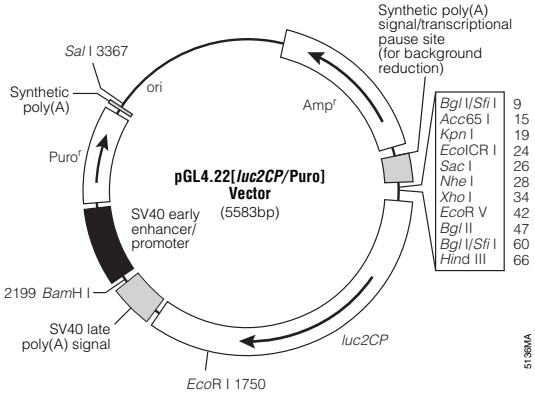
Multiple cloning region	1–70
luc2 reporter gene	100–1752
SV40 late poly(A) signal	1787–2008
SV40 early enhancer/promoter	2056–2474
Synthetic puromycin-N-acetyltransferase (Puro^r) coding region	2499–3098
Synthetic poly(A) signal	3123–3171
Reporter Vector primer 4 binding region	3238–3257
*Col*EI-derived plasmid replication origin	3495
Synthetic β-lactamase (Amp^r) coding region	4286–5146
Synthetic poly(A) signal/transcriptional pause site	5251–5404
Reporter Vector primer 3 binding region	5353–5372

Figure 20. pGL4.20[*luc2*/Puro] Vector circle map and sequence reference points.



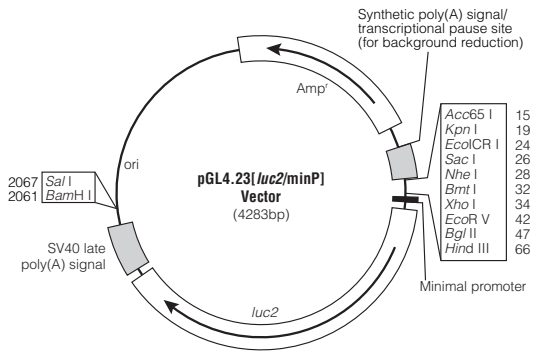
Multiple cloning region	1–70
luc2P reporter gene	100–1875
SV40 late poly(A) signal	1915–2136
SV40 early enhancer/promoter	2184–2602
Synthetic puromycin-N-acetyltransferase (Puro^r) coding region	2627–3226
Synthetic poly(A) signal	3251–3299
Reporter Vector primer 4 binding region	3366–3385
*Col*EI-derived plasmid replication origin	3623
Synthetic β-lactamase (Amp^r) coding region	4414–5274
Synthetic poly(A) signal/transcriptional pause site	5379–5532
Reporter Vector primer 3 binding region	5481–5500

Figure 21. pGL4.21[*luc2P*/Puro] Vector circle map and sequence reference points.



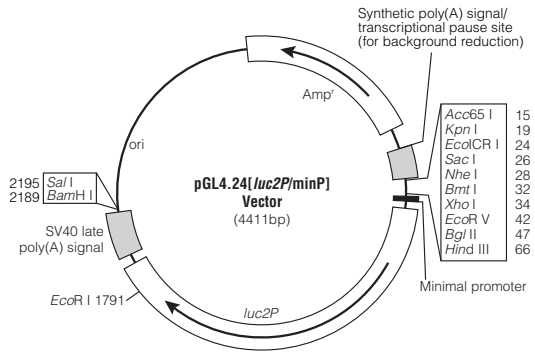
Multiple cloning region	1–70
luc2CP reporter gene	100–1929
SV40 late poly(A) signal	1966–2187
SV40 early enhancer/promoter	2235–2653
Synthetic puromycin-N-acetyltransferase (Puro^r) coding region	2678–3277
Synthetic poly(A) signal	3302–3350
Reporter Vector primer 4 binding region	3417–3436
*Col*EI-derived plasmid replication origin	3674
Synthetic β-lactamase (Amp^r) coding region	4465–5325
Synthetic poly(A) signal/transcriptional pause site	5430–5583
Reporter Vector primer 3 binding region	5532–5551

Figure 22. pGL4.22[*luc2CP*/Puro] Vector circle map and sequence reference points.



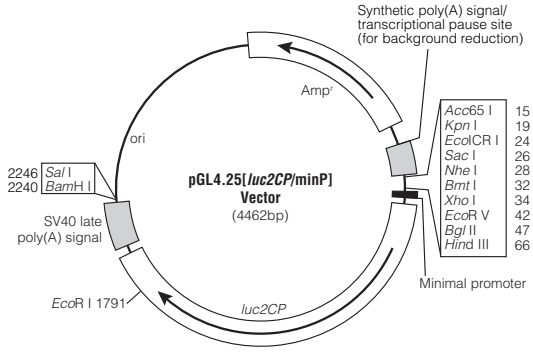
Multiple cloning region	1–70
Minimal promoter	78–108
luc2 reporter gene	141–1793
SV40 late poly(A) signal	1828–2049
Reporter Vector primer 4 binding region	2117–2136
*Col*E1-derived plasmid replication origin	2374
Synthetic β-lactamase (Amp^r) coding region	3165–4025
Synthetic poly(A) signal/transcriptional pause site	4130–4283
Reporter Vector primer 3 binding region	4232–4251

Figure 23. pGL4.23[*luc2*/minP] Vector circle map and sequence reference points.



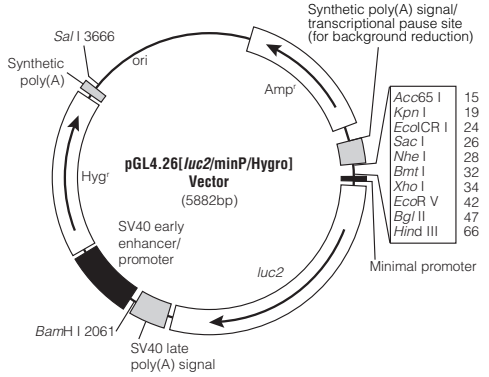
Multiple cloning region	1–70
Minimal promoter	78–108
luc2P reporter gene	141–1916
SV40 late poly(A) signal	1956–2177
Reporter Vector primer 4 binding region	2245–2264
*Col*E1-derived plasmid replication origin	2502
Synthetic β-lactamase (Amp^r) coding region	3293–4153
Synthetic poly(A) signal/transcriptional pause site	4258–4411
Reporter Vector primer 3 binding region	4360–4379

Figure 24. pGL4.24[*luc2P*/minP] Vector circle map and sequence reference points.



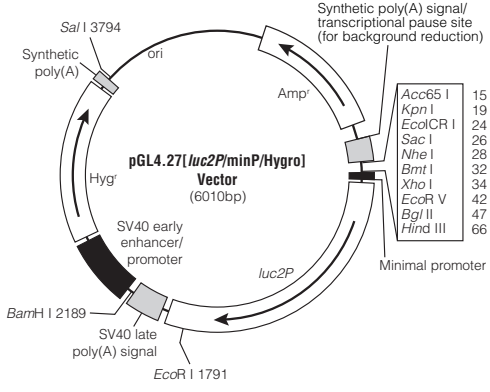
Multiple cloning region	1–70
Minimal promoter	78–108
luc2CP reporter gene	141–1970
SV40 late poly(A) signal	2007–2228
Reporter Vector primer 4 binding region	2296–2315
*Col*E1-derived plasmid replication origin	2553
Synthetic β-lactamase (Amp^r) coding region	3344–4204
Synthetic poly(A) signal/transcriptional pause site	4309–4462
Reporter Vector primer 3 binding region	4411–4430

Figure 25. pGL4.25[*luc2CP*/minP] Vector circle map and sequence reference points.



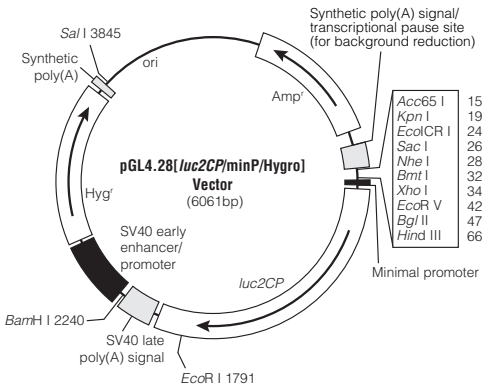
Multiple cloning region	1–70
Minimal promoter	78–108
luc2 reporter gene	141–1793
SV40 late poly(A) signal	1828–2049
SV40 early enhancer/promoter	2097–2515
Synthetic hygromycin (Hyg^r) coding region	2540–3577
Synthetic poly(A) signal	3601–3649
Reporter Vector primer 4 binding region	3716–3735
*Col*E1-derived plasmid replication origin	3973
Synthetic β-lactamase (Amp^r) coding region	4764–5624
Synthetic poly(A) signal/transcriptional pause site	5729–5882
Reporter Vector primer 3 binding region	5831–5850

Figure 26. pGL4.26[*luc2*/minP/Hygro] Vector circle map and sequence reference points.



Multiple cloning region	1–70
Minimal promoter	78–108
luc2P reporter gene	141–1916
SV40 late poly(A) signal	1956–2177
SV40 early enhancer/promoter	2225–2643
Synthetic hygromycin (Hyg^r) coding region	2668–3705
Synthetic poly(A) signal	3729–3777
Reporter Vector primer 4 binding region	3844–3863
*Col*E1-derived plasmid replication origin	4101
Synthetic β-lactamase (Amp^r) coding region	4892–5752
Synthetic poly(A) signal/transcriptional pause site	5857–6010
Reporter Vector primer 3 binding region	5959–5978

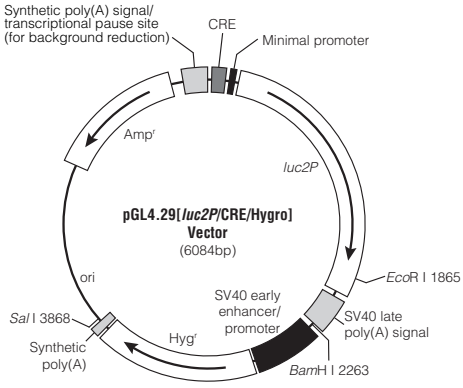
Figure 27. pGL4.27[*luc2P*/minP/Hygro] Vector circle map and sequence reference points.



Multiple cloning region	1–70
Minimal promoter	78–108
luc2CP reporter gene	141–1970
SV40 late poly(A) signal	2007–2228
SV40 early enhancer/promoter	2276–2694
Synthetic hygromycin (Hyg^r) coding region	2719–3756
Synthetic poly(A) signal	3780–3828
Reporter Vector primer 4 binding region	3895–3914
*Col*E1-derived plasmid replication origin	4152
Synthetic β-lactamase (Amp^r) coding region	4943–5803
Synthetic poly(A) signal/transcriptional pause site	5908–6061
Reporter Vector primer 3 binding region	6010–6029

5963MA

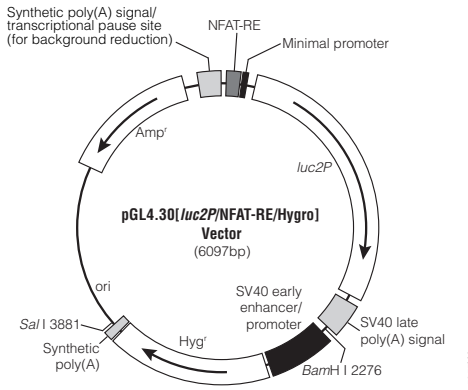
Figure 28. pGL4.28[*luc2CP*/minP/Hygro] Vector circle map and sequence reference points.



cAMP response element (CRE)	33–119
Minimal promoter	152–182
luc2P reporter gene	215–1990
SV40 late poly(A) signal	2030–2251
SV40 early enhancer/promoter	2299–2717
Synthetic hygromycin (Hyg^r) coding region	2742–3779
Synthetic poly(A) signal	3803–3851
Reporter Vector primer 4 binding region	3918–3937
*Col*E1-derived plasmid replication origin	4175
Synthetic β-lactamase (Amp^r) coding region	4966–5826
Synthetic poly(A) signal/transcriptional pause site	5931–6084
Reporter Vector primer 3 binding region	6033–6052

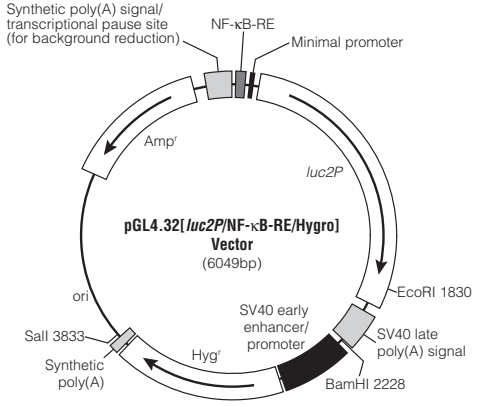
5964MA

Figure 29. pGL4.29[*luc2P*/CRE/Hygro] Vector circle map and sequence reference points.



NFAT response element	33–122
Minimal promoter	136–166
luc2P reporter gene	228–2003
SV40 late poly(A) signal	2043–2264
SV40 early enhancer/promoter	2312–2730
Synthetic hygromycin (Hyg^r) coding region	2755–3792
Synthetic poly(A) signal	3816–3864
Reporter Vector primer 4 binding region	3931–3950
*Col*E1-derived plasmid replication origin	4188
Synthetic β-lactamase (Amp^r) coding region	4979–5839
Synthetic poly(A) signal/transcriptional pause site	5944–6097
Reporter Vector primer 3 binding region	6046–6065

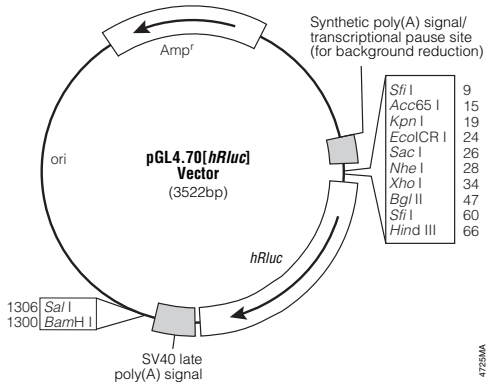
Figure 30. pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector circle map and sequence reference points.



NF-κB response element	33–84
Minimal promoter	117–147
luc2P reporter gene	180–1955
SV40 late poly(A) signal	1995–2216
SV40 early enhancer/promoter	2264–2682
Synthetic hygromycin (Hyg^r) coding region	2707–3744
Synthetic poly(A) signal	3768–3816
Reporter Vector primer 4 binding region	3883–3902
*Col*E1-derived plasmid replication origin	4140
Synthetic β-lactamase (Amp^r) coding region	4931–5791
Synthetic poly(A) signal/transcriptional pause site	5896–6049
Reporter Vector primer 3 binding region	5998–6017

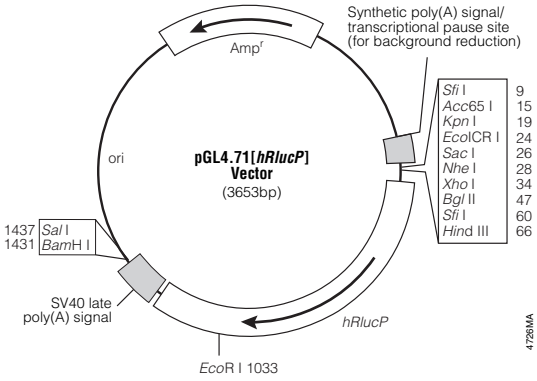
Figure 31. pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector circle map and sequence reference points.

pGL4 *hRluc* Vector Maps



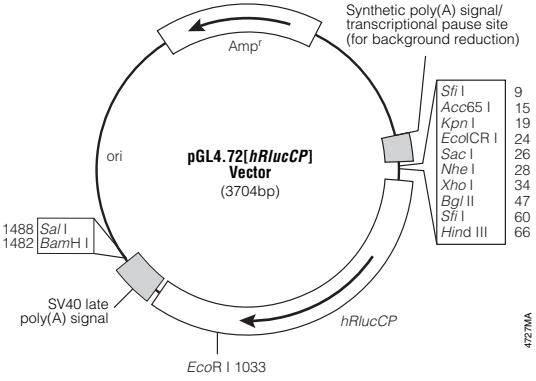
Multiple cloning region	1–70
hRluc reporter gene	100–1035
SV40 late poly(A) signal	1067–1288
Reporter Vector primer 4 binding region	1356–1375
*Col*EI-derived plasmid replication origin	1613
Synthetic β-lactamase (Amp^r) coding region	2404–3264
Synthetic poly(A) signal/transcriptional pause site	3369–3522
Reporter Vector primer 3 binding region	3471–3490

Figure 32. pGL4.70[*hRluc*] Vector circle map and sequence reference points.



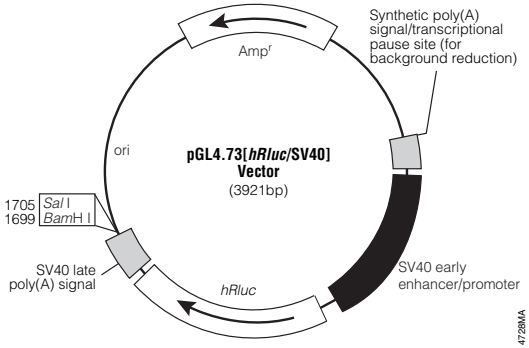
Multiple cloning region	1–70
hRlucP reporter gene	100–1158
SV40 late poly(A) signal	1198–1419
Reporter Vector primer 4 binding region	1487–1506
*Col*EI-derived plasmid replication origin	1744
Synthetic β-lactamase (Amp^r) coding region	2535–3395
Synthetic poly(A) signal/transcriptional pause site	3500–3653
Reporter Vector primer 3 binding region	3602–3621

Figure 33. pGL4.71[*hRlucP*] Vector circle map and sequence reference points.



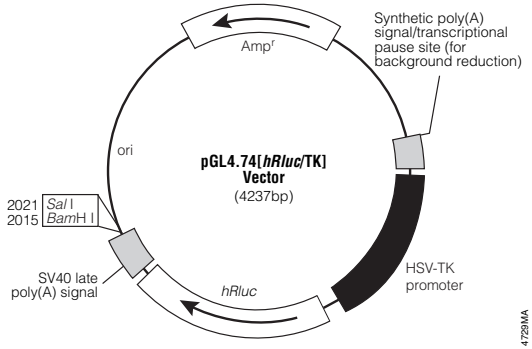
Multiple cloning region	1–70
hRlucCP reporter gene	100–1212
SV40 late poly(A) signal	1249–1470
Reporter Vector primer 4 binding region	1538–1557
*Col*EI-derived plasmid replication origin	1795
Synthetic β-lactamase (Amp^r) coding region	2586–3446
Synthetic poly(A) signal/transcriptional pause site	3551–3704
Reporter Vector primer 3 binding region	3653–3672

Figure 34. pGL4.72[*hRlucCP*] Vector circle map and sequence reference points.



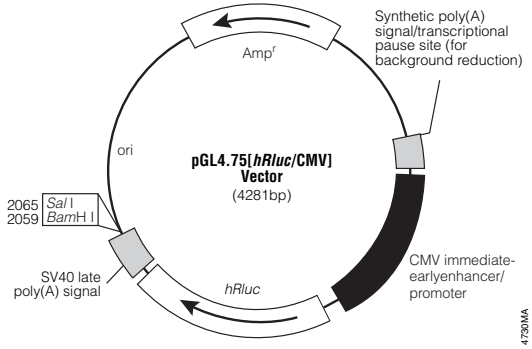
SV40 early enhancer/promoter	51–469
hRluc reporter gene	499–1434
SV40 late poly(A) signal	1466–1687
Reporter Vector primer 4 binding region	1755–1774
*Col*EI-derived plasmid replication origin	2012
Synthetic β-lactamase (Amp^r) coding region	2803–3663
Synthetic poly(A) signal/transcriptional pause site	3768–3921
Reporter Vector primer 3 binding region	3870–3889

Figure 35. pGL4.73[*hRluc*/SV40] Vector circle map and sequence reference points.



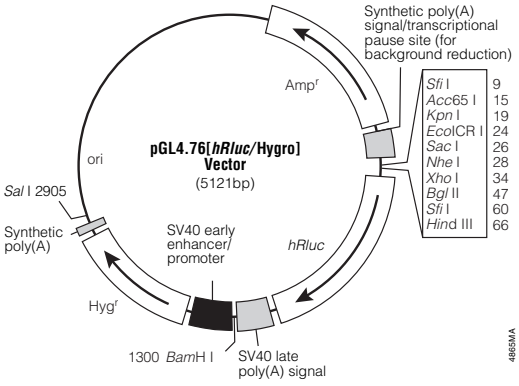
HSV-TK promoter	27–779
hRluc reporter gene	815–1750
SV40 late poly(A) signal	1784–2005
Reporter Vector primer 4 binding region	2071–2090
*Col*EI-derived plasmid replication origin	2330
Synthetic β-lactamase (Amp^r) coding region	3119–3979
Synthetic poly(A) signal/transcriptional pause site	4084–4237
Reporter Vector primer 3 binding region	4186–4205

Figure 36. pGL4.74[*hRluc*/TK] Vector circle map and sequence reference points.



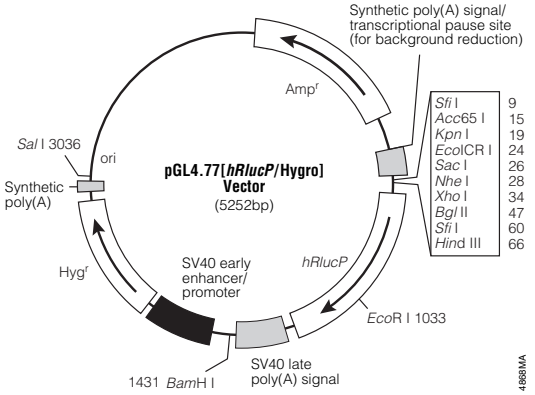
CMV immediate early enhancer/promoter	14–755
hRluc reporter gene	859–1794
SV40 late poly(A) signal	1826–2047
Reporter Vector primer 4 binding region	2115–2134
*Col*EI-derived plasmid replication origin	2372
Synthetic β-lactamase (Amp^r) coding region	3163–4023
Synthetic poly(A) signal/transcriptional pause site	4128–4281
Reporter Vector primer 3 binding region	4230–4249

Figure 37. pGL4.75[*hRluc*/CMV] Vector circle map and sequence reference points.



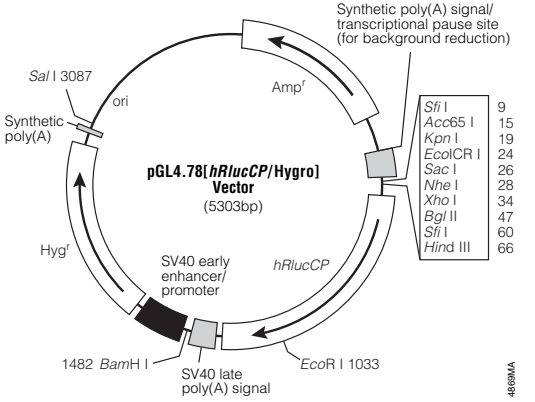
Multiple cloning region	1–70
hRluc reporter gene	100–1035
SV40 late poly(A) signal	1067–1288
SV40 early enhancer/promoter	1336–1754
Synthetic hygromycin (Hygr) coding region	1779–2816
Synthetic poly(A) signal	2840–2888
Reporter Vector primer 4 binding region	2955–2974
*Col*EI-derived plasmid replication origin	3212
Synthetic β-lactamase (Ampr) coding region	4003–4863
Synthetic poly(A) signal/transcriptional pause site	4968–5121
Reporter Vector primer 3 binding region	5070–5089

Figure 38. pGL4.76[*hRluc*/Hygro] Vector circle map and sequence reference points.



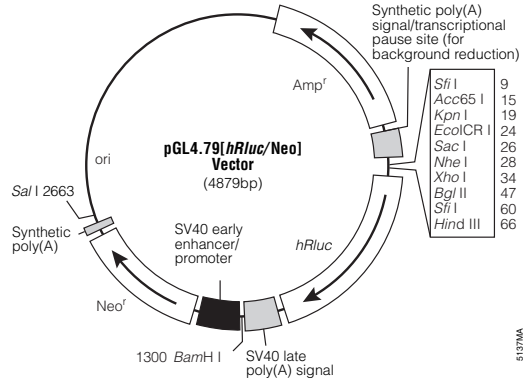
Multiple cloning region	1–70
hRlucP reporter gene	100–1158
SV40 late poly(A) signal	1198–1419
SV40 early enhancer/promoter	1467–1885
Synthetic hygromycin (Hygr) coding region	1910–2947
Synthetic poly(A) signal	2971–3019
Reporter Vector primer 4 binding region	3086–3105
*Col*EI-derived plasmid replication origin	3343
Synthetic β-lactamase (Ampr) coding region	4134–4994
Synthetic poly(A) signal/transcriptional pause site	5099–5252
Reporter Vector primer 3 binding region	5201–5220

Figure 39. pGL4.77[*hRlucP*/Hygro] Vector circle map and sequence reference points.



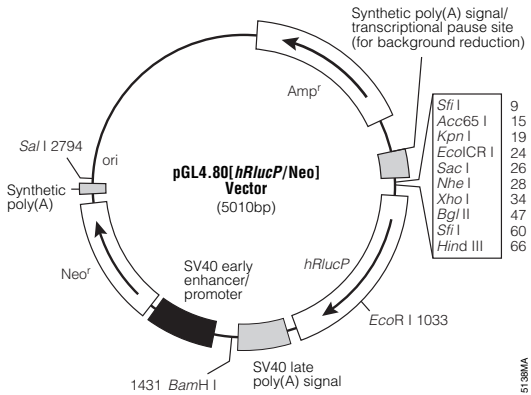
Multiple cloning region	1–70
hRlucCP reporter gene	100–1212
SV40 late poly(A) signal	1249–1470
SV40 early enhancer/promoter	1518–1936
Synthetic hygromycin (Hygr) coding region	1961–2998
Synthetic poly(A) signal	3022–3070
Reporter Vector primer 4 binding region	3137–3156
*Col*EI-derived plasmid replication origin	3394
Synthetic β-lactamase (Ampr) coding region	4185–5045
Synthetic poly(A) signal/transcriptional pause site	5150–5303
Reporter Vector primer 3 binding region	5252–5271

Figure 40. pGL4.78[*hRlucCP*/Hygro] Vector circle map and sequence reference points.



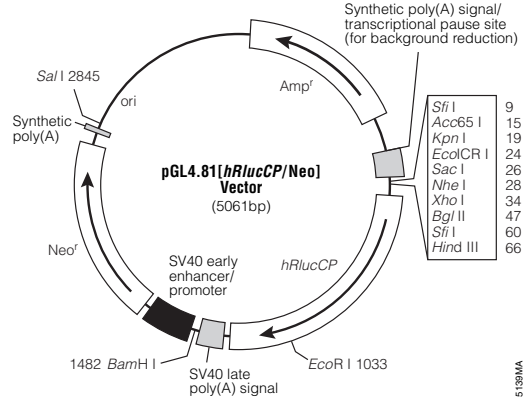
Multiple cloning region	1–70
hRluc reporter gene	100–1035
SV40 late poly(A) signal	1067–1288
SV40 early enhancer/promoter	1336–1754
Synthetic neomycin phosphotransferase (Neo^r) coding region	1779–2573
Synthetic poly(A) signal	2598–2646
Reporter Vector primer 4 binding region	2713–2732
*Col*EI-derived plasmid replication origin	2970
Synthetic β-lactamase (Amp^r) coding region	3761–4621
Synthetic poly(A) signal/transcriptional pause site	4726–4879
Reporter Vector primer 3 binding region	4828–4847

Figure 41. pGL4.79[*hRluc*/Neo] Vector circle map and sequence reference points.



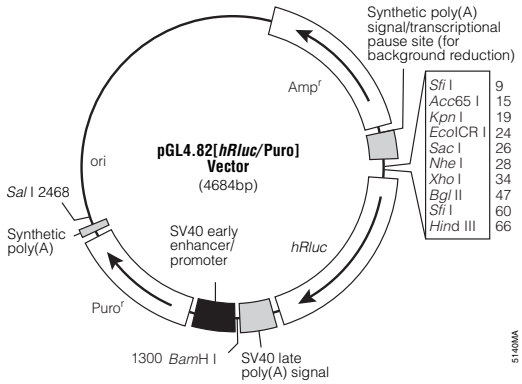
Multiple cloning region	1–70
hRlucP reporter gene	100–1158
SV40 late poly(A) signal	1198–1419
SV40 early enhancer/promoter	1467–1885
Synthetic neomycin phosphotransferase (Neo^r) coding region	1910–2704
Synthetic poly(A) signal	2729–2777
Reporter Vector primer 4 binding region	2844–2863
*Col*EI-derived plasmid replication origin	3101
Synthetic β-lactamase (Amp^r) coding region	3892–4752
Synthetic poly(A) signal/transcriptional pause site	4857–5010
Reporter Vector primer 3 binding region	4959–4978

Figure 42. pGL4.80[*hRlucP*/Neo] Vector circle map and sequence reference points.



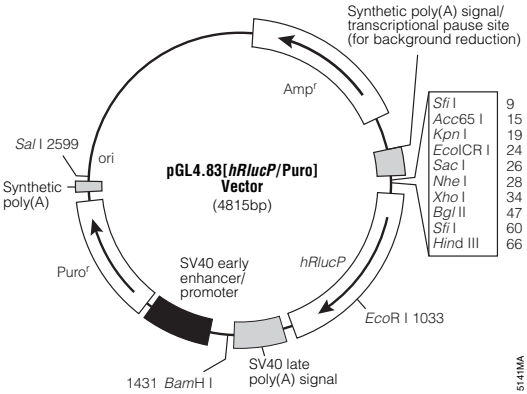
Multiple cloning region	1–70
hRlucCP reporter gene	100–1212
SV40 late poly(A) signal	1249–1470
SV40 early enhancer/promoter	1518–1936
Synthetic neomycin phosphotransferase (Neo^r) coding region	1961–2755
Synthetic poly(A) signal	2780–2828
Reporter Vector primer 4 binding region	2895–2914
*Col*EI-derived plasmid replication origin	3152
Synthetic β-lactamase (Amp^r) coding region	3943–4803
Synthetic poly(A) signal/transcriptional pause site	4908–5061
Reporter Vector primer 3 binding region	5010–5029

Figure 43. pGL4.81[*hRlucCP*/Neo] Vector circle map and sequence reference points.



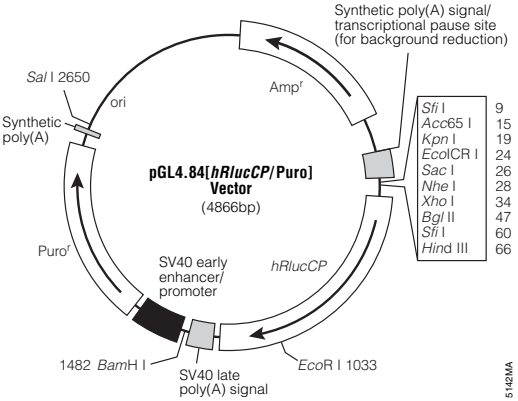
Multiple cloning region	1–70
hRluc reporter gene	100–1035
SV40 late poly(A) signal	1067–1288
SV40 early enhancer/promoter	1336–1754
Synthetic puromycin-N-acetyltransferase (Puro^r) coding region	1779–2378
Synthetic poly(A) signal	2403–2451
Reporter Vector primer 4 binding region	2518–2537
*Col*EI-derived plasmid replication origin	2775
Synthetic β-lactamase (Amp^r) coding region	3566–4426
Synthetic poly(A) signal/transcriptional pause site	4531–4684
Reporter Vector primer 3 binding region	4633–4652

Figure 44. pGL4.82[*hRluc*/Puro] Vector circle map and sequence reference points.



Multiple cloning region	1–70
hRlucP reporter gene	100–1158
SV40 late poly(A) signal	1198–1419
SV40 early enhancer/promoter	1467–1885
Synthetic puromycin-N-acetyltransferase (Puro^r) coding region	1910–2509
Synthetic poly(A) signal	2534–2582
Reporter Vector primer 4 binding region	2649–2668
*Col*EI-derived plasmid replication origin	2906
Synthetic β-lactamase (Amp^r) coding region	3697–4557
Synthetic poly(A) signal/transcriptional pause site	4662–4815
Reporter Vector primer 3 binding region	4764–4783

Figure 45. pGL4.83[*hRlucP*/Puro] Vector circle map and sequence reference points.



Multiple cloning region	1–70
hRlucCP reporter gene	100–1212
SV40 late poly(A) signal	1249–1470
SV40 early enhancer/promoter	1518–1936
Synthetic puromycin-N-acetyltransferase (Puro^r) coding region	1961–2560
Synthetic poly(A) signal	2585–2633
Reporter Vector primer 4 binding region	2700–2719
*Col*EI-derived plasmid replication origin	2957
Synthetic β-lactamase (Amp^r) coding region	3748–4608
Synthetic poly(A) signal/transcriptional pause site	4713–4866
Reporter Vector primer 3 binding region	4815–4834

Figure 46. pGL4.84[*hRlucCP*/Puro] Vector circle map and sequence reference points.

5. References

1. Klingenhoff, A. *et al.* (1999) Functional promoter modules can be detected by formal models independent of overall nucleotide sequence similarity. *Bioinform.* **15**, 180–6.
2. *pGL3 Luciferase Reporter Vectors Technical Manual*, #TM033, Promega Corporation.
3. Schimke R.T. (1973) Control of enzyme levels in mammalian tissues. *Adv. Enzymol. Mol. Biol.* **37**, 135–87.
4. Li, X. *et al.* (1998) Generation of destabilized green fluorescent protein as a transcription reporter. *J. Biol. Chem.* **273**, 34970–5.
5. Gilon, T., Chomsky, O. and Kulka, R.G. (1998) Degradation signals for ubiquitin system proteolysis in Saccharomyces cerevisiae. *EMBO J.* **17**, 2759–66.
6. Gluzman, Y. (1981) SV40-transformed simian cells support the replication of early SV40 mutants. *Cell* **23**, 175–82.
7. Wagner, E.F. *et al.* (1985) Transfer of genes into embryonal carcinoma cells by retrovirus infection: Efficient expression from an internal promoter. *EMBO J.* **4**, 663–6.
8. Stewart, C.L. *et al.* (1987) Expression of retroviral vectors in transgenic mice obtained by embryo infection. *EMBO J.* **6**, 383–8.
9. Schmidt, E.V. *et al.* (1990) The cytomegalovirus enhancer: A pan-active control element in transgenic mice. *Mol. Cell. Biol.* **10**, 4406–11.
10. Proudfoot, N.J. (1991) Poly(A) signals. *Cell* **64**, 671–4.
11. Bernstein, P. and Ross, J. (1989) Poly(A), poly(A) binding protein and the regulation of mRNA stability. *Trends Biochem. Sci.* **14**, 373–7.
12. Jackson, R.J. and Standart, N. (1990) Do the poly(A) tail and 3´ untranslated region control mRNA translation? *Cell* **62**, 15–24.
13. 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.

6. Related Products

Renilla Luciferase Assay Systems

Product	Size	Cat.#
EnduRen™ Live Cell Substrate	0.34mg	E6481
	3.4mg	E6482
	34mg	E6485
ViviRen™ Live Cell Substrate	0.37mg	E6491
	3.7mg	E6492
	37mg	E6495
Renilla Luciferase Assay System	100 assays	E2810
	1,000 assays	E2820

Luciferase Reporter Cell Lines

Product	Size	Cat.#
GloResponse™ CRE-*luc2P* HEK293 Cell Line	each	E8500
GloResponse™ NFAT-RE-*luc2P* HEK293 Cell Line	each	E8510

Firefly Luciferase Assay Systems

Product	Size	Cat.#
ONE-Glo™ Luciferase Assay System*	10ml	E6110
	100ml	E6120
	1L	E6130
Bright-Glo™ Luciferase Assay System*	10ml	E2610
	100ml	E2620
	10 × 100ml	E2650
Steady-Glo® Luciferase Assay System	10ml	E2510
	100ml	E2520
	10 × 100ml	E2550
Luciferase Assay System	100 assays	E1500
Luciferase 1000 Assay System	1,000 assays	E4550
Luciferase Assay System with Reporter Lysis Buffer	100 assays	E4030

*For Laboratory Use.

Firefly and *Renilla* (Dual-Reporter) Luciferase Assay Systems

Product	Size	Cat.#
Dual-Glo™ Luciferase Assay System	10ml	E2920
	100ml	E2940
	10 × 100ml	E2980
Dual-Luciferase® Reporter Assay System	100 assays	E1910
Dual-Luciferase® Reporter 1,000 Assay System	1,000 assays	E1980

Luminometers

Product	Size	Cat.#
GloMax® 20/20 Luminometer	each	E5311
GloMax® 20/20 Luminometer w/Single Auto-Injector	each	E5321
GloMax® 20/20 Luminometer w/Dual Auto-Injector	each	E5331
GloMax® 96 Microplate Luminometer	each	E6501
GloMax® 96 Microplate Luminometer w/Single Injector	each	E6511
GloMax® 96 Microplate Luminometer w/Dual Injectors	each	E6521
GloMax®-Multi Base Instrument*	each	E7031
GloMax®-Multi Luminescence Module	each	E7041

*E7031 cannot be sold separately. It must be purchased with a detection module such as E7041.

Lysis Buffer

Product	Size	Cat.#
Passive Lysis 5X Buffer	30ml	E1941

Antibiotic

Product	Size	Cat.#
Antibiotic G-418 Sulfate	100mg	V7981

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[d]Patents Pending.

[e]Cat.# E6881, E6891, E6901, E6911, E6921, E6931, E6941, E6951, E6961, E6971, E6981, E6991, E7501, E7511, E7521: Licensed from University of Georgia Research Foundation, Inc., under U.S. Pat. Nos. 5,292,658, 5,418,155, Canadian Pat. No. 2,105,984 and related patents.

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