pGL4 Vectors: A New Generation of Luciferase Reporter Vectors

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Abstract

Reporter gene technologies are powerful tools by which to study gene expression and regulation (1,2). Among reporters used in mammalian cells, the luciferases are preferred because they are highly sensitive, flexible, easy to quantify and detectable over a broad dynamic range (3). We have developed several new reporter designs for firefly and Renilla *luciferases, which allow a range of uses from measurement of transcriptional activity in cell biology applications to high-throughput screening in drug discovery. The resulting vectors yield higher expression with decreased signal-to-background ratios and respond more rapidly to transcriptional dynamics. The vectors are also designed to reduce the risk of anomalous effects.*

Our goal was to create an ideal reporter that:
i) expresses uniformly and optimally in host cells;
ii) minimizes off-target responses (reduces anomalous expression); and
iii) responds rapidly to transcriptional dynamics.

Introduction

We have developed a new series of reporter vectors, the pGL4 Luciferase Reporter Vectors[a,b,c,d,e]. In designing these vectors our goal was to create an ideal reporter that: i) expresses uniformly and optimally in host cells; ii) minimizes off-target responses (reduces anomalous expression); and iii) responds rapidly to transcriptional dynamics. To develop improved reporters from the native luciferases, we addressed these three criteria.

First, we increased the expression levels of the luciferase reporter in mammalian cells by redesigning the luciferase gene using codons preferred for mammalian sequence expression (4,5). Second, we removed cryptic regulatory sequences, reducing the risk of anomalous expression (4,6–8). Finally, we developed destabilized luciferase genes (Rapid Response™ Reporters), with greatly improved response rates compared to native luciferase (9).

The vectors used to deliver reporter genes into host cells are also critical for the overall performance of the reporter assay. Cryptic regulatory sequences such as transcription factor binding sites and/or promoter modules found on the vector backbone could lead to high backgrounds and anomalous responses (10,11). Although this is a common issue for all mammalian reporter vectors, little has been done until now to rectify these problems. We applied our "cleaning" strategy to the entire vector backbone, removing cryptic regulatory sequences where possible, while maintaining functionality. In addition to the redesigned vector backbone, the pGL4 Vectors incorporate a variety of features such as a choice of luciferases, including the Rapid Response™ Reporter versions, and promoterless (basic) and promoter-containing (control) vectors. Table 1 describes the components of 10 of the pGL4 Luciferase Reporter Vectors. A schematic diagram of a pGL4 Vector is shown in Figure 1.

Table 1. pGL4 Vector Components.

Vector	Multiple Cloning Region	Reporter Gene	Protein Degradation Sequence	Mammalian Promoter
pGL4.10[*luc2*]	Yes	*luc2*	No	No
pGL4.11[*luc2P*]	Yes	"	hPEST	No
pGL4.12[*luc2CP*]	Yes	"	hCL1-hPEST	No
pGL4.13[*luc2*/SV40]	No	"	No	SV40
pGL4.70[*hRluc*]	Yes	*hRluc*	No	No
pGL4.71[*hRlucP*]	Yes	"	hPEST	No
pGL4.72[*hRlucCP*]	Yes	"	hCL1-hPEST	No
pGL4.73[*hRluc*/SV40]	No	"	No	SV40
pGL4.74[*hRluc*/TK]	No	"	No	HSV-TK
pGL4.75[*hRluc*/CMV]	No	"	No	CMV

Reduced Risk of Anomalous Expression

The pGL3 Vector backbone was used as a template for the pGL4 Vector backbone. Numerous consensus transcription factor binding sites are present in the pGL3 Vector backbone (Figure 2). To reduce the risk of anomalous expression and increase the reliability of reporter gene expression, we redesigned the backbone

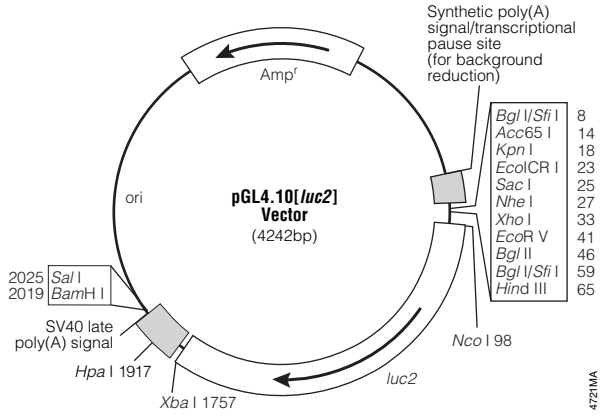

Figure 1. The pGL4.10[*luc2*] Vector.

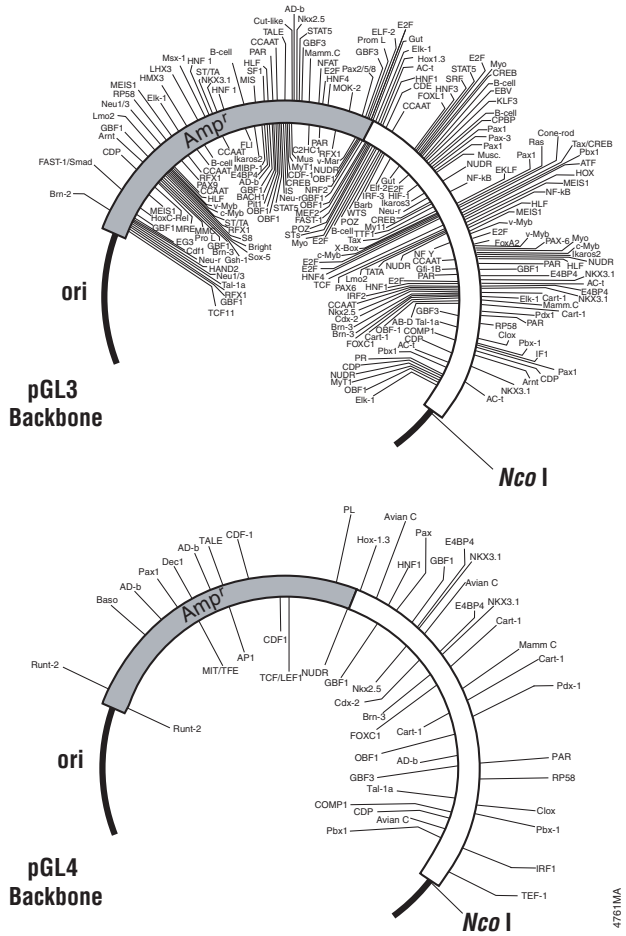

Figure 2. Comparison of consensus transcription factor binding sites in pGL3 and pGL4 Vector backbones.

of the pGL3 Vector region from the start of the reporter gene to the bacterial origin of replication sequence to greatly reduce the number of consensus transcription factor binding sites and thus the risk of anomalous expression (Figure 2). Other modifications included a redesign of the multiple cloning region, removal of the f1 origin of replication, deletion of an intronic sequence and reduction in the number of promoter modules. (Promoter modules are regulatory elements consisting of two or more transcription factor binding sites separated by a spacer.) A synthetic poly(A) signal/transcriptional pause site was retained upstream of either the multiple cloning region (in promoterless vectors) or the mammalian promoter (in promoter-containing vectors). The bacterial origin of replication and the SV40 late poly(A) signal downstream of the reporter gene were not modified.

The newly designed pGL4 Vectors behave similarly in terms of replication, selection and maintenance in *E. coli* to their pGL3 counterparts, despite significant changes in the DNA sequences.

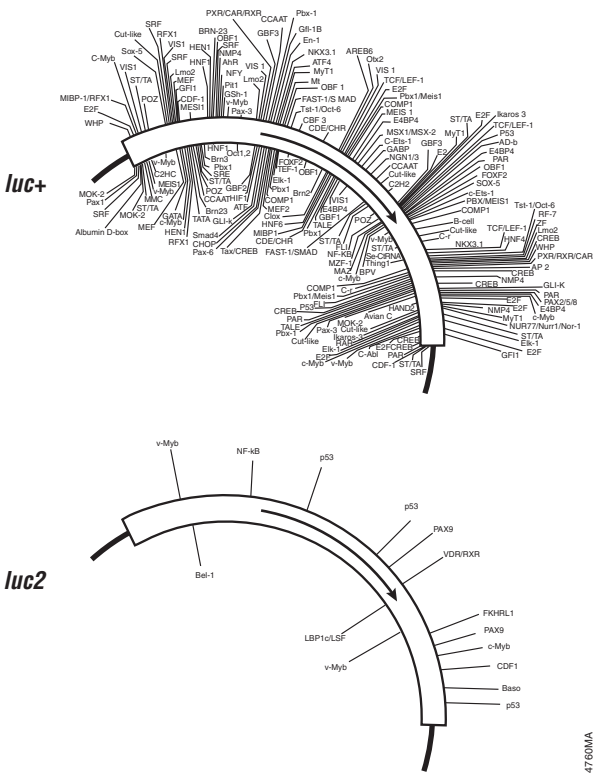

Figure 3. Comparison of consensus transcription factor binding sites in *luc+* and *luc2* genes.

Improved Luciferase Expression

The pGL4 Vectors also feature an improved firefly luciferase gene, *luc2*. Compared to its predecessor, *luc+* in the pGL3 Vectors, *luc2* is codon optimized and has fewer cryptic regulatory sequences (Figure 3). 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. Additionally, the number of predicted promoter modules have been reduced to a single module in the *luc2* gene.

In the transfection experiment shown in Figure 4, the synthetic firefly luciferase gene, *luc2*, displayed increased expression compared to *luc+*. To ensure that the synthetic construction affected only expression, both the *luc+* and *luc2* genes were cloned into the pGL3-Control Vector. The vectors were cotransfected with a control and, after 24 hours, the relative light units measured and normalized to the control. When compared to *luc+*, the *luc2* gene demonstrated a 4.1- to 11.8-fold increase in expression for the four mammalian cell lines tested.

Table 2. Background Comparison Among the pGL4, pGL3 and phRL Vectors.

Vector	Signal-to-Background Ratio[1]	Percent Increase (pGL4 Vectors)
pGL4.13[*luc2*/SV40] vs.pGL4.10[*luc2*]	3,162 ± 337	373
pGL3-Control vs. pGL3-Basic	668 ± 57	
pGL4.73[*hRluc*/SV40] vs. pGL4.70[*hRluc*]	628 ± 78	3,205
phRL-SV40 vs. phRL-null	19 ± 1.7	
pGL4.74[*hRluc*/TK] vs. pGL4.70[*hRluc*]	79 ± 8	3,335
phRL-TK vs. phRL-null	2.3 ± 0.9	
pGL4.75[*hRluc*/CMV] vs. pGL4.70[*hRluc*]	1,103 ± 115	6,388
phRL-CMV vs. phRL-null	17 ± 1.5	

[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". To calculate the signal-to-background ratio, the normalized signal from transfection of the promoter-containing construct was divided by the normalized signal from the corresponding promoterless vector. The experiment was repeated in CHO, HeLa, NIH/3T3 and HEK 293 cells, generating similar results.

Improved Signal-to-Background Ratios

Modifications to the pGL4 Vector backbone combined with synthetic reporter genes have resulted in an improved signal-to-background ratio compared to their pGL3 and phRL Vector counterparts. In Table 2 these vectors are compared to their corresponding promoterless controls and the percent increase in the signal-to-background ratios reported. At the low end, the pGL4.13[*luc2*/SV40] Vector displayed a 373% increase in signal-to-background ratio, while the greatest increase in signal-to-background ratio was demonstrated for the pGL4.75[*hRluc*/CMV] Vector at 6,388%.

Easy Transfer Between Vectors

Another feature of the pGL4 Vectors is the redesigned multiple cloning region for easy transfer of promoters and other transcriptional elements from one vector to another within the pGL4 Vector series or from pGL3 to pGL4 Vectors (Figure 5). The new multiple cloning region retains most of the key restriction enzyme sites from pGL3 Vectors, while introducing two *Sfi* I sites at either end of the multiple cloning region (*Sfi* I recognizes the DNA sequence GGCCNNNNNGGCC, where N is A, T, G or C). Each site generates unique cohesive ends. This property allows directional transfer of elements (without an internal *Sfi* I site) from the multiple cloning region of one pGL4 Vector to another with a single *Sfi* I digestion.

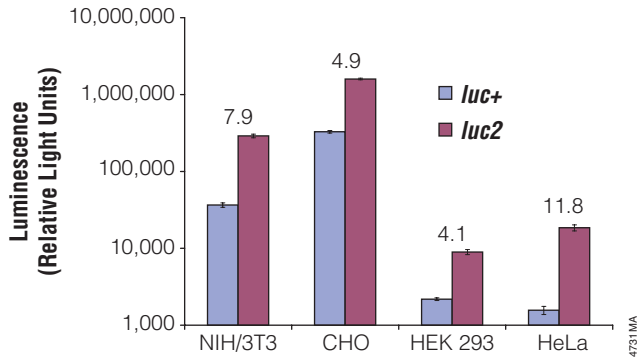

Figure 4. The firefly *luc2* gene displays higher expression than *luc+*. The *luc2* gene was cloned into the pGL3-Control Vector (Cat.# E1741), replacing the *luc+* gene. Thus both firefly luciferase genes were in the same pGL3-Control Vector backbone. The two vectors containing either of the firefly luciferase genes were transfected into NIH/3T3, CHO, HEK 293 and HeLa cells using the phRL-TK Vector (Cat.# E6241) as 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). Relative light units were normalized to the *Renilla* luciferase expression from the phRL-TK Vector transfection control. The fold increase in expression value is listed above each pair of bars.

Conclusion

The pGL4 Vector series represents a new generation of luciferase reporter vectors. There are a variety of gene and promoter configurations to choose from. Compared to their predecessors, the pGL3 Vectors, the pGL4 Vectors reduce the risk of anomalous expression, have better luciferase expression and improved signal-to-background ratios. These features plus the ease of transfer from one vector to another make the pGL4 Vectors the best choice in luciferase reporter vectors.

Editor's Note: Additional pGL4 Vectors are being developed to assist with your reporter needs, including vectors containing selectable markers. Watch for the release of these and additional pGL4 Reporter Vectors in 2005.

References

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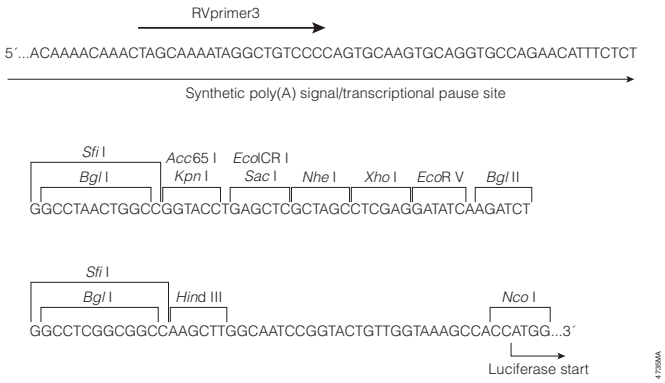

Figure 5. The multiple cloning region of the pGL4 Vectors. The *Bgl* I and *Eco*R V sites in this figure are also present in the *hRluc* gene and should not be used for cloning into the *hRluc-* (*Renilla-*) based pGL4 Vectors.

Protocol

- *pGL4 Luciferase Reporter Vectors Technical Manual* #TM259, Promega Corporation.
  www.promega.com/tbs/tm259/tm259.html

Ordering Information

Product	Size	Cat.#
pGL4.10[*luc2*] Vector	20µg	E6651
pGL4.11[*luc2P*] Vector	20µg	E6661
pGL4.12[*luc2CP*] Vector	20µg	E6671
pGL4.13[*luc2*/SV40] Vector	20µg	E6681
pGL4.70[*hRluc*] Vector	20µg	E6881
pGL4.71[*hRlucP*] Vector	20µg	E6891
pGL4.72[*hRlucCP]* Vector	20µg	E6901
pGL4.73[*hRluc*/SV40] Vector	20µg	E6911
pGL4.74[*hRluc*/TK] Vector	20µg	E6921
pGL4.75[*hRluc*/CMV] Vector	20µg	E6931

(a) Patent Pending.

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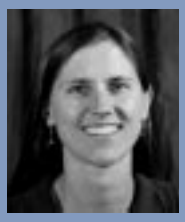
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