ENHANCED RESPONSE DYNAMICS FOR TRANSCRIPTION ANALYSIS USING NEW pGL4 LUCIFERASE REPORTER VECTORS

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Here we introduce new pGL4 luciferase reporter vectors that facilitate investigation of physiological pathways associated with gene expression, suitable for both transient and stable transfection experiments.

Introduction

Reporter gene assays that measure genetic response element (RE) activity are useful for measuring cell signaling processes that lead to specific transcription factor activation. Luciferase is an ideal reporter for applications such as RE studies, high-throughput drug screening, and target validation due to its broad dynamic range, sensitivity, ease of quantification, and lack of endogenous activity (1). The pGL4 Vectors incorporate several advances in genetic reporter technology, including codon optimization of the reporter gene to enhance expression in mammalian cells, incorporation of reporter genes that respond rapidly to transcriptional dynamics and incorporation of mammalian selection markers that facilitate stable cell line generation (2). For optimal results when analyzing RE activity, the reporter vector must contain a promoter that allows a high level of reporter gene induction by the RE (dynamic range) while maintaining low basal activity (background).

Here we describe a new series of pGL4 Vectors (pGL4.23–pGL4.28), which contain a minimal promoter that facilitates investigation of RE activity in both transient and stable transfection experiments with high dynamic range. These vectors maintain low basal expression levels and produce robust levels of luciferase expression upon RE activation. We also introduce the pGL4.29 and pGL4.30 Vectors, which contain a cAMP response element (CRE) or NFAT-RE coupled with the minimal promoter and the *luc2P* synthetic firefly luciferase reporter gene. Use of these minimal promoter and RE-containing vectors facilitates development of the high-quality transient transfection assays and stable reporter cell lines required for RE activity studies and high-throughput screening.

Optimize Expression, Minimize Off-Target Responses, and Detect Transcriptional Changes Quickly

The pGL4 Vectors were developed to optimize luciferase expression, minimize off-target responses, and respond rapidly to transcriptional dynamics. Until recently, assays analyzing RE activity with pGL4 Vectors required cloning of both the RE of interest and a promoter to obtain optimal reporter expression. The new minimal promoter pGL4 Vectors contain a minimal promoter sequence with a TATA box that maintains low background levels of luciferase expression and produces high levels of expression when induced through the RE of interest. The response kinetics of the reporter assay can be optimized through the use of rapid response luciferases, and stable reporter cell lines can be generated by using a vector that contains a hygromycin B selectable marker. Table 1 summarizes the features of the new pGL4 Vectors. The options provided in this series of vectors allow you to customize your reporter vector to your response element of interest to generate high-quality transient transfection and/or stable cell line reporter assays.

When developing the minimal promoter pGL4 Vectors, we compared the activity of the minimal promoter to a portion of the commonly used Herpes Simplex virus thymidine kinase (HSV-TK) promoter to determine which one provided the highest dynamic range while maintaining low uninduced luciferase levels. This analysis was performed using pGL4 reporter vectors in which a CRE and either the minimal promoter or a portion of the HSV-TK promoter were cloned upstream of the *luc2P* reporter gene. HEK 293 cells

Table 1. Features of Minimal Promoter and Response Element-Containing pGL4 Vectors.

Vector	Multiple Cloning Region	Reporter Gene*	Response Element	Mammalian Selection Marker
pGL4.23[*luc2*/minP][a,b]	Yes	*luc2*	No	None
pGL4.24[*luc2P*/minP][a,b]	Yes	*luc2P*	No	None
pGL4.25[*luc2CP*/minP][a,b]	Yes	*luc2CP*	No	None
pGL4.26[*luc2*/minP/Hygro][a,b]	Yes	*luc2*	No	Hygromycin B
pGL4.27[*luc2P*/minP/Hygro][a,b]	Yes	*luc2P*	No	Hygromycin B
pGL4.28[*luc2CP*/minP/Hygro][a,b]	Yes	*luc2CP*	No	Hygromycin B
pGL4.29[*luc2P*/CRE/Hygro][a,b]	No	*luc2P*	CRE	Hygromycin B
pGL4.30[*luc2P*/NFAT-RE/Hygro][a,b,c]	No	*luc2P*	NFAT-RE	Hygromycin B

* *luc2*: synthetic firefly luciferase gene; *luc2P* and *luc2CP*: Rapid Response™ firefly luciferase genes containing PEST (*luc2P*) or CL1 and PEST (*luc2CP*) degradation sequences.

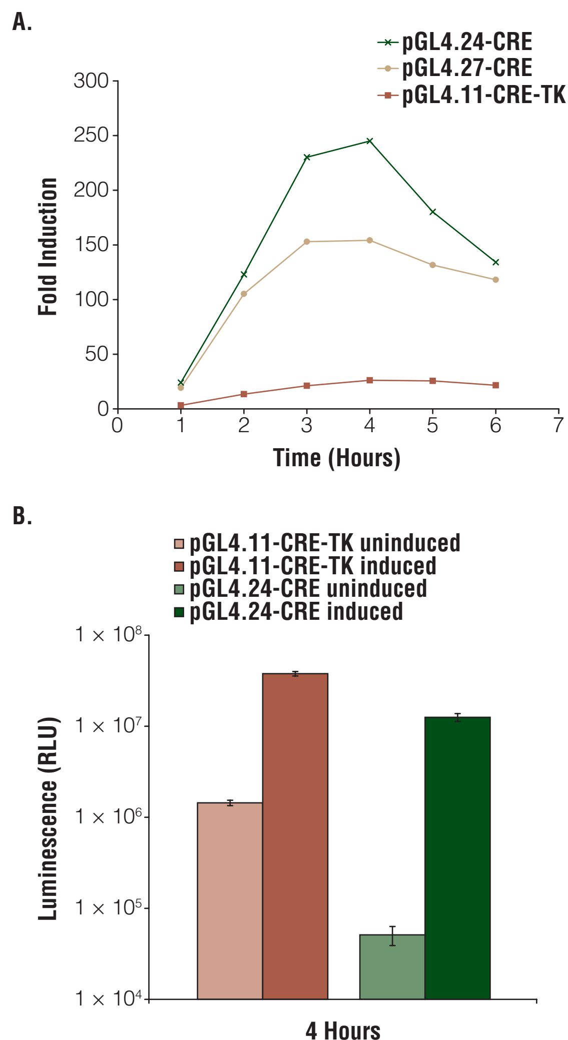

Figure 1. Minimal promoter reporter vectors produce higher luciferase induction when activated by CRE. Panel A. pGL4.11 containing the CRE and a portion of the HSV-TK promoter upstream of the *luc2P* reporter gene (pGL4.11-CRE-TK), pGL4.24 containing the CRE (pGL4.24-CRE), and pGL4.27 containing the CRE (pGL4.27-CRE) were transiently transfected into HEK 293 cells with the pGL4.74[*hRluc*/TK] *Renilla* luciferase control vector. After 24 hours, reporter gene expression was induced by adding 1µM isoproterenol to activate endogenous receptors. Uninduced and induced cells were treated with 100µM of the phosphodiesterase inhibitor Ro 20-1724. Samples were collected each hour for six hours by lysis in Passive Lysis Buffer (Cat.# E1941), and firefly and *Renilla* luciferase activities were measured using the Dual-Luciferase® Reporter Assay System (Cat.# E1960) on a GloMax™ 96 Microplate Luminometer (Cat.# E6501). Parental vectors lacking the CRE showed no reporter gene induction after isoproterenol stimulation (data not shown).
Panel B. Firefly luciferase Relative Light Units (RLU) from the 4-hour time point shown in Panel A illustrate that the higher fold of induction from the pGL4.24-CRE vector is a result of lower levels of uninduced luciferase activity. Fold induction was calculated by dividing the average normalized luciferase activity (firefly RLU/*Renilla* RLU) for the induced samples by the average normalized luciferase activity of the uninduced cells (n = 5; standard deviation bars are shown).

co-transfected with either of these vectors and the *Renilla* luciferase control vector pGL4.74 (Cat.# E6921) were induced through endogenous receptors using isoproterenol. Luciferase activity was quantified using the Dual-Luciferase® Reporter Assay System (Cat.# E1960). The results in Figure 1 show that the minimal promoter vector (pGL4.24-CRE) generated much higher induction values than the HSV-TK promoter vector (pGL4.11-CRE-TK). Further analysis of the firefly luciferase reporter activity from the time point of maximal induction (4 hours) demonstrates that the higher fold induction generated by the minimal promoter vector is due to lower uninduced levels of luciferase activity (Figure 1, Panel B) while maintaining similar levels of induced luciferase expression compared to the vector with the HSV-TK promoter. Therefore, the low basal activity of the minimal promoter in combination with the optimized luciferase expression engineered into the pGL4 Vectors results in superior reporter vectors with broad dynamic range and high sensitivity.

Study GPCR Pathways Using CRE and NFAT-RE pGL4 Vectors

The pGL4.29[*luc2P*/CRE/Hygro] and pGL4.30[*luc2P*/NFAT-RE/Hygro] Vectors contain CRE or NFAT-RE upstream of the minimal promoter. CRE and NFAT-RE reporter assays are of particular interest to researchers studying GPCR signaling pathways because a significant portion of GPCRs activate genes containing either CREs or NFAT-REs upon agonist activation of the receptor. $G\alpha_s$-coupled GPCRs activate genes containing CREs, and $G\alpha_q$-coupled receptors activate genes containing NFAT-REs (1,3). The optimized pGL4 Vector reporter expression coupled with the low level of uninduced luciferase expression from the minimal promoter allows the pGL4.29 and pGL4.30 Vectors to induce high levels of reporter gene expression with broad dynamic range. Both vectors use the *luc2P* reporter gene for rapid assay kinetics and contain a hygromycin B selectable marker to facilitate stable cell line generation. These turnkey solutions allow you to integrate the benefits of the pGL4 reporter family into your cell-based GPCR drug discovery platform with minimal effort and are also highly suitable for transient transfection assays.

Transient transfection assays in HEK 293 cells with the pGL4.29 and pGL4.30 reporter vectors show a large dynamic range of luciferase expression upon induction (Figure 2). HEK 293 cells transfected with pGL4.29 induced for 5 hours with 100µM forskolin showed a 420-fold induction. Similar experiments where pGL4.30 was transfected into HEK 293 cells and induced for 17 hours with 1µM ionomycin and 10ng/ml PMA increased luciferase activity 60-fold. The large dynamic range and high light output from induced luciferase expression make these vectors highly suitable for use in the higher density assay formats preferred for high-throughput screening.

Enhanced Response Dynamics with pGL4 Vectors

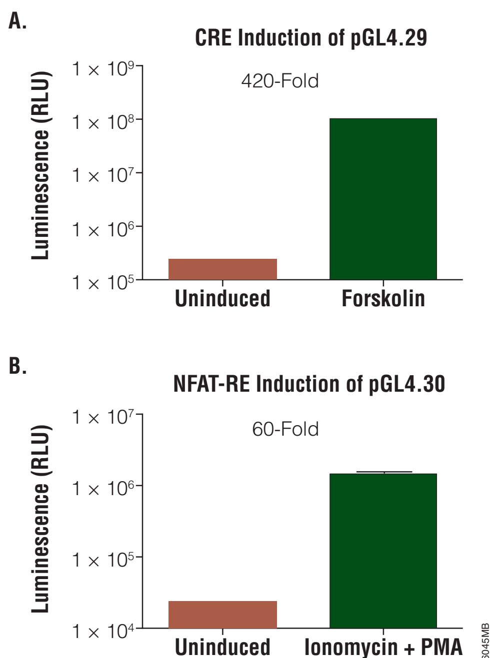

Figure 2. High levels of reporter induction for pGL4.29[*luc2P*/CRE/Hygro] and pGL4.30[*luc2P*/NFAT-RE/Hygro] Vectors in transient transfection of HEK 293 cells. pGL4.29 (**Panel A**) and pGL4.30 (**Panel B**) Reporter Vectors were transiently transfected into HEK 293 cells in a 96-well plate format. After 24 hours, reporter gene expression was induced by adding 100µM forskolin to the pGL4.29 transfected cells for 5 hours and by adding 1µM ionomycin plus 10ng/ml PMA to the pGL4.30 transfected cells for 17 hours. Uninduced controls were treated with an equivalent amount of DMSO vehicle for both pGL4.29 and pGL4.30 transfected cells. Luciferase activity was measured using the Bright-Glo™ Luciferase Assay System (Cat.# E2610). The numbers shown above the graph bars represent fold induction for the respective vectors, calculated by dividing the RLU obtained for the induced wells divided by the RLU obtained from uninduced wells (n = 15; standard deviation bars are shown).

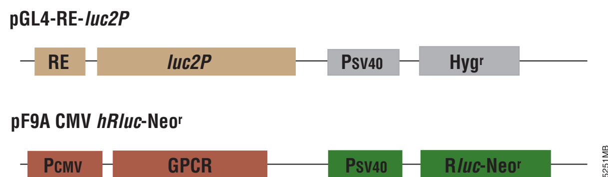

Figure 3. A diagram of two plasmids involved in the dual-reporter GPCR assay. RE, response element/promoter; *luc2P*, destabilized firefly luciferase with PEST sequence (proline, glutamate, serine, threonine); PSV40, SV40 promoter; Hygʳ, hygromycin resistance gene; PCMV, CMV promoter; R*luc*-Neoʳ, *Renilla* luciferase and neomycin resistance gene fusion. PEST sequences are associated with rapidly degraded proteins.

Stable cell lines generated with these vectors can be further customized by stably expressing the target GPCR of interest using the pF9A CMV *hRluc*-neo Flexi® target expression vector (4; Figure 3). These doubly transfected, stable cell lines constitutively express both the target GPCR of interest and *Renilla* luciferase from the pF9A vector as an internal control. Measuring *Renilla* luciferase activity in parallel with that of firefly luciferase allows normalization of the firefly luciferase reporter activity and identification of anomalous samples. This multiplexing capability provides an advantage over single-reporter assays.

Summary

The new pGL4 Vectors, incorporating a minimal promoter or containing specific response elements, offer researchers simpler, better tools for analysis of response elements. Once the function of a specific response element is defined, it can be readily configured into a cell-based assay to monitor the effects of RNAi or expression of a specific cDNA, and to identify lead compounds during high-throughput screening.

References

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4. Paguio, A. *et al.* (2006) *Cell Notes* **16**, 22–5.

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Protocol

pGL4 Luciferase Reporter Vectors Technical Manual #TM259 (www.promega.com/tbs/tm259/tm259.html)

Ordering Information

Product	Size	Cat.#
pGL4.23[*luc2*/minP] Vector	20µg	E8411
pGL4.24[*luc2P*/minP] Vector	20µg	E8421
pGL4.25[*luc2CP*/minP] Vector	20µg	E8431
pGL4.26[*luc2*/minP/Hygro] Vector	20µg	E8441
pGL4.27[*luc2P*/minP/Hygro] Vector	20µg	E8451
pGL4.28[*luc2CP*/minP/Hygro] Vector	20µg	E8461
pGL4.29[*luc2P*/CRE/Hygro] Vector	20µg	E8471
pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector	20µg	E8481

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