MOLECULAR BIOLOGY

CHOOSING THE RIGHT LUCIFERASE TO LIGHT YOUR WAY

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The wide variety of luciferase assays and genes available enable you to choose the best combination for your experimental goals. Here we discuss some of the features of the luminescent reporter technologies offered by Promega and describe how you can tailor these technologies to meet your needs.

Introduction

Reporter genes are a widely used and convenient means for measuring the effects of experimental conditions on cellular physiology. The range of applications is quite enormous, including analysis of gene transcription and regulation, receptor function and intracellular signaling, protein folding and metabolism, pathogen interactions with host cells, and RNAi suppression of gene function. The conceptual foundation of genetic reporters is simple: Association of a readily observable parameter with gene expression. Yet, as technological capabilities continue to advance, the choice of the most suitable reporter gene and assay is not always obvious.

The purpose of this review is to outline the options for bioluminescent reporter technologies available from Promega. In general, bioluminescent reporters are preferred when experiments require high sensitivity, accurate quantitation, or rapid analysis of multiple samples. As described below, dual bioluminescent assays can be particularly useful for efficiently extracting information from cultured cells.

This review is intended to help guide the reader in selecting an optimal reporter methodology to meet anticipated experimental requirements. However, as a brief overview, it may not provide all information relevant to specific circumstances. Readers are encouraged to seek further information as needed from additional Promega literature and technical support. Table 1 outlines the various available luciferase genes and assay reagents and may provide a useful reference as you read.

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Choosing Between a Single and a Dual Assay

Assays based on a single reporter provide the quickest and least expensive means for acquiring data from cells pertaining to gene expression. However, because cells are inherently complex, the quantity of data available from a single reporter may be insufficient for achieving reliable results. So, one of the first considerations in choosing a reporter methodology is deciding whether a single reporter is sufficient for the task or if the greater information density of a dual assay is preferred. Fortunately, the operational efficiency of dual assays has greatly advanced, requiring scarcely more effort than single assays.

Generally, the additional information afforded by a dual assay can improve experimental outcomes by two means: 1) reducing random variability that can obscure meaningful correlations; and 2) normalizing interfering phenomena that may be inherent in the biological systems.

Reducing random variability. Because cells can be difficult to handle reproducibly, significant variability may occur between samples within an experiment and between experiments performed at different times. This is most evident in trying to maintain uniform cell density and viability between samples and for reproducible transfection of exogenous DNA. When working with multiwell plates, variability may result from "edge effects" brought about through differences in heat capacity and humidity across the plate. Dual assays can control for much of this variability, leading to more accurate and meaningful comparisons between samples (1–4).

Normalizing interfering phenomena. When making correlations between experimental conditions and the expression of a reporter gene, often other events associated with cell physiology may affect reporter gene expression. Of particular importance are cytotoxicity effects, which may appear as genetic down regulation when using a single assay. Dual assays allow independent monitoring of both reporter expression and cell viability to avoid misinterpretation of the data. Dual assays can also allow explicit correlation of interrelated events within cells, such as the coupling of target suppression by RNAi to its consequences on cell physiology (5, Figure 1).

Distinguishing Among Single Assays

Most commonly, the expression of genetic reporters is measured using an endpoint assay. In such assays, cells are cultured for a prescribed period of time under various experimental conditions and then lysed to measure the accumulated reporter synthesized within the cells. Nondestructive reporter assays on living cells are also available. These assays allow continuous monitoring of reporter expression within living cells.

Table 1. Summary of Luciferase-Reporter Genes and Assay Systems.

Single Reporter Assays	Gene	Assay
Endpoint		
Firefly	*luc*+ (enhanced firefly luciferase gene)	Luciferase Assay System[a,b,c] (requires prelysis of cells)
	h*luc*+ (codon optimized)	Steady-Glo® Luciferase Assay System[a,b,c] (add and read, $t_{1/2} \geq 5$ hours)
	h*luc*P+ (codon optimized; low intracellular stability)	Bright-Glo™ Luciferase Assay System[a,b,c] (add and read, $t_{1/2} \geq 30$ minutes)
	h*luc*CP+ (codon optimized; lowest intracellular stability)	
Renilla	R*luc* (requires prelysis of cells)	*Renilla* Luciferase Assay System[c,d]
	hR*luc* (codon optimized)	
	hR*luc*P (codon optimized; low intracellular stability)	
	h*Rluc*CP (codon optimized; lowest intracellular stability)	
Non-destructive		
Renilla	Same *Renilla* gene configurations as above.	EnduRen™ Live Cell Substrate[c,d] (add to culture medium)
Dual Reporter Assays	**Gene**	**Assay**
Dual-luciferase		
Firefly & *Renilla*	Same gene configurations as above.	Dual-Luciferase® Reporter Assay System[a,b,c,e] (requires prelysis of cells, two-reagent addition)
		Dual-Glo™ Luciferase Assay System[a,b,c,e] (add and read)
Dual-color		
Click Beetle	*luc*RD (red luminescence)	Chroma-Glo™ Luciferase Assay System[a,b,c,f,] (add and read)
	*luc*GR99 (green luminescence; high gene homology to *luc*RD)	
	*luc*GR68 (green luminescence; high gene homology to *luc*RD)	
Genetic Reporter & Cell Viability		
Renilla	Same *Renilla* gene configurations as above.	EnduRen™ Live Cell Substrate combined with CellTiter-Glo® Luminescent Cell Viability Assay[a,g]

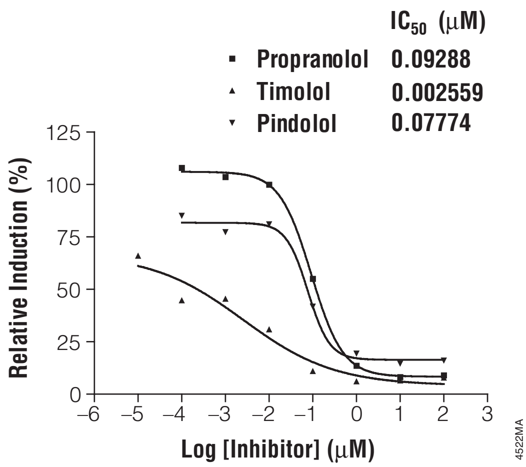

Figure 1. Analysis of receptor agonists using intracellular bioluminescence. Known inhibitors of isoproterenol induction (propranolol, timolol, and pindolol) of endogenous β–adrenergic receptors in HEK293 cells were titrated into culture medium containing agonist (6µM isoproterinol/1µM Ro-20-1724). Receptor activity was monitored by the reporter gene encoding PEST-destabilized *Renilla* luciferase (R*luc*P) coupled to a CRE transcription modulator. Nondestructive quantitation of luminescence was achieved using EnduRen™ Live-Cell Substrate in the culture medium.

Endpoint assays. The most commonly used bioluminescent reporter for endpoint assays is firefly luciferase. This reporter can be measured with very high sensitivity, down to just a few molecules per cell, and with very broad linearity. These features result in successful reporter assays that do not require prior optimization to meet specific experimental circumstances. Sometimes the *Renilla* luciferase is preferred for endpoint assays, but the assay options for this reporter are more limited. More generally, *Renilla* luciferase is used in dual assays and in nondestructive assays. Codon-optimized versions of both reporters have been created to maximize expression efficiency in mammalian cells.

The speed by which a genetic reporter can respond to changes in the transcriptional rate is correlated to the stability of the reporter within cells. Highly stable reporters accumulate to greater levels in the cells, but their concentrations change slowly with changes in transcription. Conversely, lower stability yields less accumulation but a much faster rates of response. To provide reporters designed to meet different experimental needs, families of luciferase genes have been developed yielding differing intracellular stabilities. The genes conferring the lower stabilities are referred to as the Rapid Response™ reporters[c,h,i,j,k]. Although the highest response rates generally correlate with lower sensitivity, this effect is often mitigated when creating stable cell lines.

Traditional assays for reporter genes are designed to measure one sample at a time. They are configured to quantify reporter that has been released from cells by prior treatment with a lysis buffer. They are also optimized to yield maximum light intensity. However, because the duration of the luminescence reaction is relatively short, these reagents are generally not suited for multiwell plates unless used with an automated reagent injector in the luminometer. For firefly luciferase, the traditional assay is the Luciferase Assay System; for *Renilla* luciferase, the assay is the *Renilla* Luciferase Assay System.

Using an assay reagent that produces stable luminescence is more convenient when performing assays in multiwell plates. Unfortunately, because bright reactions fade relatively quickly, a trade-off is necessary between luminescence intensity and duration. The Bright-Glo™ Reagent is designed for firefly luciferase to yield maximum luminescence intensity and sufficient duration for analysis in a multiwell plate. The Steady-Glo® Reagent provides even greater luminescence duration but with lower intensity. Both reagents are designed to work directly in culture medium for mammalian cells, so prior cell lysis is not necessary. This allows the user to grow cells in multiwell plates and then measure expression with a single step.

Nondestructive assays. Although endpoint assays allow simple and reliable quantitation of reporter expression, they are inherently destructive to the sample. In some cases, a non-destructive assay is preferred to allow repeated measurements over a period of time. The EnduRen™ Live Cell Substrate allows measurement of intracellular *Renilla* luciferase for at least 24 hours in culture. It is also useful in multiwell plates where controlling total assay volume is important or where adding assay reagent may be problematic. Because EnduRen™ Substrate is added as a component of the culture medium, no additional reagent additions are necessary when quantifying the luminescence.

Distinguishing Among Dual Assays

The dual assays allow users either to measure expression of two different reporter genes or one reporter gene and cell viability. In all cases, the assays allow both measurements to be made sequentially from each sample. Most assays are optimized for use in multiwell plates.

Dual luciferase. The most commonly used dual assay is based on combining the chemistries for firefly and *Renilla* luciferases. This works on the principle that these luciferases use different substrates and thus can be differentiated by their enzymatic specificities. The method comprises adding two reagents to each sample, with a measurement of luminescence following each addition. The first reagent activates the firefly luminescence reaction; the second extinguishes the firefly luciferase and initiates the *Renilla* luciferase reaction.

The Dual-Luciferase® Assay reagent relies on lysis of the cells before performing the assay and thus requires the use of reagent injectors if used for multiwell plates. The Dual-Glo™ Reagent is optimized for multiwell plates, providing longer luminescence duration. As with other reagents designed for

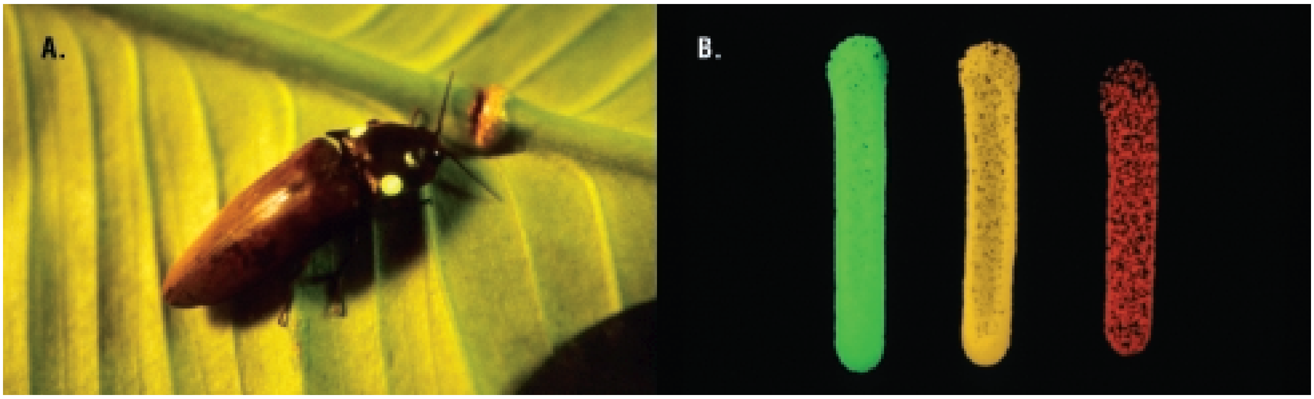

Figure 2. *Pyrophorus* sp. click beetle and different luminescent colors from the cloned luciferase genes. Panel A. The pair of light organs on the head of the click beetle, which emit a green luminescence, are easily viewed in this photograph. The light organ on the ventral cleft of the abdomen, which emits a yellow-orange luminescence, only luminesces during flight. **Panel B**. (left to right) Green and orange luminescence from genes clone from the click beetle; red luminescence generated from a mutant luciferase gene.

use in multiwell plates, the Dual-Glo™ Assay works directly in the culture medium for mammalian cells without requiring prior cell lysis.

Dual-color. In some cases, researchers may prefer to activate both luciferase assays simultaneously by adding a single reagent. This reduces total assay volume and liquid handling requirements. The light emission of the two luciferases can be differentiated by the color of the luminescence. We have developed click beetle luciferases, which are related to the firefly luciferase, to yield red and green luminescence (Figure 2). The structures of these luciferases are nearly identical, containing only a few amino acid substitutions necessary to create the different colors. This structural similarity means that both the control reporter and the experimental reporter are likely to respond similarly to biochemical changes within the cell, resulting in even more accurate normalization to the control.

The genes encoding these reporters, the Chroma-Luc™ genes[c,f,h,i], are codon optimized for mammalian cells. We developed two genes encoding the green-emitting reporter, one which is nearly identical and one which is maximally divergent but encoding the same protein. The divergent gene may be useful under circumstances where genetic recombination is a concern.

The Chroma-Glo™ Assay reagent is designed for use in multiwell plates. Its formulation supports optimal reaction kinetics for both reporters simultaneously, and it works directly in the culture medium. Because color differentiation is required for the Chroma-Glo™ Assay, a luminometer capable of using colored optical filters is required. Since the light is transmitted through the optical filters, sensitivity relative to other assay methods is reduced. Both luciferases may be detectable using optical filters when the relative concentrations differ by about 100-fold. This is less than dual-luciferase assays using chemical differentiation, where the relative concentration may differ by over 1000-fold.

Genetic reporter and cell viability. Although dual assays based on two genetic reporters may be used to implicitly monitor effects on cell viability, sometimes a more direct measure is preferred. The CellTiter-Glo® Luminescent Cell Viability Assay provides a rapid and sensitive assay of cell viability based on bioluminescent detection of cellular ATP. Because the CellTiter-Glo® Assay uses a stabilized firefly luciferase in its formulation, it cannot be directly combined with a reporter assay for expression of firefly luciferase. However, it can be readily combined with non-destructive reporter assays of *Renilla* luciferase.

Expression of *Renilla* luciferase may be quantitated, or continuously monitored, by adding EnduRen™ Substrate to the culture medium. When the reporter measurements are completed, CellTiter-Glo® Reagent may be added to the sample to inactivate the Renilla luminescence and initiate the ATP-dependent luminescence. Because the CellTiter-Glo® Assay is an endpoint assay, further sample monitoring after measuring cell viability is not possible.

Summary

The brief descriptions provided here, covering the range of available bioluminescent reporter genes and assays, illustrate the breadth of experimental strategies enabled by these methodologies. We have considered whether a single or dual assay is appropriate, the use of endpoint vs. non-destructive assays, the relationship between intracellular stability of reporters and their responsiveness to transcriptional rates, and the design of assay reagents for individual samples or multiwell plates. You can find more information in the protocols and additional technical literature written for each assay system. These documents, as well as product and application updates, can be found at our web site (www.promega.com/applications/genexp_reptr). Or, contact Promega Technical Services scientists with any questions you may have (techserv@promega.com). ■

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Protocols

Luciferase Assay System Technical Bulletin #TB281 (www.promega.com/tbs/tb281/tb281.html)

Renilla *Luciferase Assay System Technical Manual* #TM055 (www.promega.com/tbs/tm055/tm055.html)

EnduRen™ Live Cell Substrate Technical Manual #TM244 (www.promega.com/tbs/tm244/tm244.html)

Dual-Luciferase® Reporter Assay System Technical Manual #TM040 (www.promega.com/tbs/tm040/tm040.html)

Dual-Glo™ Luciferase Assay System Technical Manual #TM058 (www.promega.com/tbs/tm058/tm058.html)

Chroma-Glo™ Luciferase Assay System Technical Manual #TM062 (www.promega.com/tbs/tm062/tm062.html)

Steady-Glo® Luciferase Assay System Technical Manual #TM051 (www.promega.com/tbs/tm051/tm051.html)

Bright-Glo™ Luciferase Assay System Technical Manual #TM052 (www.promega.com/tbs/tm052/tm052.html)

Rapid-Response™ Reporter Vectors Technical Manual #TM242 (www.promega.com/tbs/tm242/tm242.html)

Chroma-Luc™ Reporter Vectors Technical Manual #TM059 (www.promega.com/tbs/tm059/tm059.html)

Synthetic Renilla *Luciferase Reporter Vectors Technical Manual* #TM237 (www.promega.com/tbs/tm237/tm237.html)

Ordering Information

Product	Size	Cat.#
Dual-Glo™ Luciferase Assay System[a,b,c,e]	10ml*	E2920
Dual-Luciferase® ReporterAssay System[a,b,c,e]	100 assays*	E1910
Chroma-Glo™ Luciferase Assay System[a,b,c,f]	10ml*	E4910
Steady-Glo® Luciferase Assay System[a,b,c]	10ml*	E2510
Bright-Glo™ Luciferase Assay System[a,b,c]	10ml*	E2610
Luciferase Assay System[a,b,c]	100 assays*	E1500
Renilla Luciferase Assay System[c,d]	100 assays*	E2810
EnduRen™ Live Cell Substrate[c,d]	0.34ng*	E6481
CellTiter-Glo® Luminescent Cell Viability Assay	10ml*	G7570

*Available in additional sizes.

For information about ordering luciferase vectors from Promega, visit our online catalog at: www.promega.com

(a)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.
(b)U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289, European Pat. No. 0 553 234 and Japanese Pat. No. 3171595 have been issued to Promega Corporation for a beetle luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Other patents are pending.c
(c)Certain applications of this product may require licenses from others.
(d)Patent Pending.
(e)U.S. Pat. No. 5,744,320, Australian Pat. No. 721172, Canadian Pat. No. 2,221,522 and European Pat. No. 0 833 939 have been issued to Promega Corporation for quenching reagents and assays for enzyme-mediated luminescence. Other patents are pending.
(f)U.S. Pat. Nos. 6,387,675 and 6,552,179 and Australian Pat. No. 698424 have been issued to Promega Corporation for mutants of beetle luciferases. Other patents are pending.
(g)U.S. Pat. No. 6,602,677 and Australian Pat. No. 754312 have been issued to Promega Corporation for thermostable luciferases and methods of production. Other patents are pending.
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