

LUCIFERASE REPORTER ASSAYS: POWERFUL, ADAPTABLE TOOLS FOR CELL BIOLOGY RESEARCH

SIMON T.M. ALLARD AND KEVIN KOPISH, PROMEGA CORPORATION

Luciferase reporter technology gives the researcher unparalleled sensitivity, dynamic range, versatility and ease of use when investigating questions that involve gene regulation. Here we demonstrate how luciferase technology can be a powerful tool in your research program across a wide area of focus.

Reporter Genes

Genetic reporters are used as indicators to study gene expression and cellular events coupled to gene expression. They are widely used in pharmaceutical and biomedical research and also in molecular biology and biochemistry. Typically, a reporter gene is cloned with a DNA sequence of interest into an expression vector that is then transferred into cells. Following transfer, the cells are assayed for the presence of the reporter by directly measuring the reporter protein itself or the enzymatic activity of the reporter protein. A good reporter gene can be identified easily and measured quantitatively when it is expressed (in the organism or cells of interest).

Bioluminescent reporters are ideal because they have a number of important features (1) including:

- Measurements that are almost instantaneous
- Exceptional sensitivity
- A wide dynamic range
- Typically no endogenous activity in host cells to interfere with quantitation

Bioluminescence encompasses a number of different chemistries evolved for light production and is based on the interaction of the enzyme, luciferase, with a luminescent substrate, luciferin. Bioluminescence is found in many phyla including coelenterates, echinoderms, mushrooms and insects. Only a few of these chemistries have been characterized in detail, two of which, the luciferases from firefly (*Photinus pyralis*) and *Renilla* (*Renilla reniformis*, a sea pansy), represent a large part of the Promega luminescence portfolio.

As a reporter technology, bioluminescence finds its greatest potential by helping to characterize the enormous complexity of living systems. The fact that it can deliver 10- to 1,000-fold higher assay sensitivity than fluorescent reporters such as GFP means it can improve assay performance substantially when applied to complex biological samples. The reaction constituents may exist in vanishingly small amounts enveloped within an intricate biological environment, yet they are still able deliver clear and quantitative signals (2).

1. Determine promoter response element (RE) of interest.

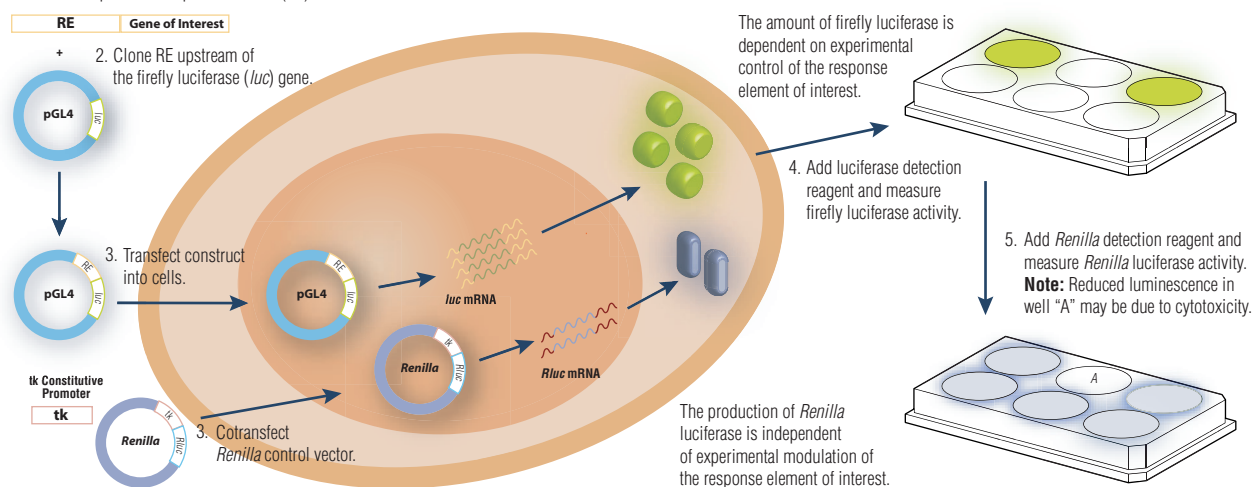


Figure 1. The DLR™ and Dual-Glo™ Assays provide rapid and convenient means for achieving greater control over the biological significance of reporter data by differentiating genetic responses of interest from nonrelevant influences in the experimental system.

REPORTER ASSAYS

APPLICATIONS OF GENETIC REPORTERS

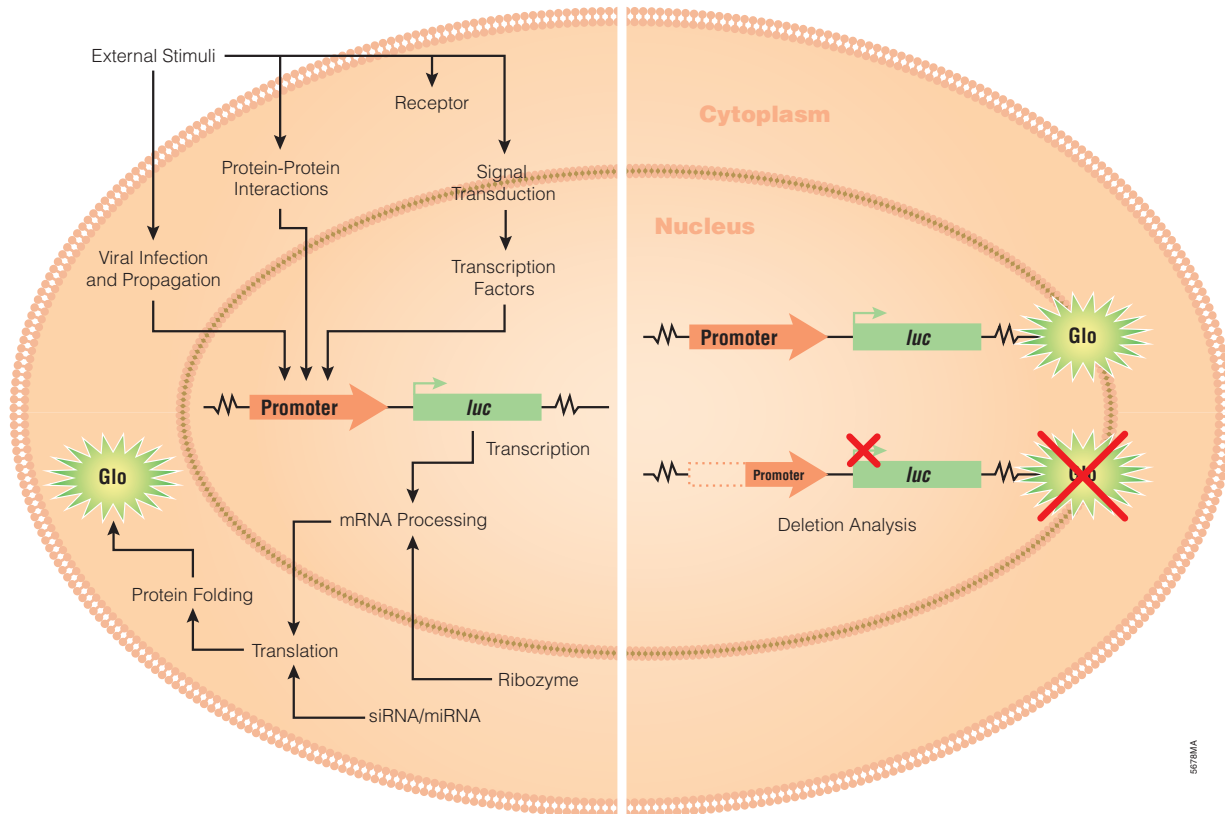


Figure 2. We have developed many simple add-mix genetic reporter assays that can be used for a variety of applications including studies of promoter structure, surface receptor activation and binding, RNA interference, cell-signaling pathways, and stem cell differentiation.

Improving the Native Protein for the Laboratory

Native luciferases such as firefly and *Renilla* are not necessarily optimal as genetic reporters. Promega has enhanced the native proteins by improving their expression in mammalian systems, reducing the risk of anomalous expression and destabilizing the reporters. To enhance mammalian expression, we have optimized codon usage for the firefly and *Renilla* luciferases. Additionally, inappropriate or unintended transcription regulatory sequences (used in mammalian cells) have been minimized. The resulting recombinant luciferase reporters show much higher specific expression in mammalian systems.

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 cells, but their concentrations change slowly with changes in transcription. Conversely, lower stability yields less accumulation but a much faster rate of response. To provide reporters designed to meet different experimental needs, families of luciferase genes have been developed yielding different intracellular stabilities. Luciferase reporters can be destabilized by genetically fusing a protein degradation sequence to the luciferase genes (3). Due to their increased

rate of degradation, these Rapid Response™ reporters respond faster and often display a greater magnitude of response to rapid transcriptional events.

Single- and Dual-Reporter Assays

Cells are inherently complex, and the data available from a single reporter may be insufficient for achieving reliable results. Dual-reporter assays enable researchers to obtain additional information from complex systems with minimal effort. The Dual-Luciferase® Reporter (DLR™) Assay and Dual-Glo™ Assay enable the sequential measurement of both firefly and *Renilla* luciferases from one sample. The DLR™ and Dual-Glo™ Assays provide rapid and convenient means for achieving greater control over the biological significance of reporter data by differentiating genetic responses of interest from nonrelevant influences in the experimental system (Figure 1).

Applications of Bioluminescent Reporters

Luciferase-based genetic reporter assays provide sensitive methods for assaying gene expression, enabling the accurate quantification of small changes in transcription resulting from subtle changes in biology. We have developed many simple add-mix genetic reporter assays that can be used for a variety

APPLICATIONS OF GENETIC REPORTERS

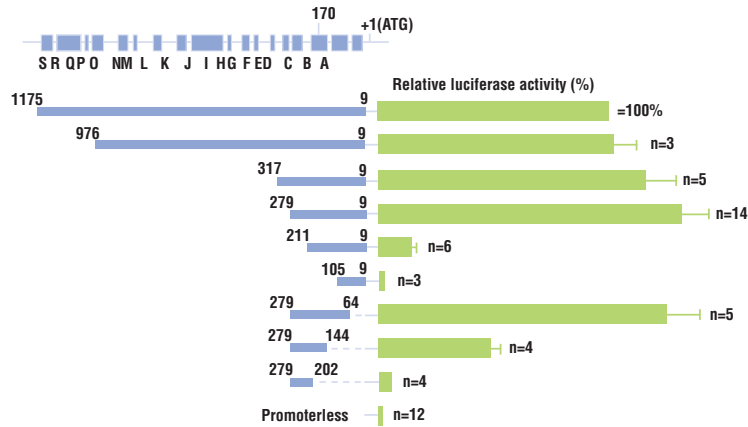


Figure 3. On the basis of observed luciferase activity, a promoter region of about 200 bp was identified that contained the necessary elements for adequate expression of the factor VIII-luciferase construct. Solid blue bars to the left indicate promoter regions that were placed upstream of the luciferase sequence. Green bars on the right indicate the level of luciferase activity relative to the intact sequence.

of applications (Figure 2). In addition, luciferase reporter gene technology has been used to create tools for screening entire genetic pathways in living cells (4,5). These ready-to-screen libraries, available from SwitchGear Genomics, represent thousands of human promoters, UTRs and other regulatory elements encompassing many different disease-related pathways.

Promoter Structural Analysis

One of the most common applications of reporter genes is analyzing the function of *cis*-acting genetic elements such as promoters (“promoter bashing”). Typically, deletions or mutations are made in a promoter region, and their effects on coupled expression of a reporter gene then are measured. One example of this application is the deletion analysis of the human factor VIII gene promoter region (6). Factor VIII is a glycoprotein that is essential for blood coagulation. By using deletion analysis, researchers measured the ability of the 5′ region of the factor VIII gene to transcribe a luciferase reporter gene. On the basis of observed luciferase activity, a promoter region of about 200 bp was identified that contained the necessary elements for adequate expression of the factor VIII-luciferase construct (Figure 3).

Functional Analysis of Promoter SNPs

In addition to studying the macro level structure of a promoter, studies of single nucleotide polymorphisms are also of interest, because they can have profound biological significance. Cytokine polymorphisms are associated with disease outcome, including interferon (IFN) treatment response in hepatitis C virus (HCV) infection. IFN- γ is a multifunctional cytokine produced by effector T and natural killer cells and is critical for host defense against a variety of intracellular pathogens, including HCV. A SNP variant located in the proximal IFN- γ promoter region, -764C/G, is significantly associated with sustained virological response (7).

To study this SNP variant, researchers purified the -764C and -764G alleles from the human IFN- γ gene and subcloned them into a promoterless firefly luciferase expression vector, pGL4.10[*Luc2P*] to create two plasmids, which then were transfected into Jurkat cells. The promoter activities of the different allele constructs were measured subsequently by assaying for the luciferase reporter gene. The studies showed that cells transfected with the variant -764G construct had a two- to threefold higher luciferase activity than the common -764C construct, indicating that the G allele conferred a higher promoter activity. The fact that the G allele is significantly associated with viral clearance and treatment response led the authors of the paper to suggest that it might be used as a genetic marker to predict the response of IFN- γ -based therapies in HCV-infected patients (7).

Studying Cell Signaling Pathways

The real power of reporter gene assays comes from their ability to be used as cellular readouts for virtually any signaling event. By coupling a response element controlled by the signaling event of interest to luciferase, investigators can quantify an intracellular response to a biological event.

G-protein-coupled receptors (GPCRs) represent one of the most important classes of drug targets, with an estimated 50% of currently marketed medicines directed against them (8). In many cases GPCRs interact with heterotrimeric G proteins, which upon stimulation by an agonist, lead to dissociation of the G-protein subunits. Major G-protein families regulate different intracellular second messenger pathways based on the type of $G\alpha$ -subunit involved. These second messenger pathways in turn activate a range of effector systems to change cell behavior, including regulation of gene transcription. This affords the means for coupling luminescence to receptor activity.

For example, the action of $G\alpha_s$ -coupled receptors, which modulate intracellular cyclic AMP (cAMP) levels via the effector adenylate cyclase, can be coupled to luciferase transcription by a cAMP response element (CRE). A rapid, accurate high-throughput GPCR-CRE assay involves using a stable cell line with a CRE/promoter-luciferase introduced transiently or integrated into the chromosome. The accuracy of the data is improved by using a second constitutively expressed *Renilla* luciferase in a Dual-Luciferase® Assay format, which removes false positives due to cell death from compound toxicity and normalizes well-to-well variations (9). Virtually any biological event that alters the activity of a transcription factor or the expression level of a gene can be monitored with a reporter gene assay.

Using Reporters to Study RNAi

RNA interference (RNAi) is a mechanism that inhibits gene expression before a gene is translated or by hindering transcription of specific genes. The two related classes of molecules that moderate RNAi processes are microRNA (miRNA) and small interfering RNA (siRNA), which are short noncoding RNAs.

Hypoxia-inducible growth factor-1 (HIF-1) is the master regulator of cellular responses to low oxygen. Over the past few years, HIF-1 has emerged as an attractive target for cancer therapy. Recent work has demonstrated that the inhibition of HIF-1 leads to slower tumor growth in vivo, resulting in a considerable amount of work to identify HIF-1 inhibitors for cancer therapy. One study used an siRNA-based loss-of-function screen to uncover druggable targets in the HIF-1 pathway (10). A stable dual-luciferase HIF-1 reporter cell line was created containing a firefly luciferase under the control of the hypoxia-responsive element (HRE) and a

constitutively expressed *Renilla* luciferase reporter. The importance of the dual-luciferase approach was realized following normalization of the results, when a number of possible siRNA target hits were ruled out due to cytotoxicity or nonspecific transcriptional/translational inhibition. The goal of this study was to find potential druggable targets from a corollary small molecule inhibitor screen. In this way RNA interference can lend specificity to the study of entire signaling networks by identifying the role of individual components of that pathway.

Summary

This short discussion highlights just a few of the broad range of applications for which luciferase reporter technology can be used. The development of the DLR™ and Dual-Glo™ Assays has provided a rapid and convenient means for achieving greater control over the biological significance of reporter data by differentiating genetic responses of interest from nonrelevant influences in the experimental system. What is clear is that luciferase reporter products from Promega are both powerful and versatile tools.

In the Literature

Examples of applications of luciferase reporters to ask fundamental biological questions can be found in the Citations Database available at: www.promega.com/citations/. To see how other researchers have used specific Promega luciferase reporters you can search by product name and number, or browse through the entire collection of “gene expression” citations.

For more information about specific Promega luciferase reporter technologies, visit: www.promega.com/reportergene/

References

1. Wood, K. (1998) *Promega Notes* **65**, 14–20.
2. Wood, K. (2007) *Promega Notes* **96**, 3–5.
3. Li, X. *et al.* (1998) *J. Biol. Chem.* **273**, 34970–5.
4. Trinklein, N.D. and Aldred, S.F. (2007) *Promega Notes* **96**, 8–10.
5. Aldred, S.F. and Trinklein, N.D. (2008) *Cell Notes* **21**, 20–2.
6. Figueiredo, M.S. and Brownlee, G.G. (1995) *J. Biol. Chem.* **270**, 11828–38.
7. Huang, Y. *et al.* (2007) *Proc. Natl. Acad. Sci. USA* **104**, 985–90.
8. Klabunde, T. and Hessler, G. (2002) *Chembiochem.* **3**, 928–44.
9. Fan, F. *et al.* (2005) *Cell Notes* **13**, 5–7.
10. Lin, X. *et al.* (2008) *Proc. Natl. Acad. Sci. USA* **105**, 174–9.

Protocols

- pGL4 Luciferase Reporter Vectors Technical Manual* #TM259 (www.promega.com/tbs/tm259/tm259.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)
- ONE-Glo™ Luciferase Assay System Technical Manual* #TM292 (www.promega.com/tbs/tm292/tm292.html)
- GloMax®-Multi Detection System Technical Manual* #TM297 (www.promega.com/tbs/tm297/tm297.html)

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

Dual-Luciferase and GloMax are registered trademarks of Promega Corporation. DLR, Dual-Glo, ONE-Glo and Rapid Response are trademarks of Promega Corporation.