Getting the Most from Your Transfections: Increasing Throughput and Sensitivity

ABSTRACT | Transfections of reporter genes are a powerful tool for the study of gene and regulatory sequence function in the context of a cell. Luminescent technologies enable the scale-down of transfection volumes, making it possible to use less material, test more variables and get more data, in less time.

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INTRODUCTION

The understanding of cellular physiology has been greatly facilitated by the ability to introduce natural or altered genes into cultured mammalian cells by transfection. The success of gene transfer depends on optimized delivery conditions and varies depending on cell type and experimental requirements. High expression levels are not always beneficial and do not always create functionally relevant systems (1). Optimal transfection conditions must be determined empirically through the use of reporters such as luciferase or green fluorescent protein (GFP).

The desire to increase throughput (e.g., understanding gene function on a whole-genome basis, or performing whole-genome-based RNAi screens) creates the need for transfections performed at increasingly smaller volumes. Luciferase assays are robust, rapid and well suited for high-throughput applications. Here we compare luciferase to the standard GFP for 96-well transfections and discuss transfection optimization with luciferase in a 96-well format.

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LUCIFERASE VERSUS GFP

With advances in reporter technologies, there has been a shift away from reporters that require cumbersome or insensitive assays (e.g., chloramphenicol acetyltransferase (CAT) and beta-galactosidase) to reporter technologies that are easy to detect such as GFP and luciferase. Both have advantages over older methods in their simplicity and sensitivity of detection and are now routinely used as markers for determining transfection efficiency and gene expression. The future of transfection lies in smaller volumes and higher throughput assays, and for such assays, luciferase offers clear advantages (Table 1).

REPORTER CHARACTERISTICS

Endogenous activity and protein half-life are two distinguishing characteristics of GFP and luciferase reporter proteins. Although GFP is not native to mammalian cells, some primary cells, such as monocytes and macrophages, are autofluorescent. This can contribute background and limit fluorescent reporter detection. Autoluminescence is not an issue with mammalian cells. Also, GFP has a longer half-life ($t_{½}$~26 hours; 2) than luciferase ($t_{½}$~3–4 hours; 3), which results in a lower chance of GFP mimicking endogenous protein expression patterns.

DETECTION METHODS

Luciferase activity is assayed by adding a substrate and detecting the signal with a luminometer. Luminometers use photomultiplier tubes to detect light and are extremely sensitive with a broad dynamic

Table 1. Comparison of GFP and Luciferase Reporters.

Reporter	Typical Detection Method	Advantages	Disadvantages
Fluorescent protein (e.g., GFP)	Microscopy, Flow cytometry (plate reader not recommended)	Simple to visualize Single-cell or cell-population analysis possible Cell sorting possible No reagents needed	Low sensitivity Limited dynamic range Expensive equipment Subjective (microscopy) Significant DNA input Longer protein half-life
Luciferase	Luminometer	Analyze entire cell population Sensitive Broad dynamic range High-throughput Easy multiplexing Minimal DNA input Average protein half-life	No single-cell analysis Assay reagents needed

range (4). Alternatively, GFP does not require substrate addition, and there are a couple of options for detecting fluorescence. The two most common detection methods, microscopy and flow cytometry, require expensive equipment. Fluorescent assays are, by nature, limited in dynamic range and sensitivity. The limited sensitivity of fluorescent plate readers makes them useful only when GFP is expressed at high levels, so this detection approach is not recommended.

The high sensitivity of luciferase reporter detection means significantly less luciferase reporter vector is needed for a strong signal-to-background ratio compared to GFP.

TRANSFECTION EFFICIENCY

The reporter and assay method chosen will influence how transfection efficiency is determined. There are two approaches routinely used to determine transfection efficiency, percent transfected cells and average reporter expression in a cell population.

The first approach, determining the percent transfected cells, can be accomplished using GFP with microscopy or flow cytometry (Figure 1, Panels A and B). Cells are scored for the number of cells expressing detectable GFP compared to the total cell number. This does not, however, give a measure of the amount of reporter expression within the cells, which can vary by several orders of magnitude (Figure 1, Panels B and C). Scoring cells by microscopy is also subjective and time consuming.

The second approach, average reporter activity, can be accomplished with GFP using a flow cytometer or with luciferase using a luminometer. Although average reporter activity does not give the percent of cells transfected, it does give a measure of total reporter activity within the sample. Because most gene expression experiments are performed in cell populations, direct correlation between the expression levels of the gene of interest and the reporter can be made.

These two approaches are compared in Figure 2. Jurkat cells were transfected with both pmaxGFP® and pGL4.10[*luc2*]-CMV vectors using five different Nucleofector® programs (Lonza). Percent transfection was determined using GFP and average reporter activity using both GFP and luciferase. Both approaches gave similar trends; however, the differences in reporter activity were much greater than differences in percent transfection. For example, the 1.5-fold difference in percent transfection between G10 and S18 programs represented a 10-fold difference in reporter activity. In addition, the percent of cells transfected with S18 and T14 programs were similar, but the reporter activity with T14 was 2- to 3-fold higher. This example highlights the value of monitoring average reporter expression activity versus percent transfected cells.

Finally, the high sensitivity of luciferase reporter detection means significantly less luciferase reporter vector is needed for a strong signal-to-background ratio compared to GFP. This allows greater flexibility when including multiple vectors in a transfection. For instance, in Figure 2, 10 times more GFP than luciferase vector was used.

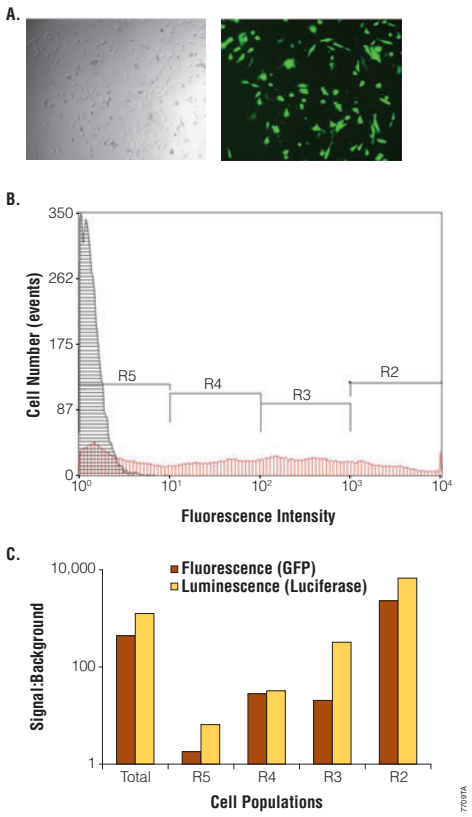

Figure 1. Determination of transfection efficiency. Panel A. HeLa cells expressing GFP reporter visualized by fluorescence microscopy (Right) compared to the total cells as determined by light microscopy (Left) give percent transfection. **Panel B.** Jurkat cells were cotransfected with a GFP reporter and pGL4.10[*luc2*]-CMV Luciferase Reporter Vector. GFP expression was analyzed by flow cytometry (red trace). Mock-transfected cells were used as a control (black trace). Transfected cells were divided into five regions based on GFP expression intensity and were resorted. Approximately 10,000 events were collected for each population (R2–R5). **Panel C.** For each sorted population shown in Panel B, mean fluorescence was measured by flow cytometry, and luminescence was measured by luminometer using the ONE-Glo™ Luciferase Assay System. Data are represented as the signal:background ratio (light from reporter-transfected cells:light from mock-transfected cells).

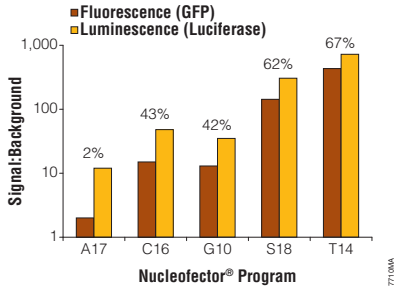

Figure 2. GFP versus luciferase for optimizing transfections. Jurkat cells were cotransfected with pGL4.10[*luc2*]-CMV and pmaxGFP® vectors at a 1:10 ratio under five different Nucleofector® programs. The levels of maxGFP® expression and percent transfected cells were determined by flow cytometry. The luciferase activity was measured by luminometer using the ONE-Glo™ Luciferase Assay System. The expression levels of both reporters are presented as a ratio of signal intensity between transfected and control cells, and the percent transfection is indicated.

OPTIMIZED 96-WELL TRANSFECTIONS

Optimizing transfections in a 96-well plate involves determining ideal cell number, transfection reagent conditions, DNA concentration and timing of treatment and recovery. These conditions must be determined empirically, and a useful place to start is to find a precedent in the primary literature or with the transfection reagent supplier for the cell line and transfection method to be used (5).

To optimize conditions, perform triplicate transfections for each condition within a single plate. Average the triplicate samples; the average value is considered an N of 1 for the experiment. Avoid putting all replicates for a given condition on the perimeter of the plate. Suboptimal incubator humidity levels can lead to volume loss and variability along the plate perimeter, termed "edge effect." By having only one of a triplicate of samples on the edge, potential variability can be either averaged or more easily identified, and the culture conditions improved as needed.

CELL VIABILITY

For the best transfection success, it is not only critical to choose the right reporter and transfection efficiency approach but also to monitor cell health. Luciferase with a 96-well format makes this simple because the luciferase assay can be multiplexed with a cell viability assay (6).

To determine the optimal Nucleofector® method and reagent for Jurkat cell transfection, the pGL4.10[*luc2*]-CMV Vector was used to test 31 different methods and three different reagents in a single 96-well plate (Figure 3). The transfected cells were assayed for luciferase activity (Figure 3, Panel B) and viability (Figure 3, Panel C). The optimal transfection condition gave the highest luciferase activity with minimal affect on cell viability: CL120 Program and SE Reagent. This correlated with the conditions recommended by Lonza as determined using the pmaxGFP® vector and flow cytometry.

The importance of monitoring viability as well as reporter activity was apparent with the CM150/SE sample. This transfection condition gave very high luciferase activity but only 65% viability, indicating that cell health under these conditions was not ideal for further experimentation.

CONCLUSION

Luciferase offers distinct advantages over GFP as a transfection and gene expression reporter. These include its short half-life, high sensitivity of detection, high signal-to-background ratio, and the relatively low expense of a luminometer. Luciferase technology enables transfection scale-down to 96-well plates, making it easier to optimize many conditions and concurrently monitor cell health. As a result, luciferase reporter technology allows scientists to get the most from their transfections and time.

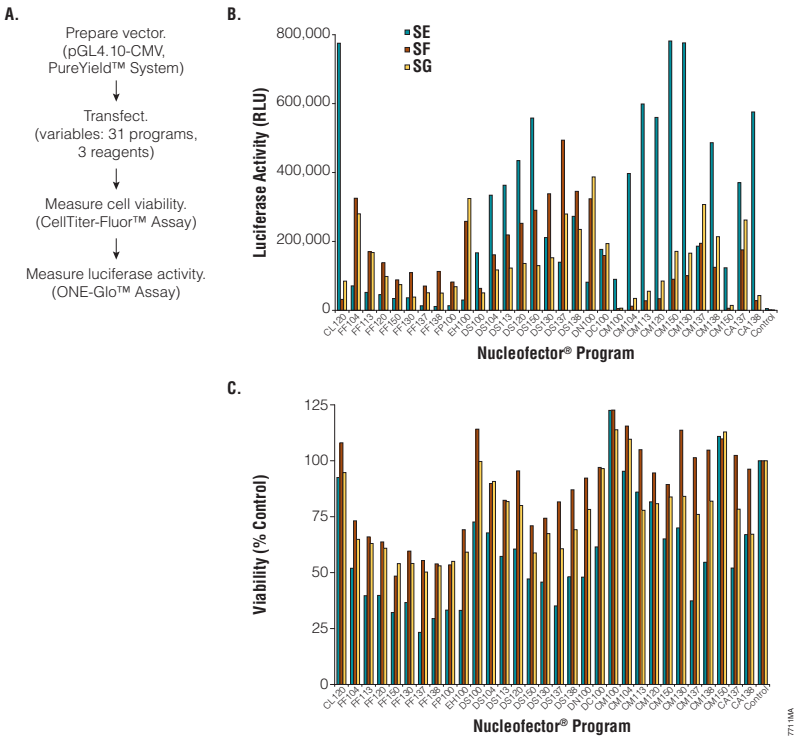

Figure 3. High-throughput testing and multiplexing for rapid transfection optimization. The Cell Line Optimization 96-well Nucleofector® Kit (Lonza) was used to optimize transfection of Jurkat cells using a luciferase reporter (pGL4.10[*luc2*]-CMV Vector) and three Nucleofector® Solutions (SE, SF and SG) in combination with 31 different programs (**Panel A**). Twenty-four hours after nucleofection, reporter activity (**Panel B**) and cell viability (**Panel C**) were analyzed using a multiplex of ONE-Glo™ Luciferase Assay and CellTiter-Fluor™ Assay, respectively, as previously described (6).

REFERENCES

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ORDERING INFORMATION

Product	Size	Cat.#
ONE-Glo™ Luciferase Assay System*†	10 ml	E6110
pGL4.10[*luc2*] Vector	20 µg	E6651
CellTiter-Fluor™ Cell Viability Assay*†	10 ml	G6080
PureYield™ Plasmid Maxiprep System †	10 preps	A2392

*For Laboratory Use. †Additional sizes available.