Reverse Transfection Using FuGENE® 6 and FuGENE® HD

ABSTRACT

In reverse transfection protocols, cells are added directly to a plate containing the transfection reagent:DNA mixture and then assayed on the second day. This approach reduces the experimental time by a day compared to standard transfection protocols. Reverse transfections are rapid, convenient and easily automated, and are frequently used for high-throughput experiments. We tested the ability of FuGENE® 6 and FuGENE® HD to "reverse" transfect three cell lines and monitored efficiency and toxicity using the ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay. We found that FuGENE® 6 and FuGENE® HD performed as well as or better than competitor transfection reagents in a "reverse transfection" experiment and were less toxic to cells than Lipofectamine™ 2000.

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INTRODUCTION

The ability to transfect a cell with DNA is key to many biological assays that study such things as protein function and intracellular events. Maximizing the expression of the encoded protein is only part of the optimization process as toxicity of the transfection reagents also can be a major factor (1) . Transfection efficiency and toxicity are cell line-dependent and require close monitoring to ensure that changes to a reporter gene or cell are not due to the transfection reagents themselves. There are a wide variety of transfection reagents available to help the researcher reproducibly transfect the DNA. The FuGENE® 6 and FuGENE® HD Transfection Reagents are nonliposomal reagents that transfect DNA into a wide variety of cell types with high efficiency and low toxicity. These reagents do not require removing the serum or cell culture medium, and there is no need to wash the cells or change the medium after introducing the reagent:DNA complex.

Monitoring cell viability along with reporter gene expression is critical for interpreting data from transfection experiments. Some reagents are more toxic than others and can kill cells. Cell death reduces the signal output and can potentially cause the data to be misinterpreted. Here we used the ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay, which combines luminescent and fluorescent assay chemistries, to better understand firefly luciferase reporter gene expression in the context of cell viability. The assay uses a two-step, addition-only process to make these measurements in a single well, negating the need to run parallel assays.

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In a standard transfection protocol, the cells are plated on day 1, transfected on day 2 and assayed on day 3 or 4 (Figure 1). In a reverse transfection protocol, cells are added directly to a plate containing the transfection reagent:DNA mix and assayed on day 2 or 3. Because the cells are added directly to the DNA, this process reduces the experimental time by one day and allows for high-throughput transfection of DNA in a plate- or microarray-format.

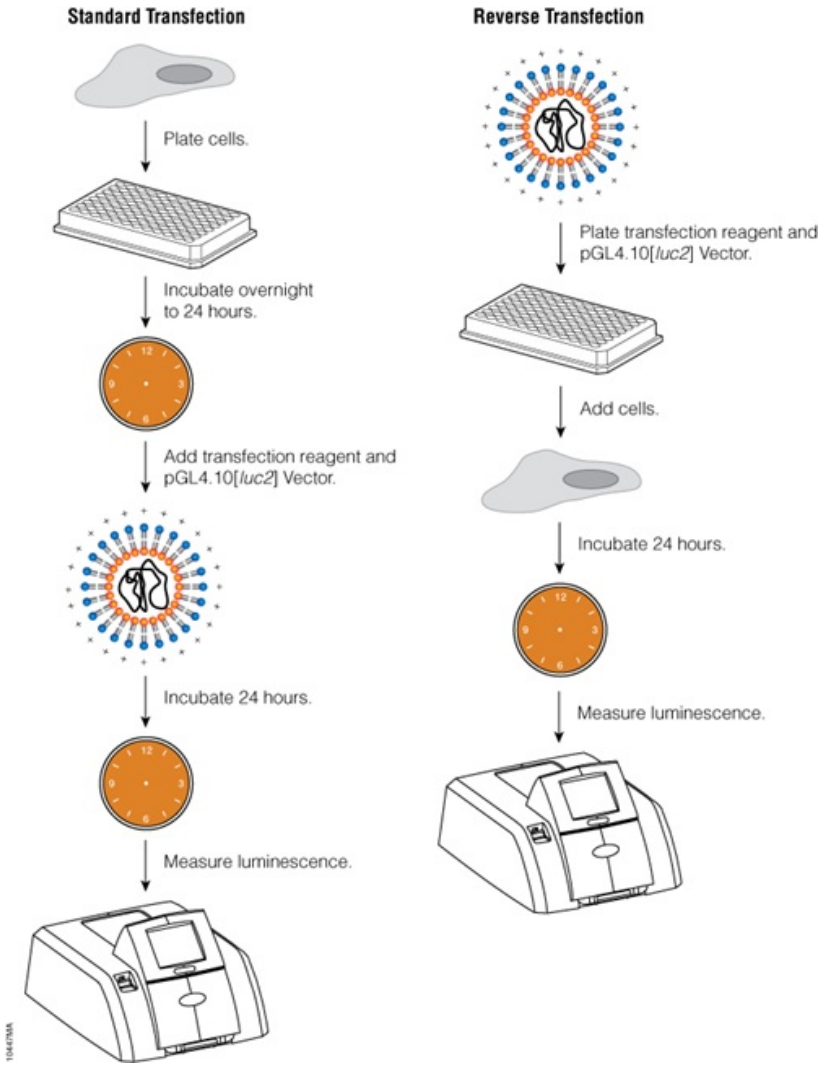

Figure 1. Diagram comparing "Standard" to "Reverse" transfection of cells.

In this report we compare luciferase expression and viability in three cell lines that we transfected using four different transfection reagents, the Promega FuGENE® 6 and FuGENE® HD Transfection Reagents, *Trans*IT®-LT1 transfection reagent (Mirus Bio LLC) and Lipofectamine™ 2000 transfection reagent (Life Technologies). We found that the FuGENE® 6 and FuGENE® HD reagents perform as well as or better than the other transfection reagents in a "reverse transfection" experiment and were less toxic to cells than Lipofectamine™ 2000.

MATERIALS

Template: pGL4.13[*luc2*/SV40] Vector (Cat.# E6681) was used in all transfection experiments. This vector encodes the luciferase reporter gene *luc2* (*Photinus pyralis*) and is designed for high expression and reduced anomalous transcription. The

pGL4 Vectors are engineered with fewer consensus regulatory sequences and a synthetic gene that has been codon optimized for mammalian expression.

Transfection Reagents: We used FuGENE® 6 Transfection Reagent (Cat.# E2691) and FuGENE® HD Transfection Reagent (Cat.# E2311), *Trans*IT®-LT1 transfection reagent (Mirus Bio LLC, Cat.# MIR2304) and Lipofectamine™ 2000 transfection reagent (Life Technologies, Cat.# 11668-027).

Cell Lines: All cell lines were purchased from ATCC: HEK293 (Cat.# CRL-1573), Hep G2 (Cat.# HB-8065) and Jurkat (Cat.# TIB-152).

Detection Reagents: To test both viability and reporter gene (*luc2*) expression, the ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay (Cat.# E7110) was used in conjunction with the GloMax®-Multi+ Detection System with Instinct™ Software (Cat.# E8032).

METHODS

Preparing HEK293 and Hep G2 Cells

1. The cells were treated with trypsin, and then collected and counted.
2. Cells were diluted to 2 x 10^5 cells/ml in DMEM + 10% FBS.

Preparing Jurkat Cells

1. Cells were collected and counted.
2. Cells were diluted to 1 x 10^6 cells/ml in DMEM + 10% FBS.

Standard Transfection Protocol

1. The cells were plated in a volume of 100µl per well onto a 96-well white plate (Costar) and incubated for 24 hours at 37°C with 5% CO_2.
2. The DNA and transfection reagents were prepared according to manufacturers' protocols. The reagent:DNA incubation was done according to the reagent manufacturers' protocols. All dilutions and complex formations used Opti-MEM® Reduced Serum Medium. For each transfection reagent we used two concentrations of reagent:DNA as follows: FuGENE® 6, 4:1 and 3:1; FuGENE® HD, 3:1 and 2.5:1; *Trans*IT®-LT1, 3:1 and 2:1; and Lipofectamine™ 2000, 3:1 and 2:1. For each combination we included two controls: a no-DNA control and a no-transfection reagent control.
3. The appropriate amount of each reagent:DNA complex was added to the wells. For FuGENE® 6, FuGENE® HD and *Trans*IT®-LT1, 2µl, 5µl and 10µl was added. For Lipofectamine™ 2000, 5µl, 25µl and 50µl was added. All conditions were performed in replicates of 4.
4. After the reagent:DNA complex was added to the cells, the plates were mixed on a plate shaker for 1 minute.
5. The plates were incubated at 37°C for 24 hours with 5% CO_2.

Reverse Transfection Protocol

1. DNA and transfection reagents were prepared according to the manufacturers' protocols. The reagent:DNA incubation was done according to the reagent manufacturers' protocols. All dilutions and complex formations used Opti-MEM® Reduced Serum Medium. For each transfection reagent we used two concentrations of reagent:DNA as follows: FuGENE® 6, 4:1 and 3:1; FuGENE® HD, 3:1 and 2.5:1; *Trans*IT®-LT1, 3:1 and 2:1; and Lipofectamine™ 2000, 3:1 and 2:1. For each combination we included two controls: a no DNA control and a no-transfection reagent control
2. The appropriate amount of each reagent:DNA complex was added to the wells. For FuGENE® 6, FuGENE® HD and TransIT®-LT1, aliquots of 2µl, 5µl or 10µl was added. For Lipofectamine™ 2000, aliquots of 5µl, 25µl or 50µl was added. All conditions were performed in replicates of 4.
3. After the reagent:DNA complex was added to the wells, 100µl of the prepared cells was added, and the plates were mixed on a plate shaker for 1 minute.
4. The plates were incubated at 37°C for 24 hours with 5% CO_2.

Detection Protocol

1. The CellTiter-Fluor™ Reagent was prepared as a 5X reagent, and the ONE-Glo™ Reagent was prepared as described in TM356.

2. To each well, 20µl of CellTiter-Fluor™ Reagent was added, and the plate was mixed on a plate shaker for 1 minute followed by a 2-hour incubation at 37°C with 5% CO_2.
3. Fluorescence was detected using a GloMax®-Multi+ Instrument with Instinct™ Software, AFC filter and the CellTiter-Fluor™ protocol.
4. Following fluorescence detection, the plate was equilibrated to room temperature, and 100µl of the ONE-Glo™ Reagent was added to each well, mixed on a plate shaker for 1 minute, and incubated for 3 minutes at room temperature.
5. Luminescence was detected using a GloMax®-Multi+ Instrument with Instinct™ Software and the ONE-Glo™ protocol.

RESULTS

Transfection Efficiency of Reverse Transfections

To test the transfection efficiency of the reverse transfection approach, a luciferase-encoding plasmid was "reverse" transfected into HEK293, Hep G2 and Jurkat cells using FuGENE® 6, FuGENE® HD, *Trans*IT®-LT1 and Lipofectamine™ 2000 transfection reagents. Luciferase activity was measured after 24 hours (Figures 2–4). Two ratios of transfection reagent:DNA concentration and three volumes of transfection reagent:DNA mix were selected based on the optimal ranges dictated by standard transfection protocols for each reagent. Using HEK293 cells, FuGENE® HD reagent produced the most luminescence, indicating the largest production of luciferase (Figure 2). The optimal transfection reagent:DNA ratio for FuGENE® HD appears to be 3:1 with an ideal volume of 10µl. FuGENE® 6 also produced a large amount of luminescence and was greater than that produced by TransIT®-LT1. With all reagents, the no-DNA controls did not produce detectable luminescence signal. Hep G2 cells produced similar results to the HEK293 cells; however, the difference between FuGENE® HD and the competitor products was much greater (Figure 3). The overall efficiency of transfection for all reagents appears to be lower with Hep G2 cells than HEK293 cells, but there is still a significant amount of signal produced with FuGENE® 6 and FuGENE® HD. The results were different with Jurkat cells; FuGENE® 6 produced the greatest amount of luminescence signal (Figure 4). The optimal transfection reagent:DNA ratio for FuGENE® 6 appears to be 4:1 with an an ideal volume of 10µl. Both FuGENE® 6 and FuGENE® HD were able to transfect a luciferase-containing plasmid using a reverse transfection protocol resulting in high luminescence signal.

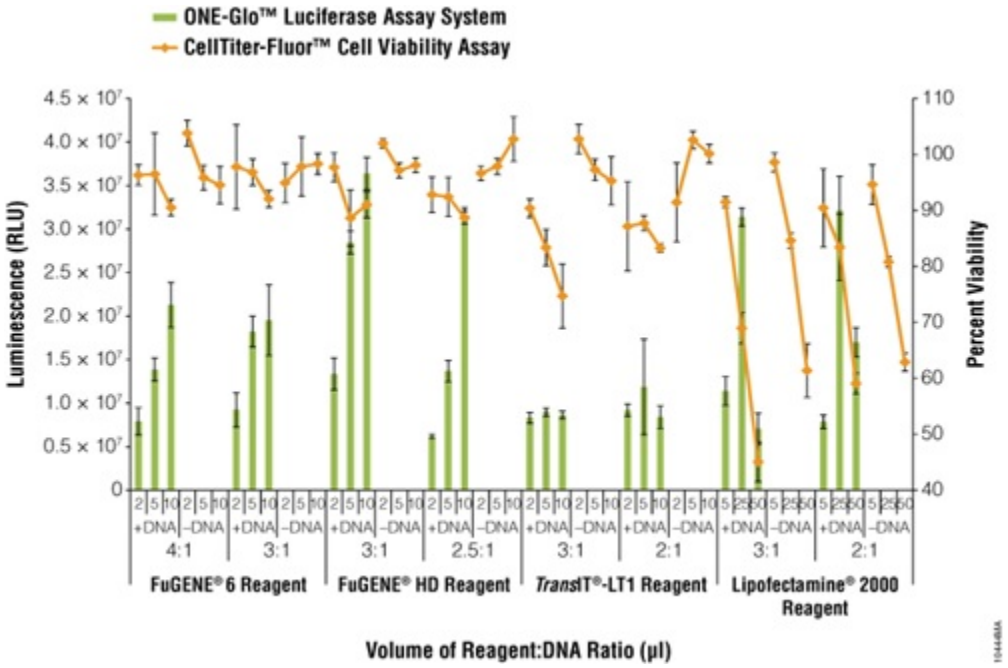

Figure 2. Reverse transfection of HEK293 cells with pGL4.13[*luc2*/SV40] DNA. Four different transfection reagents were used at two different transfection reagent:DNA ratios as indicated below the chart. Three different volumes were used in the experiment also indicated below the chart (X axis). "Luc" represents transfection of pGL4.13[*luc2*/SV40] DNA, and control represents transfection reagent without DNA. Bars indicate relative light units (RLU) as determined by ONE-Glo™ Luciferase Assay System (left axis). Lines indicate % viability (from control with no transfection reagent) as determined by CellTiter-Fluor™ Cell Viability Sssay (right axis). Error bars correspond to replicates of four data points.

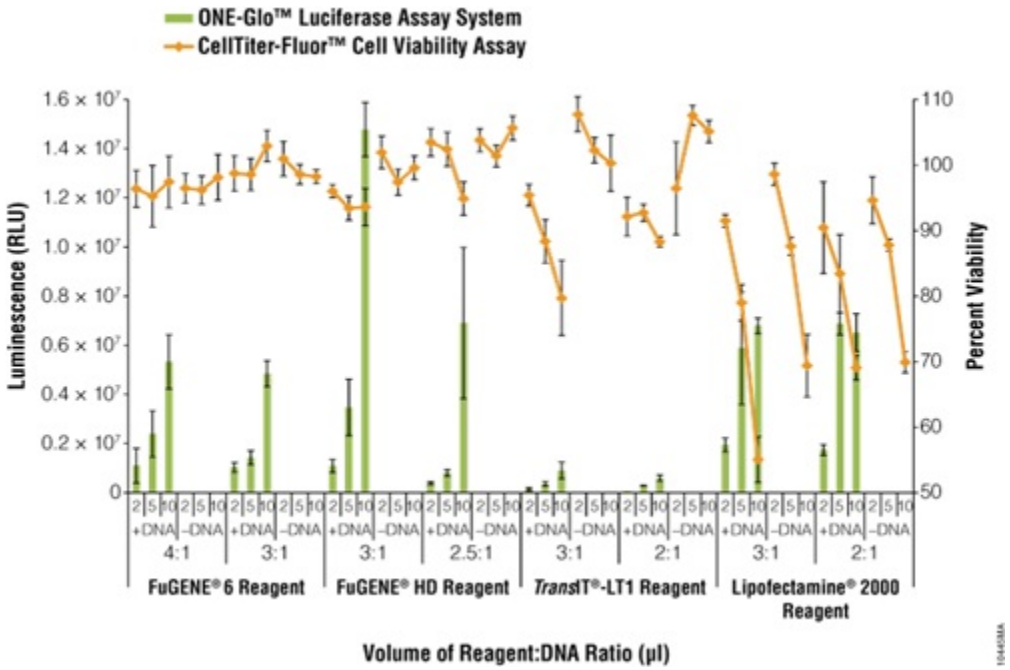

Figure 3. Reverse transfection of Hep G2 cells with pGL4.13[*luc2*/SV40] DNA. Four different transfection reagents were used at two different transfection reagent: DNA ratios as indicated below the chart. Three different volumes were used in the experiment also indicated below the chart (X axis). "Luc" represents transfection of pGL4.13[*luc2*/SV40] DNA, and control represents transfection reagent without DNA. Bars indicate relative light units (RLU) as determined by ONE-Glo™ Luciferase Assay System (left axis). Lines indicate % viability (from control with no transfection reagent) as determined by CellTiter-Fluor™ Cell Viability Assay (right axis). Error bars correspond to replicates of four data points.

Transfection Reagent Toxicity

To test toxicity of FuGENE® 6, FuGENE® HD, *Trans*IT®-LT1 and Lipofectamine™ 2000 with HEK293, Hep G2 and Jurkat cells, we multiplexed our luminescent assay with a live cell viability assay. The CellTiter-Fluor™ Reagent measures viability of cells by detecting the production of a fluorophore through live-cell protease activity (2) . Multiplexing these two assays allows for luminescent detection and live-cell detection in the same well. FuGENE® 6 and FuGENE® HD Transfection Reagents resulted in the lowest decrease in viability for all three cell types tested with greater than 90% viability (Figure 2–4). Lipofectamine™ 2000 transfection reagent was the most toxic, and produced as high as a 55% drop in viability in HEK293 cells (Figure 2). Jurkat cells appear to be less sensitive to toxicity than HEK293 and Hep G2 cells, as only slight decreases in viability were seen with all transfection reagents in these cells (Figure 4).

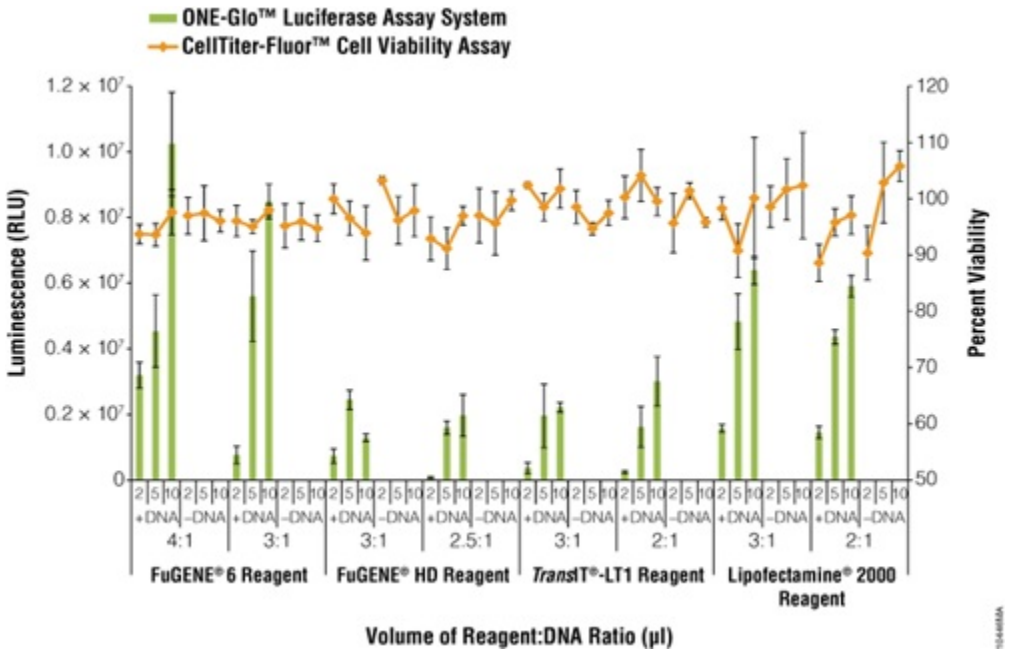

Figure 4. Reverse transfection of Jurkat cells with pGL4.13[*luc2*/SV40] DNA. Four different transfection reagents were used at two different transfection reagent:DNA ratios as indicated below the chart. Three different volumes were used in the experiment also indicated below the chart (X axis). "Luc" represents transfection of pGL4.13[*luc2*/SV40] DNA and control represents transfection reagent without DNA. Bars indicate relative light units (RLU) as determined by ONE-Glo™ Luciferase Assay System (left axis). Lines indicate % viability (from control with no transfection reagent) as determined by CellTiter-Fluor™ Cell Viability Assay (right axis). Error bars correspond to replicates of four data points.

Cell Line Variation

One important factor to consider when choosing a transfection reagent is that transfection efficiency and toxicity can vary among different cell lines. In this experiment, transfection with FuGENE® HD resulted in the greatest luminescent signal in both the HEK293 and Hep G2 cell lines; however, FuGENE® 6 had the best results in the Jurkat cell line (Figures 2–4). The FuGENE® 6 and FuGENE® HD transfections resulted in low cell toxicity in all three cell lines tested (Figures 2–4). Lipofectamine™ 2000 was the most toxic reagent, with the highest cell death in the HEK293 and Hep G2 cell lines (>55% loss in viability); however, a much lower toxicity was observed in the Jurkat cell line (~10 loss in viability) (Figures 2–4).

Standard versus Reverse Transfection

To compare "standard" versus "reverse" transfection methods, we performed parallel experiments using HEK293 cells (Figure 5). On day 1, cells were trypsinized, counted and plated onto a 96-well plate; then the cells were placed at 37°C for ~24 hours

(standard protocol). Also on day 1 the same trypsinized and counted cells were added to a 96-well plate with transfection reagent:DNA mixture already on the plate (reverse protocol). This plate was placed at 37°C overnight. On day 2 of the standard protocol, the transfection reagent:DNA mixture was added to the plated HEK293 cells and placed at 37°C for 24 hours. On day 2 of the reverse transfection protocol, the plate was assayed for luciferase activity and cell viability using the ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay. The standard transfection protocol plate was measured on day 3 using the same assay. The overall efficiency of the reverse transfection protocol was about 30% of that of the standard transfection protocol for all the transfection reagents tested. For the luciferase assay, there was still a significant amount of signal from the reverse transfected cells. The reverse transfected cells also appeared to be more sensitive to toxicity, especially in the case of Lipofectamine™, where the viability dropped from 65% for the standard transfection protocol to 45% for the reverse transfection protocol.

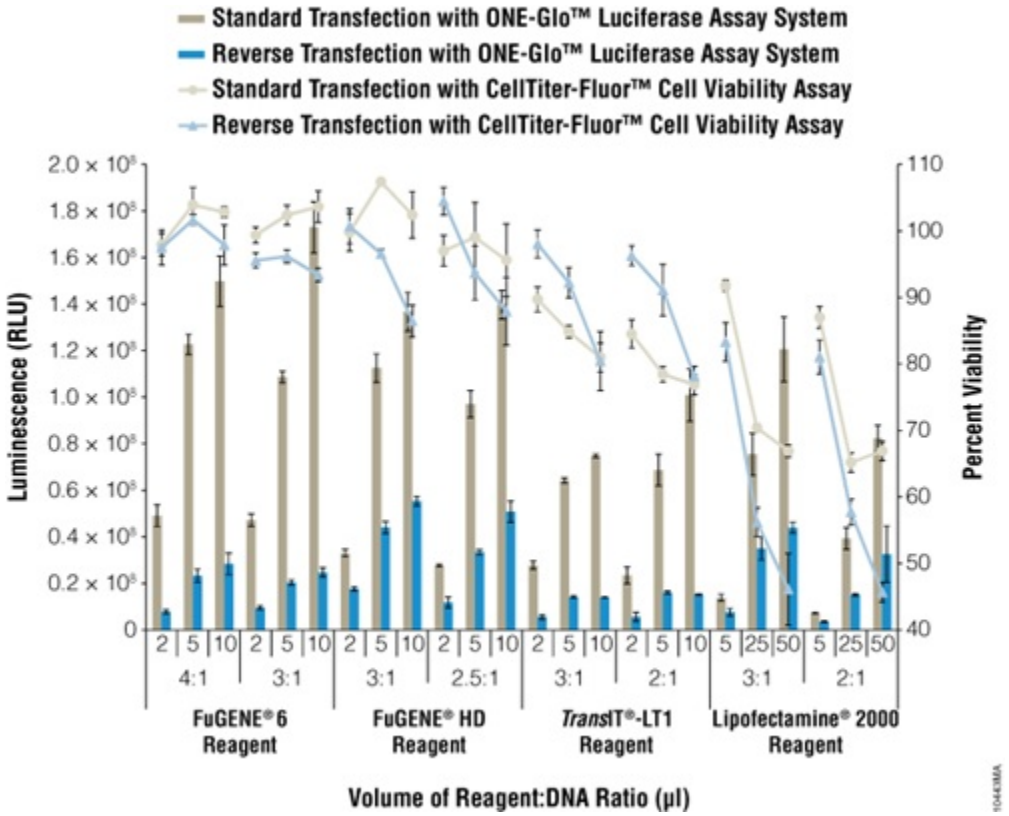

Figure 5. Comparison of standard to reverse transfection in HEK293 cells with pGL4.13[*luc2*/SV40] DNA. Four different transfection reagents were used at two different transfection reagent:DNA ratios as indicated below the chart. Three different volumes were used in the experiment also indicated below the chart (X axis). Bars indicate relative light units (RLU) as determined by ONE-Glo™ Luciferase Assay System (left axis). Lines indicate % viability (from control with no transfection reagent) as determined by CellTiter-Fluor™ Cell Viability Assay (right axis). Error bars correspond to replicates of four data points.

CONCLUSIONS

We compared luciferase expression, cell viability and transfection efficiency in three cell lines that we transfected using four different transfection reagents, the Promega FuGENE® 6 and FuGENE® HD Transfection Reagents, *Trans*IT®-LT1 transfection reagent and Lipofectamine™ 2000 transfection reagent using both standard and reverse transfection protocols. We found that transfection efficiency with the different reagents was cell line-dependent. Cell toxicity for the different transfection reagents also varied, with FuGENE® 6 and FuGENE® HD being less toxic to the cells than Lipofectamine™

2000. However, the reverse transfected cells were more sensitive overall to the toxicity of the transfection reagents than the cells transfected using the standard protocol. For the reverse transfection protocol, FuGENE® 6 and FuGENE® HD perform as well as or better than competitor transfection reagents in terms of both transfection efficiency and cell viability. Finally, although reverse transfection saves time (around 1 day), there is also a significant drop in transfection efficiency that must be considered before choosing this approach.

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