A Highly Efficient Method of Transfecting Hep G2 Cells

SUMMARY

Genlantis has developed an improved lipid-based transfection reagent, GenePORTER® 3000 (GP3K), which exploits a novel, proprietary, delivery technology called Advanced Conjugation Enhancement (ACE). This technology provides a significant increase in delivery efficiency of the difficult-to-transfect cell line Hep G2. Here we demonstrate that using GP3K in a rapid, same-day transfection protocol with Hep G2 cells will result in a 3-fold improvement in transfection efficiency over the most popular commercially available transfection reagent, Lipofectamine® 2000 (LF2K) (Life Technologies). This result shows that simple, lipid-mediated transfection of Hep G2 cells is a viable alternative to gene delivery methods such as electroporation and viral infection.

INTRODUCTION

Hep G2 is a Human hepatocellular liver carcinoma cell line (1). It has been the subject of numerous studies focusing on hepatic metabolism, tumor progression, endocrinology, apoptosis, protein secretion, lipid biochemistry, and many other areas. The transfer of exogenous DNA into Hep G2 is a central part of these experiments.

Lipid-mediated transfection is the simplest and most versatile method to introduce exogenous DNA into mammalian cells. However, Hep G2 an exceptionally difficult cell line to transfect. As a result, researchers often turn to electroporation or viral infection as more efficient gene delivery methods. Such alternatives typically provide higher efficiency than transfection but involve limitations that make them less than ideal. For example, electroporation requires expensive instrumentation. The process of electroporation itself results in undesirably high cell mortality rates - and cells that do survive are likely to have undergone physiological changes in response to the process. On the other hand, viral infection frequently imposes inconvenient size limitations on transgenic nucleic acids. Viral transfer requires more stringent containment facilities, and carries health risks that other methods do not. Thus, neither electroporation nor viral infection is particularly well suited for high throughput applications.

Improving the efficacy of lipid-based transfection into Hep G2 cells would alleviate the limitations of electroporation and viral infection. We will show that the GP3K reagent achieves this purpose.

MATERIALS AND METHODS

In one experiment, healthy, low passage number, log phase Hep G2 cells were detached using Detachin™ Cell Detachment Solution (Genlantis Cat. No. T100100) and replated at high density (300,000 cells/500µl/well in complete medium) in a 24-well plate. The cells were incubated at 37ºC with 5% CO_2 for 30 minutes, sufficient to allow the cells to settle and adhere. Meanwhile, duplicate

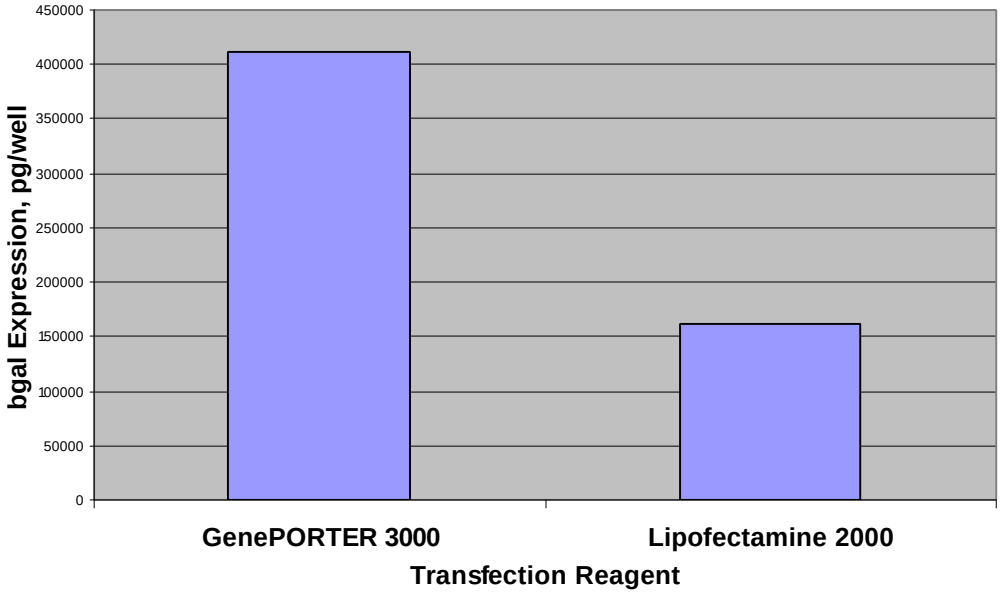
transfection reactions were prepared using 1µg/well gWiz ß-galactosidase plasmid DNA (Genlantis Cat. No. P010200), and either GP3K or LF2K, according to the manufacturers' recommended protocols. Just prior to adding the transfection complexes, the complete medium was removed from the cells and replaced with an equal volume of serum-free medium. The transfection reactions (lipoplexes) were then added directly to the cells and incubated at 37ºC with 5% CO_2. After 4 hours, an equal transfection volume of complete medium containing 20% serum was added, and the cells were allowed to continue incubation.

Following the identical transfection protocol in a second experiment, freshly detached Hep G2 cells were plated directly on glass coverslips in 12-well tissue culture plates at 300,000 cells/well. The reporter plasmid, gWiz GFP (Genlantis Cat No. P040400), was transfected following the GP3K protocol. Cellular expression of GFP was determined with a fluorescent microscope using a FITC laser (Ex 480/30, DM 505, BA 535/40).

RESULTS AND DISCUSSION

The transfected cells from the first experiment were lysed and assayed for β-galactosidase activity approximately 48 hours post-transfection (ONPG Assay Kit. Genlantis Cat. No. A10200K). The results show that the β-galactosidase activity for cells transfected with GP3K was approximately three times higher than that of cells transfected with LF2K (**Figure 1**).

Figure 1: Hep G2 ONPG Assay Results



For the second experiment, photographs of the gWiz-GFP-transfected Hep G2 cells were taken at both a 20X magnification, to see overall transfection efficiency, and at 60X magnification, to determine post-transfection cellular health and morphology. The results (Figures 2 & 3) indicate excellent transfection efficiency, robust GFP expression, and healthy cell morphology.

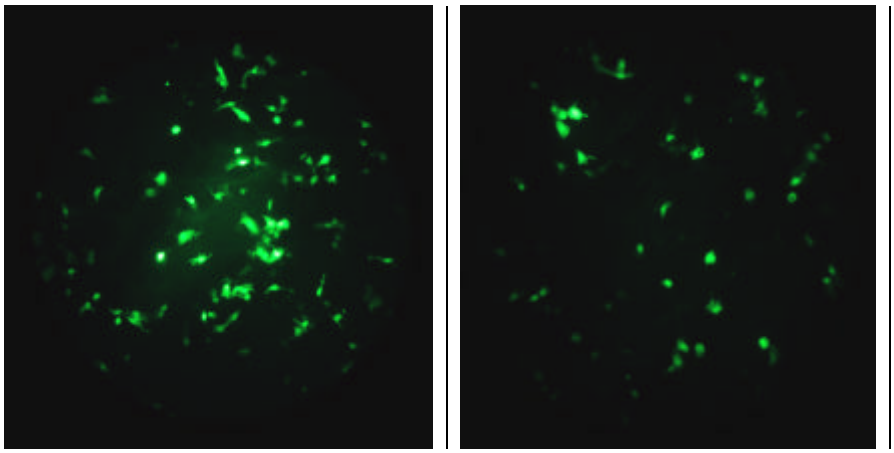


Figure 2: Whole-field 20X view of Hep G2 cells transfected with gWiz GFP plasmid DNA and **A).** GenePORTER 3000 and **B)** Lipofectamine 2000

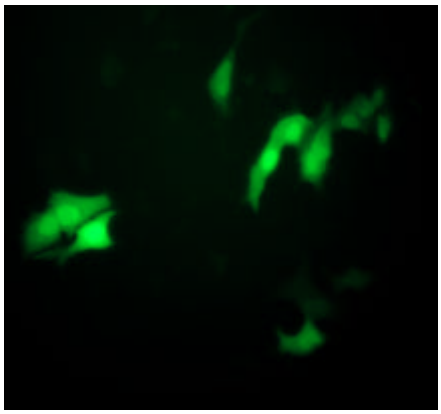


Figure 3: Whole-field 60X view of Hep G2 cells transfected with gWiz GFP plasmid DNA and GenePORTER 3000

The intense GFP expression, combined with the 3X increase in ß-galactosidase activity clearly demonstrates improved transfection mediated by the GP3K reagent.

Exploiting a new transfection technology – Advanced Conjugation Enhancement (ACE) - we have demonstrated that the efficiency of lipid-mediated transfection into Hep G2 can be significantly increased when using GP3K as opposed to the most commonly used cationic lipid, LF2K. The improved efficiency combined with very low cytotoxicity makes GP3K an ideal alternative for a wide range of applications requiring gene delivery into Hep G2 cells.

REFERENCES

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