

A Highly Efficient Method of Transfecting NIH-3T3 Cells

GenePORTER® 3000 Transfection Reagent

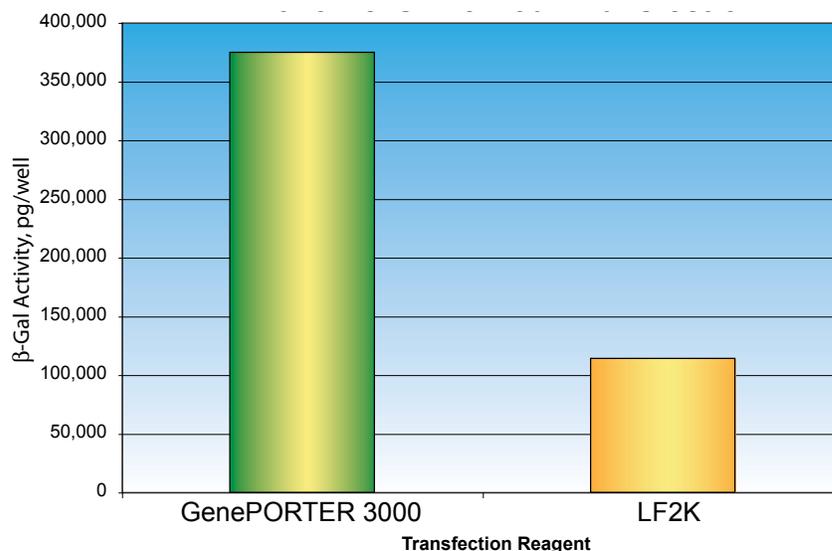
Genlantis has developed a new, highly efficient, lipid-based transfection reagent, GenePORTER® 3000 using a novel, proprietary technology called advanced conjugation enhancement (ACE). This new reagent is able to deliver plasmid DNA into the fibroblast cell line, NIH-3T3, with three fold greater efficiency than the popular commercial transfection reagent, Lipofectamine 2000 (LF2K, Life Technologies). Importantly, this improved efficiency does not come at the expense of cell viability since cells transfected using GenePORTER 3000 reagent are significantly healthier and exhibit higher viability than cells transfected using LF2K. Moreover, the increased transfection efficiency and lower cytotoxicity afforded by GenePORTER 3000 reagent largely eliminates the need to use alternate, cumbersome gene delivery methods such as electroporation or viral infection.

Introduction

The mouse fibroblast cell line, NIH-3T3, is used to study many aspects of cell biology. Established from a NIH Swiss mouse embryo, these cells are highly contact inhibited, and are sensitive to sarcoma virus focus formation and leukemia virus propagation. Introduction of exogenous nucleic acids into NIH-3T3 cells and evaluation the subsequent physiological effect are critical elements of many studies. High delivery efficiency with the least disruption to cell physiology is the goal in transfection experiments. NIH-3T3 cells are moderately difficult to

transfect with lipids and polymers. The highest efficiency nucleic acid delivery into NIH-3T3 is usually obtained using electroporation or viral infection. However, these methods are often less desirable since they have limitations that transfection does not. Electroporation requires expensive instrumentation and results in very high cell mortality. Viral infection often carries safety issues as viral vectors are potentially infectious to humans. Furthermore, the cargo DNA in viral infections must conform to the size constraints of the viral system, making it more cumbersome and less versatile than transfection. Neither electroporation nor viral delivery is readily adaptable for many high throughput projects. Therefore, improvement of lipid-mediated transfection of NIH-3T3 cells would be highly advantageous.

Figure 1. NIH 3T3 cells 48 hours after transfection



Same day transfection of freshly plated NIH-3T3 cells with 1ug of gWiz™ B-Gal and GenePORTER 3000 versus LF2K in serum-free medium for 4 hours followed by addition of equal volume complete medium + 20% FBS in a 24-well plate. B-galactosidase expression was measured 48 hours post-transfection.

Methods and Materials

In one experiment, healthy, low passage number, log phase NIH-3T3 cells were detached from culture dishes using the Detachin™ Cell Detachment Solution (Genlantis Cat. No. T100100) and plated at high density (300,000 cells /500ml/well in serum-free medium) in a 24-well plate. The cells were incubated at 37°C with 5% CO₂ for 30 minutes, a sufficient time to allow the cells to settle and adhere to the plate surface. Meanwhile, duplicate transfection reactions were prepared using 1ug/well gWiz β-Galactosidase plasmid (Genlantis Cat. No. P010200) and either GP3K or LF2K according to the manufacturers' recommended protocols.

Just prior to adding the DNA/transfection reagent complexes (lipoplexes) to the cells, the complete medium was removed and replaced

with an equal volume of serum-free medium. The lipoplexes were added directly to the cells and incubated at 37°C with 5% CO₂. After 4 hours, an equal transfection volume of complete medium containing 20% serum was added.

Approximately 48 hours post-transfection, the cells were lysed and assayed for β-galactosidase activity (ONPG Assay Kit, Genlantis Cat. No. A10200K).

In an identical experiment, freshly detached NIH-3T3 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 GenePORTER 3000 protocol. The cells were screened for GFP expression with a fluorescent microscope using a FITC laser (Ex 480/30, DM 505, BA 535/40).

Results

The results of the first experiment with a β-galactosidase-encoding vector show that reporter gene activity for cells transfected with GenePORTER 3000 reagent was at least three fold higher than that of cells transfected with LF2K (Figure 1).

The results of the second experiment with a GFP-encoding vector indicate

excellent transfection efficiency and robust transgene expression, as well as healthy cell morphology (Figures 2 and 3).

The 3X increase in β-galactosidase activity, together with the high level GFP expression, clearly demonstrates improved transfection mediated by the GenePORTER 3000 reagent.

Conclusions

The choice of nucleic acid delivery method for any cell line must factor in the efficacy of the delivery agent and the collateral effect that the method has on the host cell. Generally, the most effective delivery methods have been the most cytotoxic.

With the development of Advanced Conjugation Enhancement (ACE) technology, Genlantis has created a uniquely efficient transfection reagent, GP3K. As shown above, GP3K will deliver DNA to the moderately difficult to transfect NIH-3T3 cell line with a much higher efficiency than LF2K, while maintaining very low cytotoxicity. This makes GP3K an attractive alternative to LF2K, viral infection and electroporation.

References

1. Jainchill, J.L., Aaronson, S.A., and Todaro, G.J. (1969). *J. Virology* 4: 549-553.

Figure 2. 20 X view of NIH 3T3 Cells transfected with GenePORTER 3000 reagent.

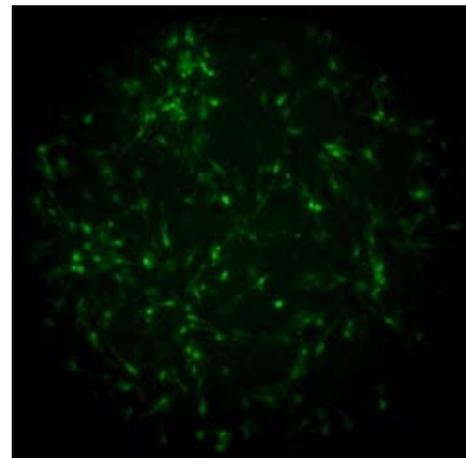


Figure 2: Whole-field 20X view of NIH 3T3 cells transfected with gWiz GFP plasmid DNA and GenePORTER 3000

Figure 3. 60 X view of NIH 3T3 Cells transfected with GenePORTER 3000 reagent.

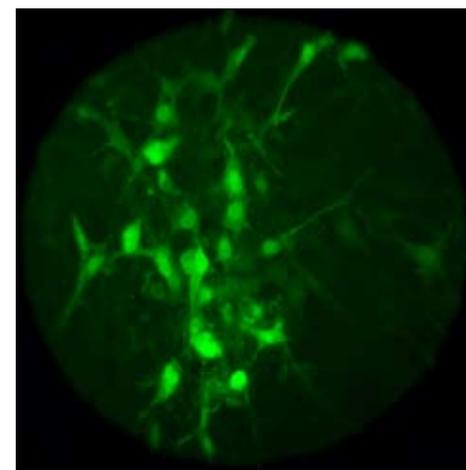


Figure 3: 60X magnification view of GFP expression in NIH 3T3 cells transfected with gWIZ GFP and GenePORTER 3000

GenePORTER® 3000 Transfection Reagent Kits		
0.75 ml kit	75 reactions	T203007
1.5 ml kit	150 reactions	T203015
15 ml kit	1,500 reactions	T203115
Bulk applications	Custom	Inquire