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Comparison and optimization of transfection methods for human lymphoblast TK6 cell line

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Method Article

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Abstract

Transfection has recently gained attention in the field of biomedical research due to its ability to manipulate gene expression. Every mammalian cell type has a characteristic set of requirements for optimal transfection. Some cells can be difficult to transfect and requires optimization for successful transfection. Human lymphoblast TK6 cell line, an important cell line for genotoxic studies, are known to be extremely hard to transfect. Transfection methods for human lymphoblast TK6 is increasingly important. To accomplish this, TK6 human lymphoblasts were transfected with plasmid constructs that expressed Green fluorescent Protein (GFP) and NanoLuc® activity. We compared the transfection efficiencies of three commercially available transfection reagents including Amaxa 96-well Nucleofection procedure using various solutions (SF, SE and SG), Lipofectamine LTX, and Metafectene Pro®. The transfection efficiencies, and toxicity of various reagents were tested by fluorescence microscopy, luciferase activity, and cell viability assays. Amaxa 96-well Nucleofection Solution SF was identified as the best transfection reagent due to its relatively high luciferase activity, acceptable cell viability (80%), and GFP transfection efficiency (80%). Efficient conditions for transfection utilized with this reagent included 0.4μg of plasmid DNA, 1.8×10^5 cells, and the DS 137 nucleofector program.

Introduction

Transfection is an important analytical tool to study the function of genes and proteins in a cellular environment.[1,2] Successful transfection is influenced by the quality of the nucleic acid, duration of transfection, transfection reagent and cell lines.[3,4] Many cell lines can be easily transfected. However, others such as some human primary cells are traditionally proven to be very difficult to transfect .[5–7] Cells considered to be difficult or hard-to-transfect include, inter alia, stem cells, primary cells, mast cells, natural killer cells, macrophages and TK6 human lymphoblasts .[8–11]

Successful transfecting TK6 human lymphoblasts is a crucial step for studying cell biology at the molecular level through gene expression. Various methods have been developed for introducing exogenous constructs into cells with each differ by their properties and having advantages and limitations.[12] Viral methods use modified viruses to deliver nucleic acid while non-viral method uses nanocarriers for delivery. Viral vectors are associated with high gene transfer efficiencies, however, are labor intensive and required certain biosafety measures. [13,14]Non-viral transfection methods are relatively safer but have several drawbacks which include inefficiency and toxicity.[15]

Nonviral transfection can be explored using physical and chemical approaches. Physical transfection approach uses a wide range of physical tools (e.g., needle injection, electroporation, gene gun, ultrasound, and laser-based transfection) to deliver nucleic into cells.[16] In a chemical approach naturally and synthetic chemicals, such as DEAE-dextran, cationic lipids, and cationic polymers are used to facilitate the delivery of nucleic acid the cell membrane.[17,18]

METAFECTENE® PRO is a poly cationic transfection reagent based on liposome technology.[19] Metafectene® Pro ensures easy entry of plasmid DNA into cells by condensing DNA into compact structures.[20,21] METAFECTENE® PRO exhibits high transfection efficiency and low toxicity in multiple cell lines and/or primary cells including, HEK 293T cells, THP-1 (RCB1189), BV2 cells, primary T-cells and Jurkat; HEK293 cells.[20, 22–26]

Lipofectamine™ LTX with PLUS™ reagent is an origin-free liposomal transfection reagent. Lipofectamine is effective, easy to use and relatively less expensive compared to the other methods and generally achieve relatively high transfection efficiency.[27,28] The popularity of the use of a liposome-based transfection, Lipofectamine™ LTX with PLUS™ is based on the number of scientists using this technique for a variety of cell lines such as Human Mesenchymal Stem Cells, jurkat cells, HEK293T and MCF-7 cells.[29–33 29-33]

The Amaxa Nucleofector™ shuttle system is an electroporation technique that utilizes a combination of electrical parameters, generated by a device called Nucleofector with cell-type specific reagents.[34] Nucleofection are famously known to overcome the lower transfection efficiency by chemical methods. Nucleofection system is a significant advance over standard electroporation systems for its high transfection efficiency (optimized nucleofection parameters yielded survival rates above 60%) in a multitude of cell lines such as, primary neurons, dendritic cells, T cells, leukemia cells, peripheral blood mononuclear cells, ovarian cancer cell lines, human myeloma cell lines (HMCLs) and Eimeria .[35–44]

Optimal transfection conditions are those that yield maximal reporter gene expression with minimal detrimental impact on cell viability. No single delivery method or transfection reagent can be applied to all types of cells; cellular cytotoxicity and transfection efficiencies vary dramatically depending on the reagent, protocol, and cell type being utilized.[45] Our cell line for this study is the TK6 human lymphoblasts cell line, a traditionally-difficult-to-transfect cell type like Jurkat cells (another hard-to-transfect cell type) due to mainly due to their fragility and slow-dividing rates. Transfection of TK6 human lymphoblasts is an essential tool for scientific and therapeutical applications. Herein, we examine the transfection efficiency of three commercially available transfection reagents, METAFECTENE® PRO, Lipofectamine™ LTX and Amaxa Nucleofector Shuttle System using different buffers. These transfections reagents were selected due to their high transfection efficiency and minimal cell toxicity. [26–35] Results from this study will lead to the development of optimized protocols for transfection efficiency of hard-to-transfect-cell-line such as TK6 human lymphoblasts.

Materials And Methods

Chemicals and reagents

The Amaxa nucleofection solution SF was obtained from Lonza (Allendale, New Jersey). The Lipofectamine LTX and Metafectene Pro were purchased from Life Technologies and Biontex Inc.,

respectively.

Plasmid DNAs

The pGFPmax was obtained from Lonza (Allendale, New Jersey). The pGFPmax contains the lac promoter and drives the expression of an enhanced green fluorescent protein. pNL1.2[Nluc] and pNL1.1.CMV were purchased from Promega™ Corporation (Madison, wisconsin). The pNL1.1.CMV contains the CMV promoter and expresses nanoluciferase and pNL1.2[Nluc] is a luminescent reporter.

Cell culture

Human p53-proficient B-lymphoblastic TK6 cells were generously provided by Dr. Howard Liber, Colorado State University. Cells were passaged at 2.0 $x10^5$ cells/ml in RPMI 1640 supplemented with 2mM L-glutamine and 10% fetal bovine serum (FBS, Life Technologies, Inc.). Cells were incubated at 37°C with 5% CO_2, and the media was changed every 36h. Cells were passaged into fresh media 12–14hr prior to each experiment.

Metafectene transfection

Optimization was done by following the optimized protocols for the Jurkat cell line. The TK6 cells were seeded in a 6-well plate at a density of 1 x 10^5 cells/ml. Thereafter, the cells were transfected with 0.5 and 1.0µg pGFPmax using Metafectene Pro (Biontex, Germany) at ratio from 1:2 to 1:6; control cells were not transfected with DNA. Transfection was accomplished by adding the DNA and Metafectene to solutions A and B respectively containing OptiMEM. The two solutions A (DNA + OptiMEM + Glutamax) and B (Metafectene Pro + OptiMEM + Glutamax) were mixed and incubated at room temperature for 20 min. After incubation, the DNA-lipid complexes were added dropwise to the cells and swirled with extreme care to avoid breaking up the complexes. The samples were kept in a CO_2 incubator at 37°C. GFP and cell viability were assessed at 24 and 36h post transfection.

In a parallel experiment, the cells were transfected with pNl1.1CMV expressing nano luciferase, and control cells were transfected with the empty pNL1.2 vector. Cells transfected with no DNA served as an additional control. The ratio of DNA (in µg) to lipid-mediated reagent varied from 1:2 to 1:6. Solutions A (DNA + OptiMEM + Glutamax) and B (Metafectene Pro + OptiMEM + Glutamax) were mixed and incubated at room temperature for 20 min. The DNA-lipid complexes were then added dropwise to the cells and swirled with extreme care. The samples were kept in a CO_2 incubator at 37°C. Nano luciferase Assay (Promega) was performed at 24 and 36h post-transfection by following the manufacturer’s protocol. Transfection efficiency using normalized luciferase activity and GFP as well as cell viability was determined at 24 and 36h post transfection. The experiment was conducted in duplicates and repeated twice.

Lipofectamine transfection

Transfection was performed according to manufacturer’s protocol by following the optimized protocol for Jurkat cells. TK6 cells were seeded in 6-well plates at a density of 1.0 x 10^5 and 1.0 x 10^6 cells/well.

Different concentrations of pGFPmax/ empty pNL1.2 (0.5-1.0µg) was diluted into 100µL optiMEM media, PLUS reagent (1.5–2.5µl) and lipofectamine (3.75-10.0µl). After 30 minutes of incubation at room temperature, 100.0 µl of the DNA/PLUS/Lipofectamine LTX complexes were added to the cells in complete growth media in the 6-well plate and incubated at 37°C in the CO_2/95% air incubator. Control cells received no DNA. Cells transfected with empty pNL1.2 vector served as an additional control. GFP transfection efficiency, normalized nano luciferase activity and cell viability were determined at 24 and 36h post transfection. The experiment was conducted in duplicates and repeated twice.

Cell nucleofection.

Initial nucleofection optimization

Nucleofection was carried out using the Jurkat Cell Line Optimization 96-well Nucleofector Kit from Amaxa (http://www.amaxa.com), according to the manufacturer's recommendations. Briefly, TK6 cells were split into three aliquots, each containing 1.0 x 10^6 cells. The aliquots were centrifuged at 0.2g (rcf) for 5 minutes at room temperature, and the media completely removed. Each of the three cell pellets was re-suspended in one of three different nucleofection solutions (SE, SF, and SG), and 0.4 µg pmaxGFP plasmid (Lonza) that encodes green fluorescent was added to each solution. Each well in the 96-well nucleofection plate contained 20.0µl of cells and DNA in one of the three nucleofection solutions; control cells received no DNA. Immediately, the mixture was transferred into an Amaxa Shuttle nucleofection conducted using the recommended program. Upon completion of the nucleofection program, 80.0µl of pre-warmed complete media was added to each well of the 96-well nucleocuvette plate. The contents (100.0µl) of each microcuvette (Lonza) well were rapidly removed and transferred to the appropriate cell culture plates. The cells were incubated for 24–36 h in a humidified 37°C/5% CO_2 atmosphere. Transfection efficiency was determined by fluorescence microscopy, and cell viability assessed using the vi-cell counter (Beckman). Unless otherwise indicated, all nucleofection experiments were carried out in duplicates and repeated twice.

Secondary nucleofection optimization

A second nucleofection optimization was performed using SF reagent, which allowed the further evaluation of this reagent. TK6 lymphoblastic cells were pelleted by centrifugation at 0.2g (rcf) for 5 minutes at room temperature. The cells were re-suspended to a density of 1.0 x 10^6 cells/20ul in the supplemented nucleofection solution SF (Lonza). The p53-proficient TK6 cells were nucleofected with 0.4µg pGFPmax (0.5 µg pGFPmax was also tested); control cells were not transfected with DNA.

In a parallel experiment, the cells were transfected with 0.4µg and 0.5µg pNL1.1.CMV and control cells were transfected with the promoter-less vector pNL1.2; additional controls were cells that did not receive DNA. Nucleofection was conducted by using the DS 137 program on the Amaxa Nucleofector 96-well Shuttle. After nucleofection, the contents of each microcuvette (Lonza) well were rapidly removed with 80.0µl of pre-warmed supplemented RPMI 1640 media and transferred to the appropriate wells in the 6-well culture plates. The cells were kept incubated for 24–36h in a humidified 37°C/5% CO_2 atmosphere.

Transfection efficiency using GFP was determined by fluorescence microscopy and also by assaying for normalized luciferase activity. Cell viability was determined by using the Vi-cell instrument. All measurements were conducted at 24 and 36h post transfection. The experiment was conducted in duplicates and repeated twice.

Determination of transfection efficiency using GFP expression

At 24 and 36 hrs post-transfection, 20.0µl of cells transfected with GFP as well as the control was added to clean glass slide. GFP fluorescence was captured using the Olympus 1X71 microscope equipped with a camera and processed with Digital Site controller software. Cells were counted in multiple randomly selected fields, and transfection efficiency was obtained by dividing the number of cells expressing GFP by the total number of cells detected by bright field microscopy. The results were expressed mean percentage of GFP transfection efficiency in different field ± standard error.

Nano-Glo luciferase assay

Nano-Glo Luciferase assay was performed by utilizing the Promega Nano-Glo Luciferase assay. Nano-Glo Luciferase Assay reagent was made by adding one volume of Nano-GloLuciferase Assay Substrate to 50 volumes of Nano-Glo Luciferase Assay Buffer as recommended by the manufacturer. Thereafter, 100.0 µl of the nanoluciferase reagent was added to 5.0×10^5 cells of each sample in a 96-well plate. Luciferase assays were performed at 24h and/or 36h post DEB exposure in Spectramax M5. Assays were done in triplicates. Assays were performed at 24h and/or 36h post transfection in duplicates.

Results

Optimizing transfection conditions for Metafectene Pro transfection reagent

To assess the transfection efficiency of Metafectene® Pro in TK6 human lymphoblasts, we tested 4 different ratios (1:2-1:6) using 1 and 0.5 µg of purified pGFPmax plasmids. Traditionally, the optimum ratio of nucleic acid [µg] to Metafectene® Pro [µl] is between 1:2 and 1:7 (Metafectene® Pro Manuel, 2013). As shown in Figure 1A cell viability (~96%) in this cell line using Metafectene® Pro was comparable regardless of the ratio (Fig 1A). No significant ($p>0.05$) differences in cell viability between control cells that received no DNA and cells that were transfected with various reagent/DNA ratios for both 24 and 36h post transfection (Fig 1A). Maximal transfection efficiency was achieved at reagent to DNA ratios of 3 for both 24 and 36h post transfection (Fig.1B). Transfection efficiency of cells that received pGFPmax was significantly ($p<0.05$) greater than the control cells that received no DNA (Fig. 1B). Collectively, the results of Fig. 1 demonstrate that optimal transfection conditions for the Metafectene Pro transfection reagent occur at reagent/DNA ratio of 3 when 1.0ug of DNA is utilized for transfection.; no significant difference in transfection efficiency and cell viability was observed between 24 and 36h transfection times.

The experiment performed in Fig.1 was repeated by utilizing the best transfection conditions (reagent to DNA ratio of 3, 1μg of DNA) obtained for the Metafectene Pro reagent. Cell viability and transfection efficiency were then assessed using GFP and nano-luciferase activity (Fig. 2). Cells transfected with pNL1.1CMV had no significant effect on cell viability (Fig 2A). Transfection efficiency of cells that received the pGFPmax plasmid was significantly ($p<0.05$) greater than control cells that received no DNA (Fig. 2B). No luciferase activity was detected (Fig. 2C). Collectively, these results demonstrate that Metafectene Pro is not toxic to cells under conditions where 80% GFP transfection efficiencies are achieved. Since no nanoluciferase activity was detected under optimal transfection conditions for this reagent, metafectene cannot be used in our system. For a transfection reagent to be useful in our system, the reagent should be able to produce high transfection efficiency and luciferase activity with minimal toxicity.

Optimizing transfection conditions for Lipofectamine LTX

To determine the toxicity and transfection efficiency of Lipofectamine LTX, cells were seeded in 6-well plates at 0.5×10^5 and 1.0×10^6 cells/well and transfected with 0.5 μg and 1.0μg of pGFPmax plasmids, respectively. Cells transfected with 1.0μg of plasmid showed no significant difference ($p>0.05$) in cell viability between the control cells that received no DNA and cells transfected with DNA at 24 and 36h post exposure (Fig 3A). Cells transfected with pGFPmax demonstrated a significantly ($p<0.05$) greater transfection efficiency than the control cells (Fig 3B). In a parallel experiment cell were transfected with 1.0 μg of pNL1.1CMV plasmid while control cells received 1.0 μg promoterless vector pNL1.2. No significant difference in cell viability between cells transfected with pNL1.1CMV and control cells that received pNL1.2 at 24 and 36h post transfection (data not shown). Luciferase activity was significantly greater ($p<0.05$) in cells transfected with pNL1.1CMV than the control cells at 24 and 36h post transfection; a significant 30.0% increase in luciferase activity was observed at 36h as compared to 24h post-transfection (Fig 3C). Collectively, these results demonstrate that Lipofectamine LTX achieves transfection efficiencies of at least 80% with no significant toxicity to TK6 cells at both 24 and 36h transfection times. Under Lipofectamine transfection conditions, cells express nanoluciferase to the order of $1.8 \text{X} 10^5$ RLU, indicating that lipofectamine LTX could potentially be utilized for our nanoluciferase promoter assays, especially at 36 post-transfections, when the promoter activity is at its highest levels; 1.0×10^6 cells/well transfected with 1.0μg plasmid DNA seems to achieve the highest number of transfected cells.

Optimizing transfection conditions for the Amaxa Nucleofector using the pGFPmax plasmid

To determine the optimal conditions for nucleofection of the TK6 cell line, an initial optimization experiment was performed as described by the manufacturer. The range of possible outcomes for the 96-well nucleofection conditions was characterized by using cell/nucleic acid mixtures combined with one of the 3 proprietary reagents: SE, SF, and SG. Of the three optimization buffers and programs, SF reagent with program, DS 137 demonstrated relatively high percent cell viability and transfection efficiency (data not shown). SF reagent with program, DS 137 was subsequently used in the secondary optimization.

Secondary optimization of nucleofection conditions by using the Amaxa nucleofector

To evaluate the most promising conditions, a second optimization was performed using nucleofector reagent SF, which gave the best overall results, as shown in Fig. 5. This was achieved by transfecting the TK6 cell line with 0.4 and 0.5µg of pmaxGFP plasmid (expressing green fluorescence protein); cell viability and transfection efficiency were then assayed at 24 and 36h post transfection (Fig. 6). Cells transfected with 0.4µg of DNA recorded significantly greater ($p<0.05$) percent cell viability than cells transfected with 0.5µg of DNA for both 24 and 36h post transfection (Fig. 5A). No significant difference ($p>0.05$) in transfection efficiency between cells transfected with 0.4µg and 0.5µg of DNA were observed (Fig. 5B).

In a parallel experiment, cells were transfected with 0.4 and 0.5µg of pNL1.1CMV (expressing luciferase activity) while control cells received pNL1.2 (promoter-less vector). Cell viability and luciferase activity were then assessed at 24 and 36h post transfection (Fig. 6). Cells transfected with 0.4µg of DNA recorded significantly greater ($p<0.05$) percent cell viability compared to cells that received 0.5 µg of DNA (Fig. 6A) at 24 and 36h post transfection. Cells transfected with pNL 1.1CMV significantly expressed luciferase with maxima expression at 36h post transfection. Luciferase activity of cells transfected with 0.4µg of pNL 1.1CMV was significantly ($p<0.05$) greater than cells transfected with 0.5 µg of pNL 1.1CMV (Fig. 6B). Collectively, these results (Fig. 6) demonstrated that cells transfected with 0.4µg yielded maximal GFP transfection efficiency with minimal toxicity. The percent transfection efficiency and cell viability were ~80 and ~85, respectively. Luciferase activity was RLU ~5.4×10^5, as opposed to 1.8×10^5 RLU obtained for lipofectamine LTX. Amaxa Nucleofection yielded the strongest luciferase signal of all the 3 reagents tested after 24 and 36hrs. However, amaxa nucleofection resulted in overall weak but acceptable cell viability.

Discussion

Most molecular biology studies are based on transfection of nucleic acid into eukaryotic cells. [46,47]These studies therefore require suitable transfection methods, with each method using different approaches depending on cell type and purpose .[48] Every method varies with respect to transfection efficiency and cell toxicity. However, the method of choice should have high transfection efficiency, and low toxicity. For the most part optimization is a requisite for best results.[49]

Optimization is typically required to arrive at the best transfection conditions for cells. TK6 human lymphoblasts cell line is a traditionally-difficult-to-transfect cell type. Optimizing TK6 human lymphoblasts with nucleic acid molecules of interest at a relatively high efficiency while maintaining cell viability is essential for studying gene function, regulation, and protein function. In this we evaluated optimum conditions for transfection of TK6 human lymphoblasts using three commonly used transfection agents: Amaxa Nucleofector Solutions, Lipofectamine LTX, and Metafectamine Pro. These reagents were selected based on information available from the respective company concerning their high transfection efficiency and low toxicity in multiple cell lines, including difficult to transfect cell

lines.[20,29,50–53]. We assessed the results to confirm that our conditions maximized both transfection efficiency and cell viability. The data demonstrated that by optimizing transfection conditions for TK6 human lymphoblasts, nucleic acid molecules can be delivered in a highly efficient manner. Nucleofection is more effective than chemical transfection reagents from several different cationic categories (Metafectamine Pro and Lipofectamine LTX) at delivering DNA into a TK6 human lymphoblast.

In our study Metafectene Pro resulted in highly effective transfection of plasma DNA with low toxicity into TK6 human lymphoblasts resulting in high percent cell viability and transfection efficiency. However, no luciferase activity was detected in TK6 cells transfected with Metafectene. The absence of luciferase activity when transfected with metafectene Pro suggests that metafectene inhibits or interferes with Nanoluciferase luciferase activity in Nano-Glo luciferase suggesting the presence of certain chemotypes acting as inhibitors of NanoLuc. Some inhibitors of NanoLuc include those with a phenyl-1,4-dihydropyridine found in the drug Isradipine and aryl sulfonamide. [54] Interestingly studies have shown that luciferase activities were detected when the same cells were co-transfection with GFP-Max.[55] Also, luciferase activities have been detected in other systems the Dual-Luciferase® Reporter Assay System, Bright-Glo reagent and rennila Luciferase Assay System.[56,57] Due to high transfection efficiency associated with low toxicity, Metafectene Pro has successfully been used to transfect a wide variety of cells lines.[58,59]

We also found that, transfecting at a reagent to DNA ratio of 3:1 was the optimum for our system after 24- and 36-hours incubation. In contrast, the reagent to DNA ratio of 6:1 was more toxic to our cells. Higher reagent to DNA ratio is known to be associated with high toxicity in cells.[60] For a transfection reagent to be useful in our system, the reagent should exhibit high percent cell viability and transfection, and high luciferase activity. Metafectene did not meet all these conditions since no nanoluciferase activity was detected under optimal transfection conditions. Due to the absence of detectable luciferase activity associated with Metafectene Pro we decided to optimize transfection conditions using lipofectamine LTX and Amaxa Nucleofection Shuttle System.

Lipofectamine reagents are associated with relatively high transfection efficiency in many different cell types including lymphocytes.[61–63] These reagents are noninfectious, easy to use and can transfer DNA of various sizes. High cellular transfection efficiency is attributed to the interaction between cationic lipids and DNA which facilitate the delivery of DNA into the cells.[64,65] In our study we found that transfecting TK6 cells with lipofectamine LTX yielded a relatively high percent transfection efficiency, cell viability and luciferase activity at 24 and 36h post transfections suggesting that lipofectamine could be used in our system. High luciferase activity at 36h post transfection of lipofectamine LTX has also been observed in primary human umbilical vein endotherlial and mice cells.[60,66]

The Amaxa Nucleofector 96-Well Shuttle system is a fully automated high throughput system to transfect difficult-to-transfect cell lines and primary cells in the 96-well format. Amaxa Nucleofector 96-Well Shuttle system is an attractive primary experimental tool because of its simplicity and reproducible results.

Amaxa Nucleofector Shuttle has been shown to deliver successfully high transfection efficiencies in several cell lines.[67–70] However, Amaxa Nucleofector Shuttle has shown some very poor transfection results in other cells suggesting that cell type does have a major effect influence transfection efficiency of cells transfected with nucleofector.[71–74] In this study transfecting TK6 with 0.4μg of plasmid DNA using reagent SF, program DS 137 as was associated with high transfection efficiency (~ 80%) and luciferase activity (RLU = 5.1 X 10^5) with acceptable cell toxicity (~ 15%). Chicaybam et al., [75] observed similar results when T-lymphocytes were nucleofected at the same transfection conditions. However, nucleofection was more toxic to our cells compared to the other transfection methods used due to long lasting pulses or polarization of the cells from the electric field.[76,77] Amaxa nucleofection was identified as the optimal transfection reagent for transfecting TK6 cells because of its higher luciferase activity, high transfection efficiency and acceptable percent cell viability.

Taken together, our results show that of the three tested reagents, Amaxa 96-well Nucleofection Shuttle System using Solution SF delivered the best transfection results in traditionally hard-to transfect cell lines such as TK6 human lymphoblasts. Transfection with Amaxa Nucleofection Shuttle System using Solution SF yields high luciferase activity and transfection efficiency, and acceptable cell toxicity.

Declarations

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AUTHORS’ CONTRIBUTIONS

Akamu Jude Ewunkem: Conceptualization, Methodology and was responsible for conducting experiments. Akamu Jude Ewunkem: Contributed conception and design of the study in addition to the analysis and interpretation of all data, performed all the statistical analysis. Akamu Jude Ewunkem: Review & Editing. Agee Kyle: Review & Editing. All authors reviewed the manuscript. The author(s) read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

There are no conflicts of interest to declare.

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Figures

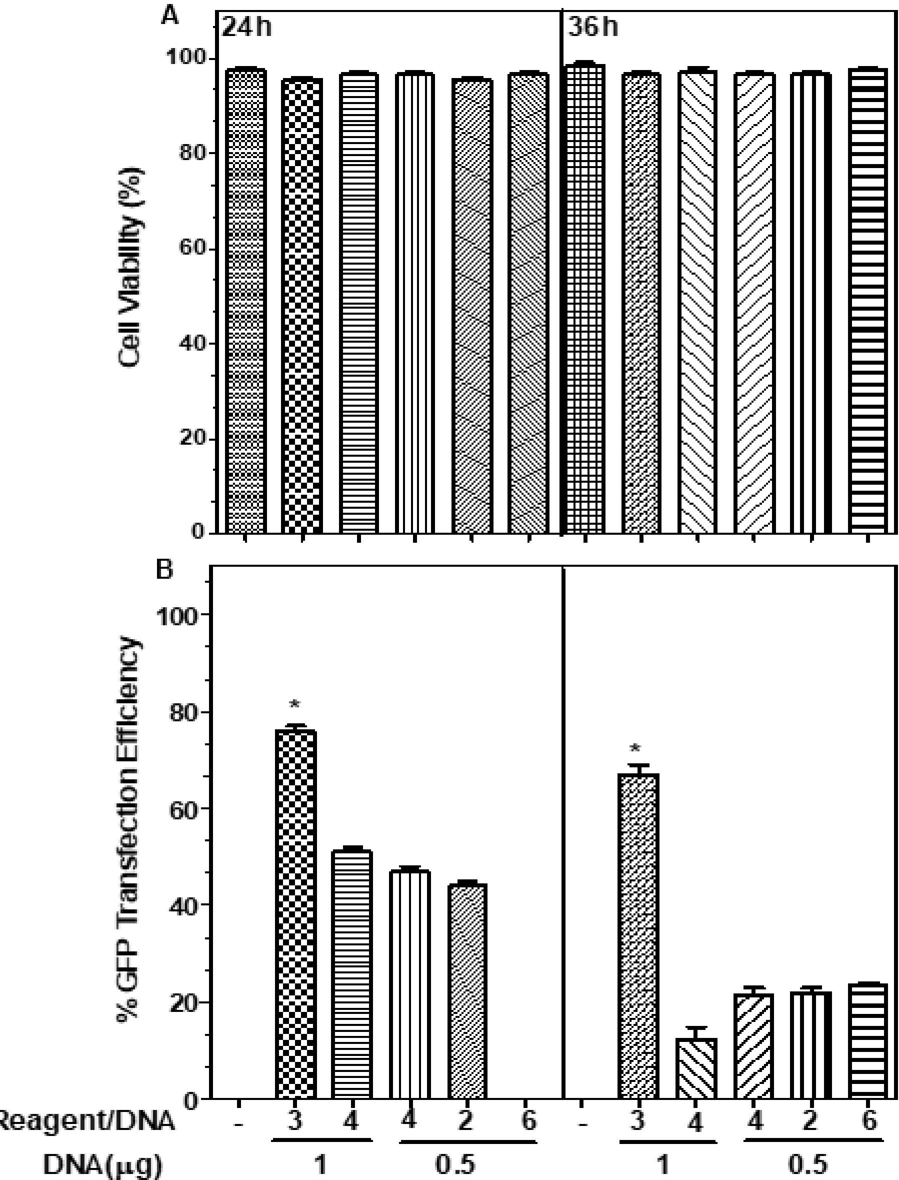

Figure 1

Transfection efficiency of Metafectene Pro. TK6 human lymphoblast cells were subjected to transfection using 1 and 0.5μg of pGFPmax in the ratios of 1:2-1:6 METAFECTENE PRO in a 6-well plate. A. Cell viability and B. GFP transfection efficiency was determined 24 and 36h post transfection (*: p-value < 0.05)

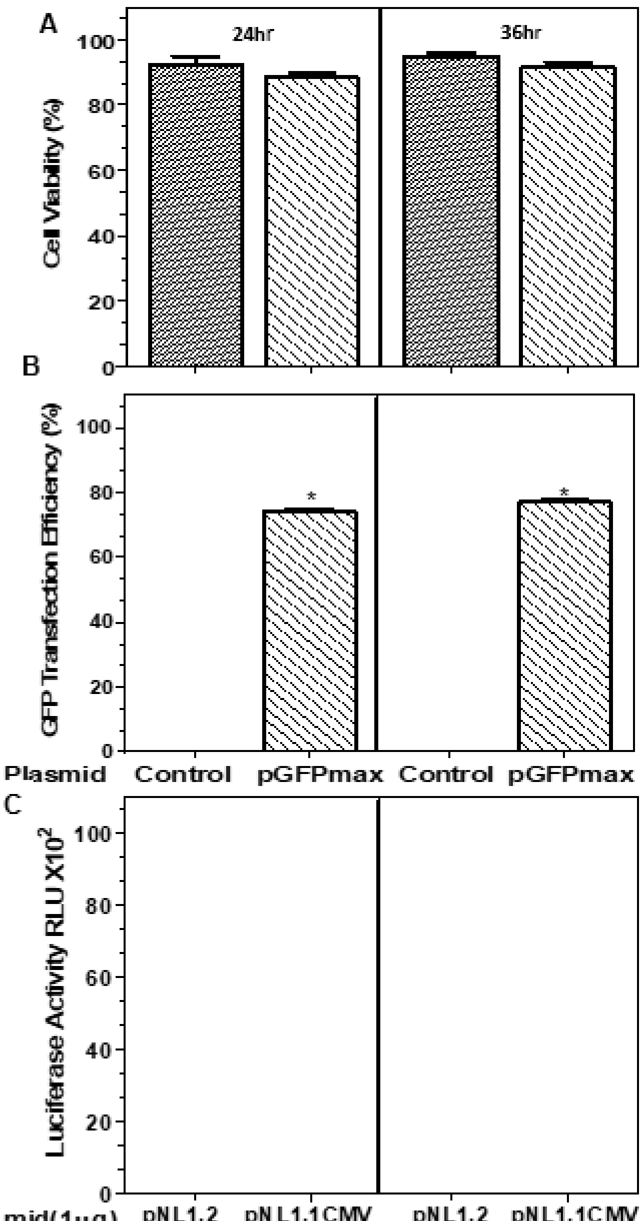

Figure 2

Transfection efficiency of Metafectene Pro. TK6 human lymphoblast cells were subjected to transfection using 1.0μg of pGFPmax in the ratios of 1:3 METAFECTENE PRO in a 6-well plate. A. Cell viability: B. GFP transfection efficiency and C. luciferase activity were determined 24 and 36h post transfection (*: p-value < 0.05)

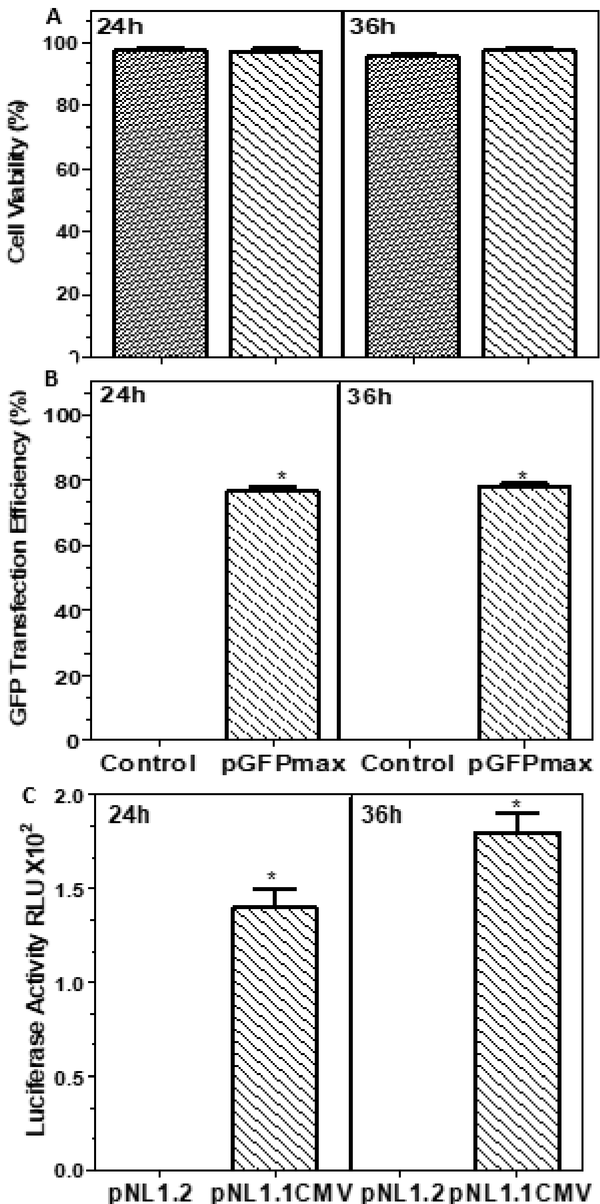

Figure 3

Determination of cell viability, and GFP and nanoluciferase transfection efficiencies of the Lipofectamine LTX in TK6 cells using the pGFPmax and pNL1.1CMV plasmids. TK6 cells were seeded in 6-well plates at a density of 1.0 x 106 cells/well and transfected with 1.0 µg. Cell viability and transfection efficiency were assessed at 24 and 36h post-transfection. A. Cell viability; B. GFP transfection efficiency and C. luciferase activity were determined 24 and 36h post transfection (*: p-value < 0.05)

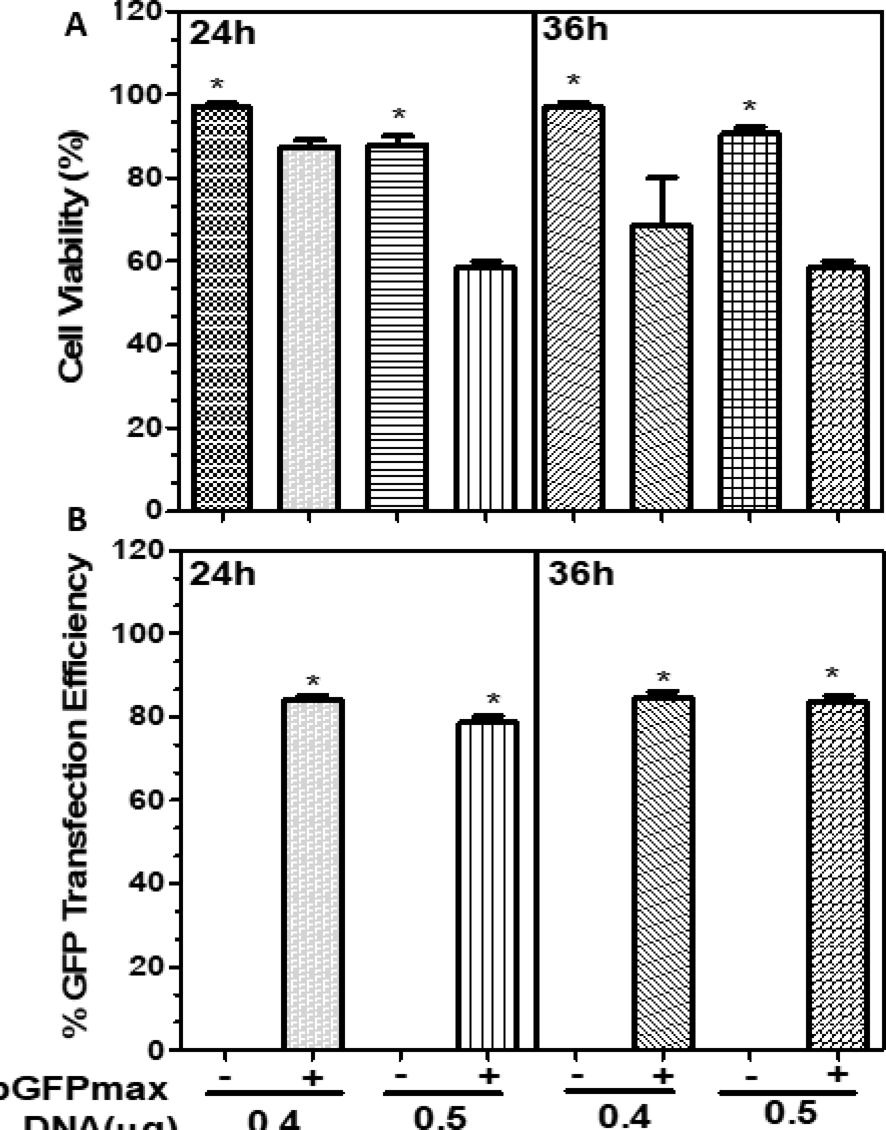

Figure 4

FIGURE 5.

Optimization of conditions to minimize cell toxicity while maximizing GFP transfection efficiency of the Amaxa Nucleofector using 0.4µg and 0.5µg of pGFPmax at 24h and 36h post-transfection. A. Cell viability: B. GFP transfection efficiency. (*: *p*-value <0.05).

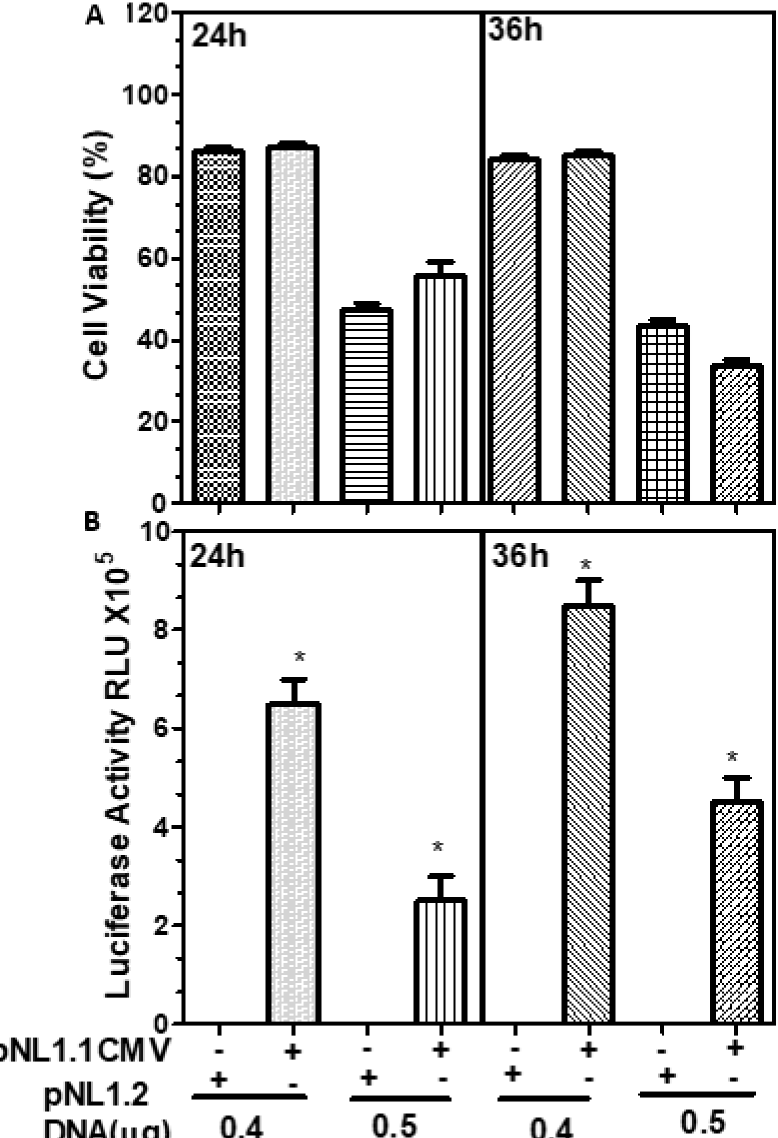

Figure 5

FIGURE 6.

Optimization of conditions for transfection efficiency of amaxa using luciferase activity. TK6 cells were transfected with pNL1.1CMV and pNL1.2 and the cell mixtures were combined with SF proprietary reagent. A. Cell viability and B. luciferase activity was measured at 24h and 36h Post transfection. (*: p-value <0.05).