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Optimization of transfection methods for human lymphoblast TK6 cell line

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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 require optimization for successful transfection. Human lymphoblast TK6 cell line, an important cell line for genotoxic studies, is known to be extremely hard to transfect. Thus, optimizing transfection methods for human lymphoblast TK6 are 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 efficiency 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 efficiency 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%). Optimal conditions for transfection utilized with this reagent included 0.4 μg of plasmid DNA, 1.8×10^5 cells, and using the DS 137 Nucleofector program.

Keywords: Optimization; Nucleofection; Lipofectamine LTX; Metafectene Pro; Transfection; Cell viability

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1. Introduction

Transfection is an important analytical tool for studying 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 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 transfection of thymidine kinase heterozygous cell line of human lymphoblasts (TK6 human lymphoblasts) is a crucial step for studying cell biology at the molecular level through gene expression. Many transfection methods have been developed. Each transfection method utilizes different approaches. However, each transfection method has 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, viral-mediated transfections are labor-intensive and require certain biosafety measures. In addition, viral vectors generally transduce reticuloendothelial organs, which dramatically decreases the delivery efficiency of viruses into their target organs[13,14]. Non-viral transfection methods are relatively safer but have several drawbacks, which include inefficiency and toxicity[15]. Non-viral transfection can be explored using physical and chemical approaches. The 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 acid into cells[16]. In a chemical approach, natural and synthetic chemicals, such as diethylaminoethyl-dextran, cationic lipids, and cationic polymers, are used to facilitate the delivery of nucleic acid into the cell membrane[17,18].

Metafectene Pro® is a polycationic transfection reagent based on liposome technology[19]. Metafectene® Pro ensures easy entry of plasmid DNA into cells by condensing DNA into compact structures[19,20]. Metafectene Pro® exhibits high transfection efficiency and low toxicity in multiple cell lines and primary cells, including human embryonic kidney 293 cells (HEK 293), human leukemia monocytic cell line (THP-1), immortalized murine microglial cells derived from C57/BL6 (BV2), primary T-cells, and Jurkat cells[20,21-25].

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 transfection methods. Furthermore, lipofectamine consistently produces high transfection efficiency[26,27]. 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, transformed HEK293T cells and Michigan Cancer Foundation-7 (MCF-7) cells[28-32].

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[33]. Nucleofection is famously known to overcome the lower transfection efficiency by chemical methods. The 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, and *Eimeria*[34-43].

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 efficiency vary dramatically depending on the reagent, protocol, and cell type being utilized[44]. The cell line used in this study is TK6 human lymphoblasts, which, like Jurkat cells, is traditionally difficult to transfect due to its 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[25-34]. 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.

2. Materials and methods

2.1. Nucleofection systems and reagents

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

2.2. 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 (GFP). NanoLuciferase reporter vectors pNL1.2 (NlucP) and pNL1.1 (Nluc) under the control of Cytomegalovirus promoter (pNL1.1.CMV) were purchased from Promega™ Corporation (Madison, Wisconsin). The pNL1.1.CMV contains the CMV promoter and expresses NanoLuciferase, and pNL1.2 (NlucP) is a luminescent reporter.

2.3. 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 $\times 10^5$ cells/mL in RPMI 1640 supplemented with 2 mM 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 36 h. Cells were passage into fresh media 12 – 14 h before each experiment.

2.4. 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 1×10^5 cells/mL. After that, the cells were transfected with 0.5 μg and 1.0 μg pGFPmax using Metafectene Pro (Biontex, Germany) at a 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, with both containing minimal essential medium (MEM) with a reduced serum 5% (OptiMEM) instead of the standard 10% FBS. 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 36 h post-transfection.

In a parallel experiment, the cells were transfected with pNl1.1 CMV expressing NanoLuciferase, 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 added dropwise to the cells and swirled with extreme care. The samples were kept in a CO_2 incubator at 37°C. NanoLuciferase Assay (Promega) was performed at 24 and 36 h 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 36 h post-transfection. The experiment was conducted in duplicate and repeated twice.

2.5. Lipofectamine transfection

Transfection was performed according to the manufacturer's protocol by following the optimized protocol for Jurkat cells. TK6 cells were seeded in 6-well plates at a density of 1.0×10^5 and 1.0×10^6 cells/well. Different concentrations of pGFPmax/empty pNL1.2 (0.5 – 1.0 μg) were diluted into 100 μL optiMEM media, PLUS reagent (1.5 – 2.5 μL), and lipofectamine (3.75 – 10.00 μL). After 30 min of incubation at room temperature, 100 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 5% CO_2/95% air incubator. Control cells received no DNA. Cells transfected with an empty pNL1.2 vector served as an additional control. GFP transfection efficiency, normalized NanoLuciferase activity, and cell viability were determined at 24 and 36 h post-transfection. The experiment was conducted in duplicate and repeated twice.

2.6. Cell nucleofection

2.6.1. Initial nucleofection optimization

Nucleofection was carried out using the Jurkat Cell Line Optimization 96-well Nucleofector Kit from Amaxa (catalogue no: V4XC-1024), according to the manufacturer's recommendations. Briefly, TK6 cells were split into three aliquots, each containing 1.0×10^6 cells. The aliquots were centrifuged at 0.2 g RCF for 5 min at room temperature, and the media was completely removed. Each of the three cell pellets was resuspended 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 μL 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. On completion of the nucleofection program, 80 μL of pre-warmed complete media was added to each well of the 96-well Nucleocuvette plate. The contents (100 μL) of each Nucleocuvette 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 was assessed using the Vi-CELL counter (Beckman). Unless otherwise indicated, all nucleofection experiments were carried out in duplicate and repeated twice.

2.6.2. 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 0.2 g RCF for 5 min at room temperature. The cells were resuspended to a density of 1.0×10^6 cells/20 μL in the SF-supplemented nucleofection solution (Lonza). The p53-proficient TK6 cells were nucleofected with 0.4 μg pGFPmax (0.5 μg pGFPmax was also tested); control cells were not transfected with DNA.

In a parallel experiment, the cells were transfected with 0.4 μg and 0.5 μg 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 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 μL of pre-warmed RPMI 1640 media supplemented with 2 mM L-glutamine and 10% FBS and transferred to the appropriate wells in the 6-well culture plates. The cells were incubated for 24 – 36 h

in a humidified 37°C/5% CO_2 atmosphere. Transfection efficiency using GFP was determined by fluorescence microscopy and by assaying for normalized luciferase activity. Cell viability was determined using the Vi-CELL instrument. All measurements were conducted at 24 and 36 h post-transfection. The experiment was conducted in duplicate and repeated twice.

2.7. Determination of transfection efficiency using GFP expression

At 24 and 36 h post-transfection, 20 µL of cells transfected with GFP as well as the control was added to a 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 expressed a mean percentage of GFP transfection efficiency in different fields ± standard error.

2.8. Nano-Glo Luciferase assay

Nano-Glo Luciferase assay was performed by utilizing the Promega Nano-Glo™ Luciferase Assay System. Nano-Glo Luciferase Assay reagent was made by adding one volume of Nano-Glo Luciferase Assay Substrate to 50 volumes of Nano-Glo Luciferase Assay Buffer as recommended by the manufacturer. After that, 100 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 24 h and/or 36 h post-transfection using SpectraMax M5. Assays were done in triplicate.

2.9. Cell viability

Cell concentration and viability were determined using the Vi-CELL XR Cell Viability Analyzer (Beckman Coulter, Inc.). Briefly, each sample was diluted and loaded in the Vi-CELL XR and processed through the machine one at a time. After selecting the dilution factor, Vi-CELL XR automatically aspirated and mixed the samples with trypan blue. The device eventually recorded the viability of the cell population in percentage. Immediately after cell counting, the flow-through was collected from the waste bottle.

2.10. Statistical analysis

Data are shown as means±standard error (SE). The student *t*-test was performed to compare means between transfected and non-transfected cells. All statistical analyses were performed using GraphPad Prism version 8.0. Differences of $p<0.05$ were considered significant.

3. Results

3.1. Optimizing transfection conditions for Metafectene Pro transfection reagent

To assess the transfection efficiency of Metafectene® Pro in TK6 human lymphoblasts, we tested four different ratios (1:2 – 1:6) using 1 and 0.5 µg of purified pGFPmax plasmids. Conventionally, the optimum ratio of nucleic acid (µg) to Metafectene® Pro (µL) is between 1:2 and 1:7 (Metafectene® Pro Manual, 2013). As shown in Figure 1A, cell viability (~96%) in this cell line using Metafectene® Pro was comparable regardless of the ratio. No significant 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 36 h post-transfection ($p > 0.05$, Figure 1A). Maximal transfection efficiency was achieved at reagent-to-DNA ratios of 3 for both 24 and 36 h post-transfection (Figure 1B). The transfection efficiency of cells that received pGFPmax was significantly greater than the control cells that received no DNA ($p < 0.05$, Figure 1B). Collectively, the results shown in Figure 1 demonstrate that optimal transfection conditions for the Metafectene Pro transfection reagent occur at a

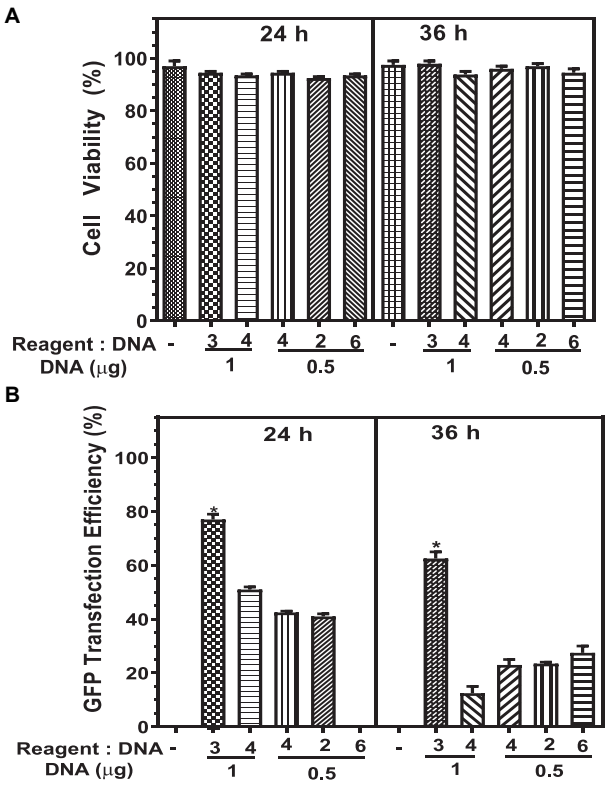

Figure 1. Transfection efficiency of Metafectene Pro. TK6 human lymphoblast cells were subjected to transfection using Metafectene Pro®. (A) Cell viability and (B) green fluorescent protein transfection efficiency. $^*p < 0.05$.

reagent/DNA ratio of 3:1 when 1.0 µg of DNA is utilized for transfection; no significant difference in transfection efficiency and cell viability was observed between 24 and 36 h transfection times.

The experiment performed in Figure 1 was repeated by utilizing the best transfection conditions (reagent to DNA ratio of 3, 1 µg of DNA) obtained for the Metafectene Pro reagent. Cell viability and transfection efficiency were then assessed using GFP and NanoLuciferase activity (Figure 2). Cells transfected with pNL1.1CMV had no significant effect on cell viability (Figure 2A). The transfection efficiency of cells that received the pGFPmax plasmid was significantly greater than control cells that received no DNA ($p < 0.05$, Figure 2B). No luciferase activity was detected (Figure 2C). Collectively, these results demonstrate that Metafectene Pro is not toxic to cells under conditions where 80% GFP transfection efficiency is achieved. Since no NanoLuciferase activity was detected under optimal transfection conditions for this reagent, Metafectene Pro 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.

3.2. 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 1.0 µg of pGFPmax plasmids, respectively. Cells transfected with 1.0 µg of plasmid showed no significant difference in cell viability between the control cells that received no DNA and cells transfected with DNA at 24 and 36 h post-exposure ($p > 0.05$, Figure 3A). Cells transfected with pGFPmax demonstrated a significantly greater transfection efficiency than the control cells ($p < 0.05$, Figure 3B). In a parallel experiment, the cells 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 36 h post-transfection (Figure 3C). Luciferase activity was significantly greater in cells transfected with pNL1.1CMV than the control cells at 24 and 36 h post-transfection ($p < 0.05$, Figure 3D); a significant 30% increase in luciferase activity was observed at 36 h as compared to 24 h post-transfection (Figure 3D). Collectively, these results demonstrate that lipofectamine LTX achieves transfection efficiency of at least 80% with no significant toxicity to TK6 cells at both 24 and 36 h transfection times. Under lipofectamine transfection conditions, cells express NanoLuciferase to the order of 1.8×10^5 RLU, indicating that lipofectamine LTX could potentially be utilized for our NanoLuciferase promoter assays, especially at 36 h post-transfections, when the promoter activity is at its highest levels; 1.0×10^6 cells/well transfected with 1.0 µg plasmid DNA seems to achieve the highest number of transfected cells.

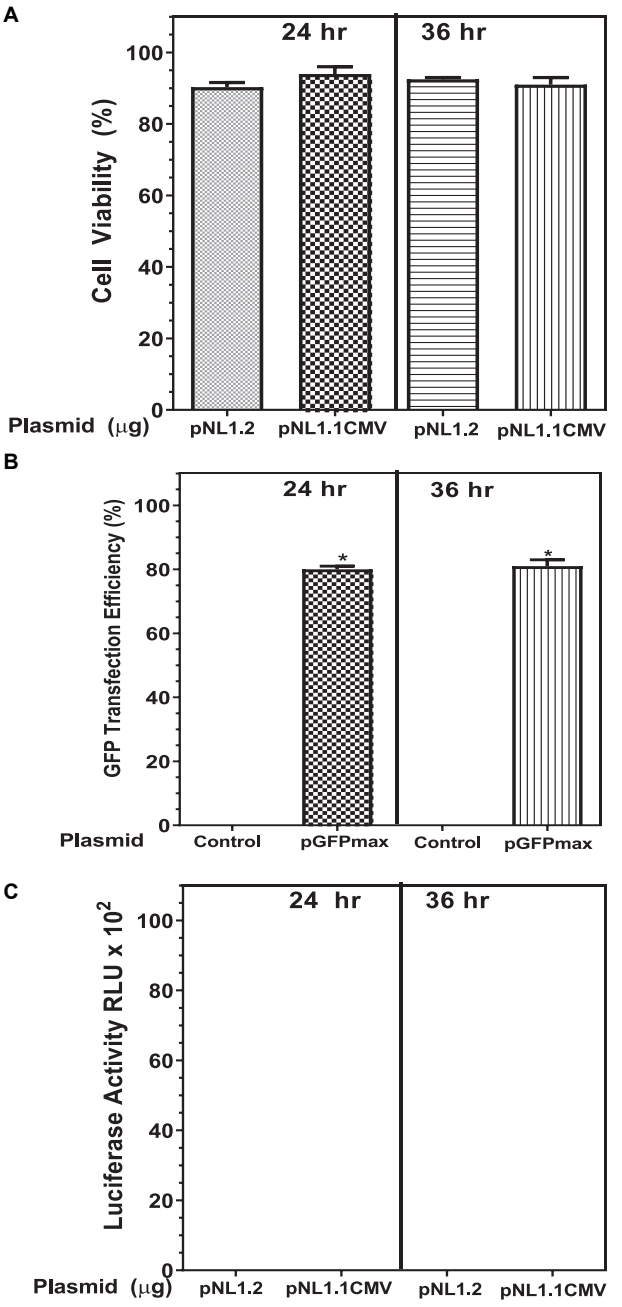

Figure 2. Transfection efficiency of Metafectene Pro. TK6 human lymphoblast cells were subjected to transfection using Metafectene Pro®. (A) Cell viability. (B) Green fluorescent protein transfection efficiency. (C) Luciferase activity. *$p < 0.05$.

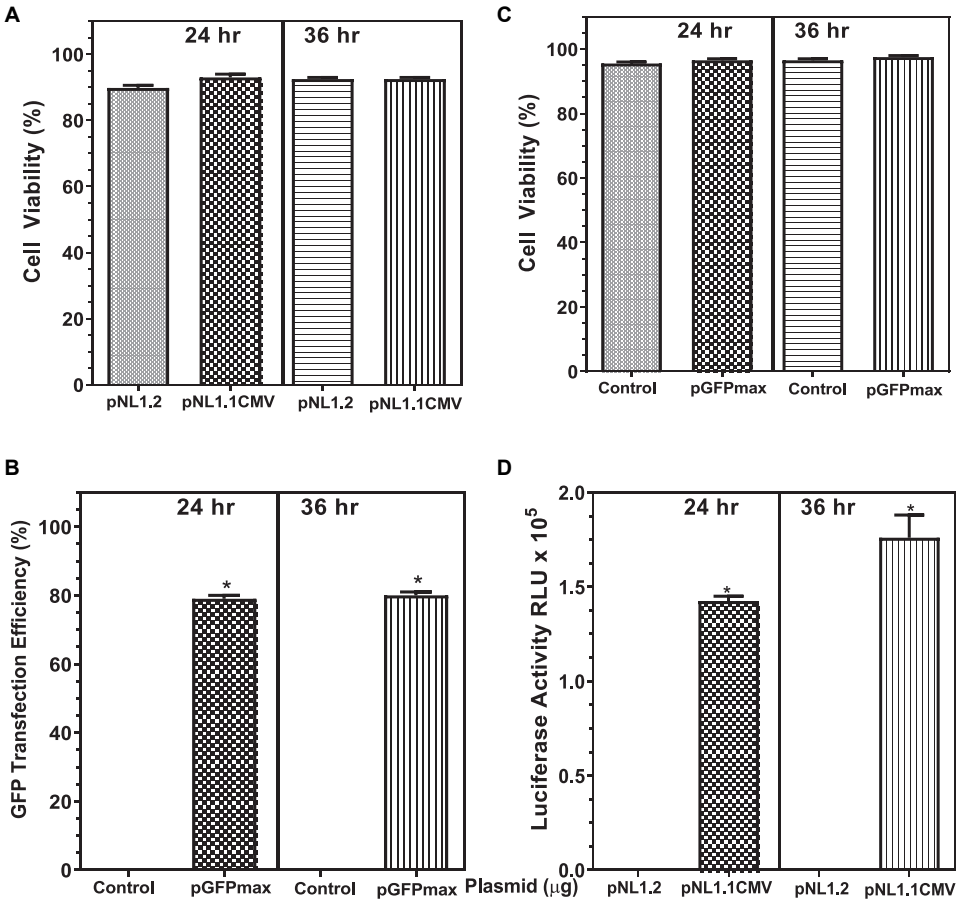

Figure 3. Determination of transfection efficiency of the lipofectamine LTX in TK6 cells using pGFPmax and pNL1.1 CMV plasmids. (A) Cell viability of control cells that received no DNA and cells transfected with DNA. (B) Green fluorescent protein transfection efficiency. (C) Cell viability of cells transfected with pNL1.1 CMV and control pNL1.2. (D) Luciferase activity. *$p < 0.05$.

3.3. 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 using cell/nucleic acid mixtures combined with one of the three 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.

3.4. Secondary optimization of nucleofection conditions 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 Figure 4. This was achieved by transfecting the TK6 cell line with 0.4 μg and 0.5 μg of pmaxGFP plasmid (expressing green fluorescence protein); cell viability and transfection efficiency were then assayed at 24 and 36 h post-transfection (Figure 4). Cells transfected with 0.4 μg of DNA recorded significantly greater percent cell viability than cells transfected with 0.5 μg of DNA for both 24 and 36 h post-transfection ($p < 0.05$, Figure 4A). No significant difference in transfection efficiency between cells transfected with 0.4 μg and 0.5 μg of DNA was observed ($p > 0.05$, Figure 5B).

In a parallel experiment, cells were transfected with 0.4 μg and 0.5 μg of pNL1.1 CMV (expressing luciferase activity), while control cells received pNL1.2 (promoter-less vector). Cell viability and luciferase activity were then assessed at 24 and 36 h post-transfection (Figure 5). Cells transfected with 0.4 μg of DNA recorded a significantly greater cell viability percentage compared to cells that

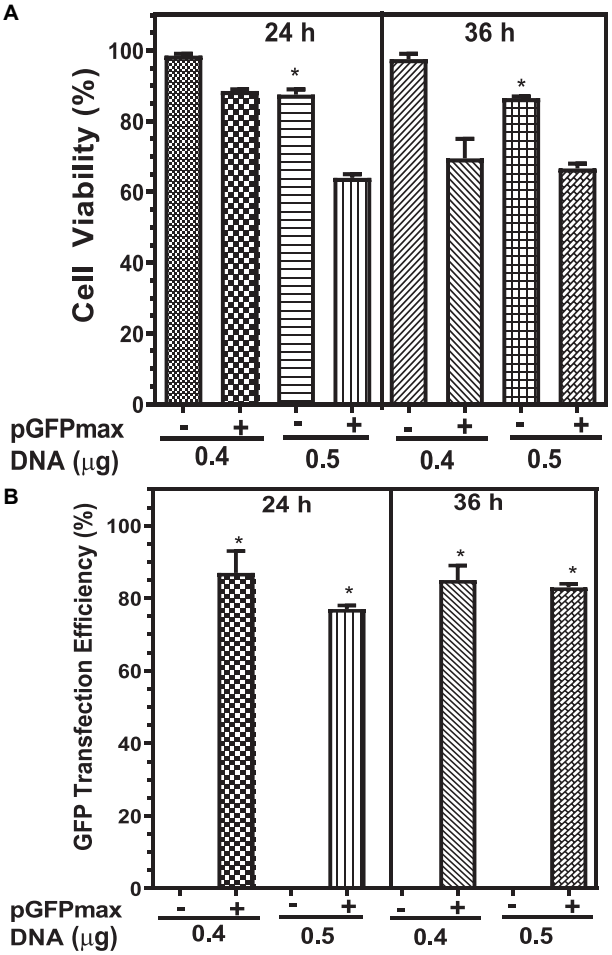

Figure 4. Optimization of conditions to minimize cell toxicity while maximizing green fluorescent protein (GFP) transfection efficiency of the Amaxa Nucleofector. (A) Cell viability. (B) GFP transfection efficiency. *$p < 0.05$.

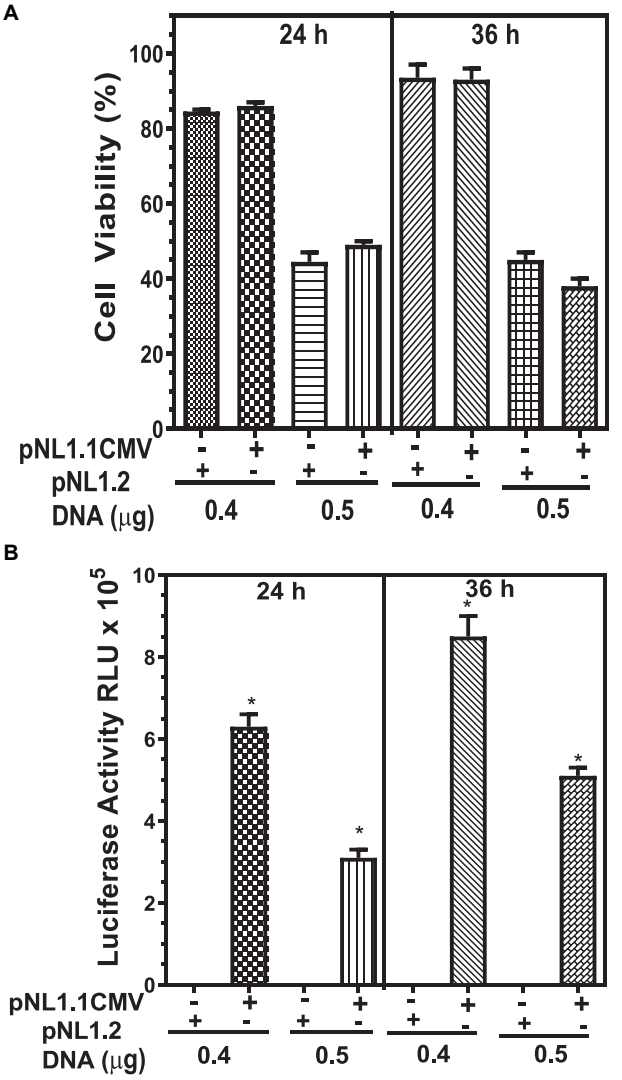

Figure 5. Optimization of conditions for transfection efficiency of Amaxa. TK6 cells were transfected and combined with SF proprietary reagent. (A) Cell viability. (B) Luciferase activity. *$p < 0.05$.

received 0.5 μg of DNA at 24 and 36 h post-transfection ($p < 0.05$, Figure 5A). Cells transfected with pNL 1.1CMV significantly expressed luciferase with maximal expression at 36 h post-transfection. Luciferase activity of cells transfected with 0.4 μg of pNL 1.1CMV was significantly greater than cells transfected with 0.5 μg of pNL 1.1CMV ($p < 0.05$, Figure 5B). Collectively, these results (Figure 5) demonstrate that cells transfected with 0.4 μg yielded maximal GFP transfection efficiency with minimal toxicity. The transfection efficiency percentage and cell viability were in the range of 80 – 85%. Luciferase activity was about 5.4×10^5 RLU, as opposed to 1.8×10^5 RLU obtained for lipofectamine LTX. Amaxa Nucleofection yielded the strongest luciferase signal of all the three reagents tested after 24 and 36 h (Table 1). However, Amaxa nucleofection resulted in overall weak but acceptable cell viability. GFP fluorescence of the various transfection methods is shown in Figure 6.

4. Discussion

Most molecular biology studies are based on nucleic acid transfection into eukaryotic cells[45,46]. These studies, therefore, require suitable transfection methods, with each method using different approaches depending on cell type and purpose[47]. 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 example, lentivirus-based transfection is an efficient method for the delivery of nucleic acids to cells; however, it is tedious, and time-consuming, and toxicity of the viral components can be a serious barrier[13,14]. For the most part, optimization is a requisite for best results[48]. In this study, we exploited different non-viral transfection

Table 1. The effects of different transfection methods of TK6 human lymphoblasts on transfection efficiency and cell viability

Transfection method	Luciferase signal (RLU)	Cell viability (%)	GFP transfection efficiency (%)
Metafectene Pro	-	90 – 90	77 – 83
Lipofectamine LTX	1.6×10^5–1.8×10^5	95 – 98	76 – 83
Amaxa Nucleofection	2.4×10^5–8.2×10^5	80 – 85	70 – 80

Abbreviations: RLU: Relative luminescence unit; GFP: Green fluorescent protein.

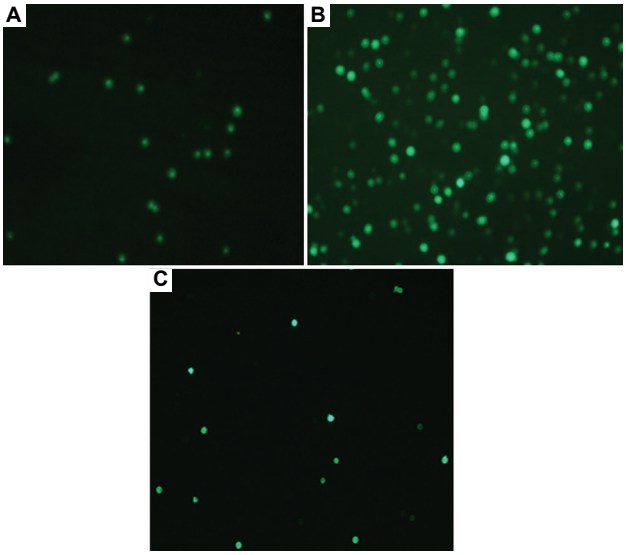

Figure 6. Green fluorescent protein fluorescence of different transfection methods determined at 24 h. (A) Metafectene Pro, (B) Lipofectatmine LTX. (C) Nucleofection. Microscope magnification: ×20.

methods to avoid the issues associated with viral-based transfection methods potentially.

Optimization is typically required to arrive at the best transfection conditions for cells. TK6 human lymphoblast 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 study, we evaluated optimum conditions for transfection of TK6 human lymphoblasts using three commonly used transfection agents: Amaxa Nucleofector Solutions, Lipofectamine LTX, and Metafectene Pro. These reagents were selected based on the available information from the respective company concerning their high transfection efficiency and low toxicity in multiple cell lines, including difficult-to-transfect cell lines[20,28,49-52]. 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 (Metafectene 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 the presence of certain chemotypes in Metafectene inhibits or interferes with NanoLuciferase luciferase activity in Nano-Glo Luciferase. Some inhibitors of NanoLuc include those with a phenyl-1,4-dihydropyridine found in the drug isradipine and aryl sulfonamide[53]. Interestingly, studies showed that luciferase activities were detected when the same cells were cotransfection with GFP-Max[54]. Furthermore, luciferase activities have been detected in other systems, such as the Dual-Luciferase® Reporter Assay System, Bright-Glo reagent, and Renilla Luciferase Assay System[55,56]. Due to high transfection efficiency associated with low toxicity, Metafectene Pro has successfully been used to transfect a wide variety of cell lines[57,58].

We also found that transfecting at a reagent-to-DNA ratio of 3:1 was the optimum for our system after 24- and 36-h incubation. In contrast, the reagent-to-DNA ratio of 6:1 was more toxic to our cells. A higher reagent-to-DNA ratio is known to be associated with high toxicity in cells[59]. For a transfection reagent to be useful in our system, the reagent should exhibit high percentage of cell viability, transfection efficiency, 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[60-62]. These reagents are non-infectious, 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 facilitates the delivery of DNA into the cells[63,64]. Our study found that transfecting TK6 cells with Lipofectamine LTX yielded a relatively high percent transfection efficiency, cell viability, and luciferase activity at 24 and 36 h post-transfections, suggesting that lipofectamine could be used in our system. High luciferase activity at 36 h post-transfection of lipofectamine LTX has also been observed in primary human umbilical vein endothelial and mice cells[59,65].

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. The system is an attractive primary experimental tool due to its simplicity and reproducible results. Amaxa Nucleofector Shuttle has been shown to deliver successfully high transfection efficiency in several cell lines[66-69]. However, Amaxa Nucleofector Shuttle has shown some very poor transfection results in other cells, suggesting that cell type has a major influence on transfection efficiency of cells transfected with Nucleofector[70-73]. In this study, transfecting TK6 with 0.4 μg of plasmid DNA using reagent SF and program DS 137 was associated with high transfection efficiency (~80%) and luciferase activity (RLU = 5.1×10^5) with acceptable cell toxicity (~15%). Chicaybam *et al.* observed similar results when T-lymphocytes were nucleofected at the same transfection conditions[74]. 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[75,76]. Amaxa nucleofection was identified as the optimal transfection reagent for transfecting TK6 cells due to its higher luciferase activity, high transfection efficiency, and acceptable cell viability percentage.

5. Conclusion

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 is accompanied by acceptable cell toxicity.

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Conflict of interest

There are no conflicts of interest to declare.

Author contributions

Conceptualization: Akamu Jude Ewunkem
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All data generated or analyzed during this study are included in this published article.

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