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TRANSFECTION OF DIFFICULT-TO-TRANSFECT ZEBRAFISH (*Danio rerio*) ZF4 CELLS USING CHEMICAL TRANSFECTION AND NUCLEOFECTION METHOD

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ABSTRACT

Transfection is a powerful tool for introducing foreign DNA into target cells. Many kits and methods are offered to simplify the process of introducing foreign DNA into cells. ZF4 is a cell type derived from zebrafish that is difficult to transfect. The majority of studies involving transfection into cells require transfection efficiency >70%. Our research demonstrates transfection using 2 kinds of methods, namely chemical transfection using X-tremeGene HP and electroporation-based transfection using a nucleofector device which will later be called Nucleofection. Our results show that transfection using electricity (Nucleofection) provides better transfection efficiency than chemical transfection (X-tremeGene HP).

***Keywords**: maxGFP, nucleofection, transfection, zebrafish (Danio rerio), ZF4 cells*

INTRODUCTION

The use of zebrafish as a research object in vertebrate model systems has been around for a long time. Currently the use of zebrafish has expanded into various fields of genetics, molecular biology, medical research, developmental biology, neurobiology, modern biotechnology, etc. The advantage of using zebrafish in research is easier monitoring of development because the embryos are transparent. However, in the introduction of foreign DNA into cells through transfection, zebrafish cells, for example ZF4, are cells that are difficult to transfect. Many reagents that offer the ability to easily transfect foreign DNA into the ZF4 zebrafish cell line (Sandbichler *et al.,* 2013).

We used a commercially available vector holding the gene for an advanced version of a green fluorescent protein (GFP) enhancer called maxGFP. This vector (pmaxGFP vector, Amaxa) works under the control of the cytomegalovirus (CMV) promoter as an indicator of successful transfer of plasmid DNA into cells. According to the manufacturers, maxGFP is able to produce an increased fluorescence quantum compared to conventionally enhanced GFP and can therefore be used as a sensitive positive control vector, while providing similar transfection efficiencies as well as cell death.

Our study aims to determine the difference in the percentage of transfection efficiency in ZF4 cells using two methods, namely chemical transfection and Nucleofection. Viral strategies are usually used to transfer plasmid DNA into cells that are difficult to transfect. However, while viral strategies are time consuming and involve security risks, nonviral methods are often inefficient for most ‘‘hard-to-transfect’’ cells. Nucleofection has been shown to overcome this limitation. Nucleofection has been widely tested to transfer plasmid DNA into ‘‘hard-to-transfect’’ cells, even Nucleofection succeeded in transferring plasmid DNA into primary cells (Aluigi *et al.,* 2006; Gresch & Altrogge, 2012). Electroporation is a physical transfection method that uses electrical voltage to transiently increase the cell membrane permeability to transfect of the foreign nucleic acid (Chong *et al.,* 2021; Sherba *et al.,* 2020). This method is commonly employed to transfect difficult-to-transfect cells such as stem cells, B cell lines, and primary cells (Canoy *et al.,* 2020). Unlike traditional electroporation, Nucleofection combines cell-specific electrical parameters and Nucleofector to deliver genetic material,

including DNA, oligonucleotides, and small-interfering RNA (siRNA) directly to the nucleus (Han *et al.,* 2008). Out of the numerous transfection reagents currently available from many major biochemical suppliers, we selected nonviral transfection reagents that can be used in serum-containing media namely X-tremeGENE HP and electroporation-based transfection namely Nucleofection by Amaxa. The transfection reagent we tested was X-tremeGene HP (Roche) which according to the manufacturer, is a sterile and nonliposomal blend of lipids and other components supplied in 80% ethanol. X-tremeGene HP is designed to transfect a broad range of eukaryotic cells, including insect cells, many cell lines not transfected well by other reagents, and hard-to-transfect cell lines, such as K-562, HepG2, and HT-1080. K-562 are lymphoblast cells isolated from the bone marrow of a 53-year-old chronic myelogenous leukemia patient. HepG2 [HEPG2] is a cell line exhibiting epithelial-like morphology that was isolated from a hepatocellular carcinoma of a 15-year-old, white, male youth with liver cancer. HT-1080 human cells are epithelial cells derived from connective tissue from a patient with Fibrosarcoma.

MATERIALS AND METHODS

Cell culture

Zebrafish ZF4 cells were obtained from the American Type Culture Collection (ATCC). Zebrafish embryonic fibroblast ZF4 cells were cultured at temperature 28°C and 5% CO_2 incubator, in DME/F-12 (Hyclone) supplemented with 1% Penicillin-streptomycin (Hyclone) and 10% FBS (Gibco).

Transfection of ZF4 cells by X-tremeGENE HP

The procedure of transfection by X-tremeGENE HP was performed according to the manufacturer’s instructions. We allowed X-tremeGENE HP DNA Transfection Reagent, DNA, and diluent to equilibrate to +15 to +25°C. We used pmaxGFP that contain *Green Fluorescense Protein* to introduced to the ZF4 cells. Briefly, we vortexed the X-tremeGENE HP DNA Transfection Reagent vial. We diluted DNA with appropriate diluent to a final concentration of 2 µg plasmid DNA/200 µl medium. We placed 200 µl of diluent, containing 2 µg DNA into each of four sterile tubes labeled 4:1. We used 8 µl of X-tremeGENE HP DNA Transfection Reagent of the total transfection complex in each well. After transfection process, the cells were incubated for 7 hours, 17 hours, and 24 hours at 28°C with 5% CO_2 incubator.

Transfection of ZF4 cells by Nucleofection

Non-viral transfection method to transfer plasmid DNA into ZF4 cells that used in this experiment was Nucleofection (Lonza). The procedure of transfection by Nucleofection (Lonza) was performed according to the manufacturer's instructions. All transfection procedures, we used 4D-Nucleofector Unit X with 100 µl Nucleocuvette vessel, Amaxa SG Cell Line 4D-Nucleofector X Kit L (Lonza), and program DS-134, the cells were put in 6-well plates (Greiner). After Nucleofection process, the cells were incubated for 7 hours, 17 hours, and 24 hours at 28°C with 5% CO_2 incubator.

Transfection efficiency

Post-transfection using two method (X-tremeGENE and Nucleofection), the fluorescence was measured using microscope Zeiss Axiocam and X-Cite Series 120 Lumen Dynamics. Transfection efficiency was calculated as percentage of transfected cells from all cells by counting transfected cells holding a maxGFP signal. Representative images of transfection variations that yielded strong maxGFP signals per cell number were used for cell counting.

RESULTS AND DISCUSSION

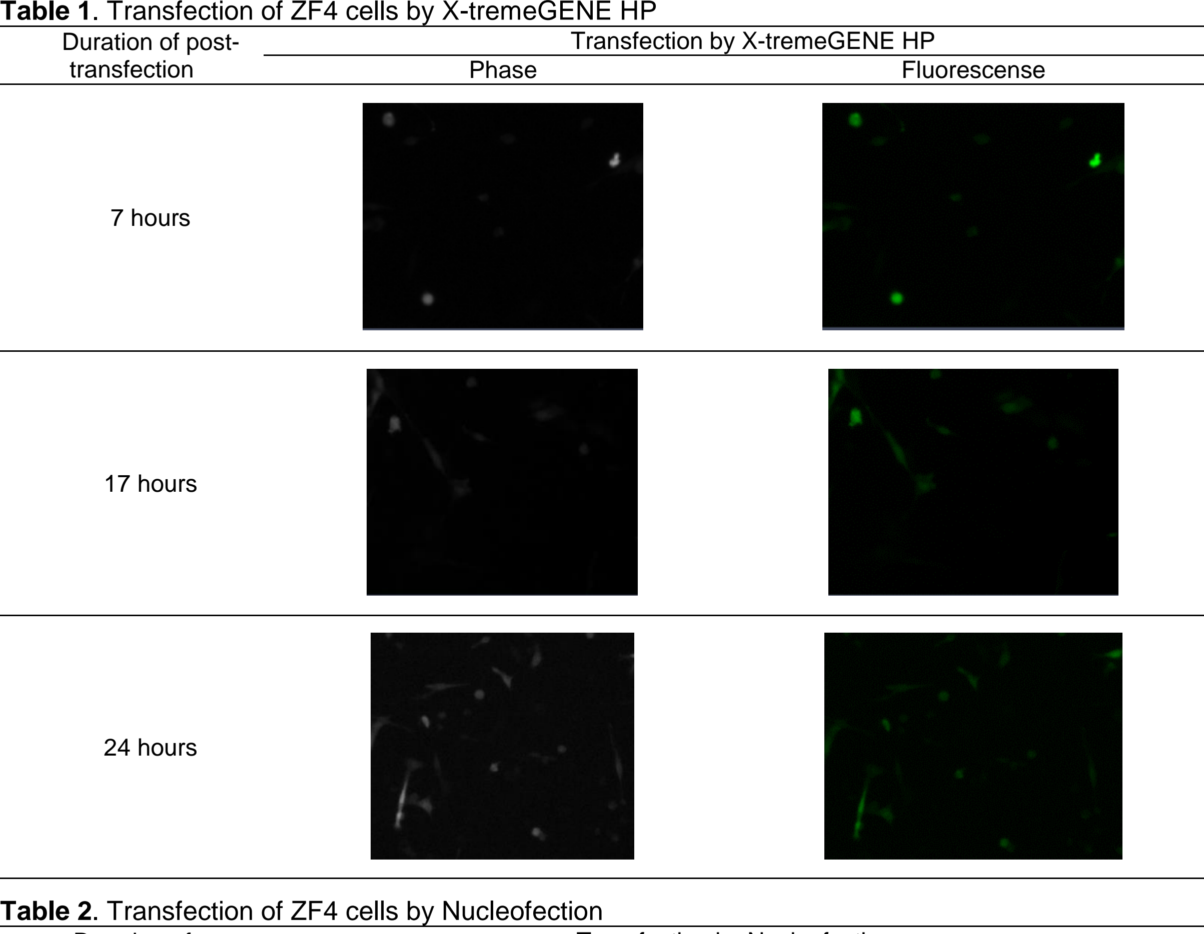
Fluorescence expression

Differences in fluorescent expression at each time point of the incubation period of 7 hours, 17 hours, and 24 hours after pmaxGFP was transfected into ZF4 cells using the X-tremeGENE reagent are shown in **Table 1**. The results showed that the transfection efficiency was very small, less than 5%. after an incubation period of 17 hours and 24 hours there was also no significant increase. Meanwhile, fluorescence expression in cells transfected using the Nucleofection method showed a significant increase from the incubation period of 7 hours to 17 hours and 24 hours (**Table 2**). however, after 24 hours of incubation period, the ability of cells to express genetic material begins to decrease, marked by the number of dead cells.

The results obtained were that at 7 hours of incubation after transfection, the efficiency of transfection in cells introduced with pmaxGFP using X-tremeGENE was very small, only around 3%. Whereas those using nucleofection had a transfection efficiency of 50%. At 17 hours of incubation after transfection, the

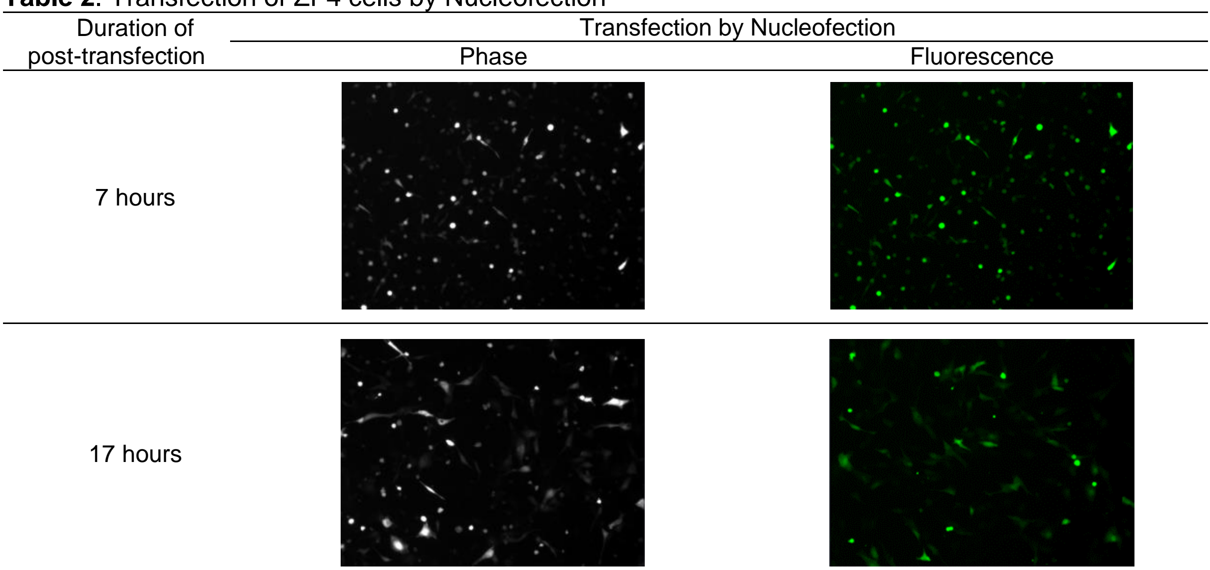
efficiency of transfection in cells introduced with pmaxGFP using X-tremeGENE slightly increased when compared to the incubation period of 7 hours after transfection, which was 4%. Whereas those using Nucleofection had a transfection efficiency of 65%. At 24 hours of incubation after transfection, there was no significant increase in the efficiency of transfection in cells introduced with pmaxGFP using X-tremegene, which was 5%. Meanwhile, those using Nucleofection had a transfection efficiency of 75%. It can be seen from the results obtained on Nucleofection, the number of cell deaths after 24 hours of transfection was higher than those 7 hours and 17 hours after transfection.

Table 1. Transfection of ZF4 cells by X-tremeGENE HP



Duration of post-transfection	Transfection by X-tremeGENE HP	
	Phase	Fluorescense
7 hours		
17 hours		
24 hours		

Table 2. Transfection of ZF4 cells by Nucleofection



Duration of post-transfection	Transfection by Nucleofection	
	Phase	Fluorescence
7 hours		
17 hours		

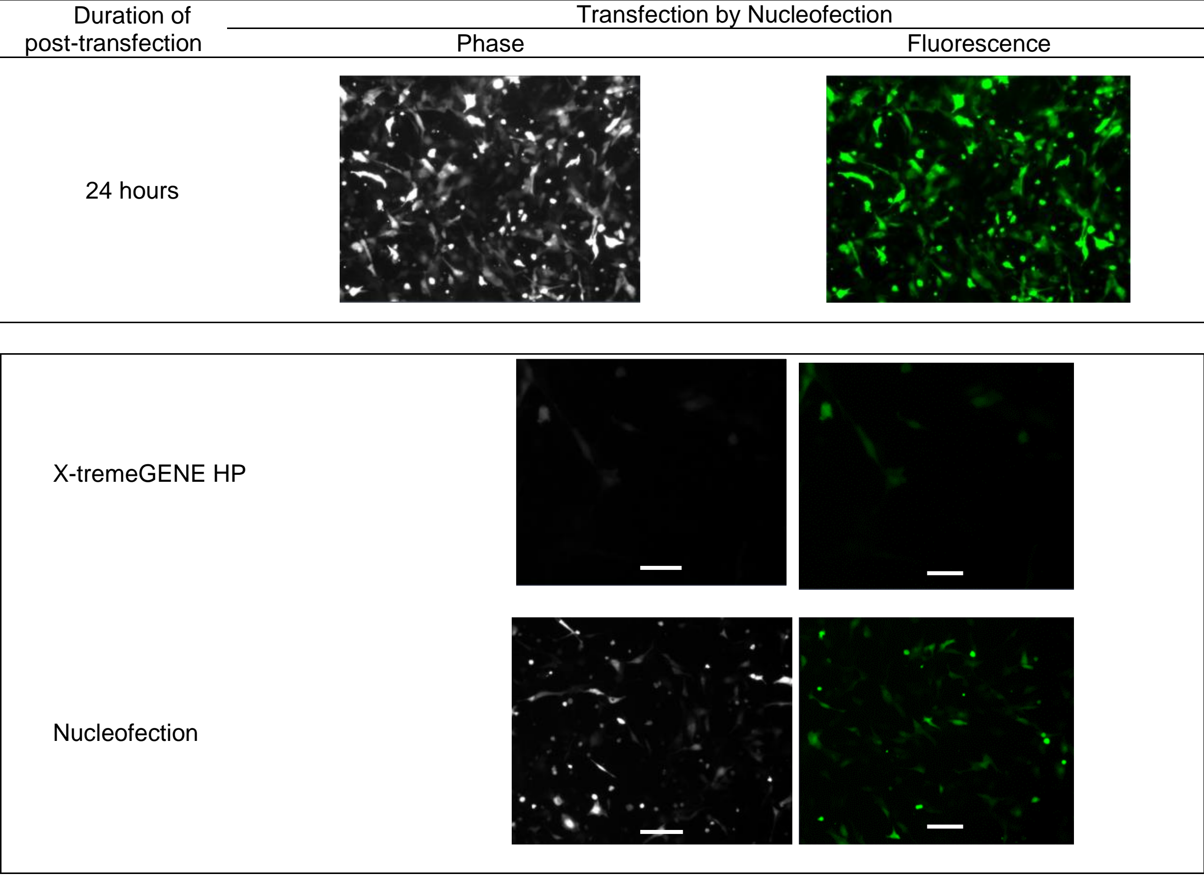


Figure 1. Fluorescence expression of transfected ZF4 cells at 17 hours post-transfection. pmaxGFP vector was transfected into ZF4 cells using X-tremeGENE HP transfection reagent and Nucleofection method with program DS-134. Scale bar 100 µm.

Transfection is an appropriate method for introducing foreign genetic material into cells. The results of chemical transfection efficiency are influenced by several things, including the use of certain types of reagents (Shi *et al.,* 2018), the origin of target cell, the nature of target cell (Wang et al., 2018), and the ratio of reagents and DNA (Gharaati-Far *et al.,* 2018). The measurement of transfection efficiency expressed in terms of the percentage of cells that were successfully transfected is a subjective measurement that is influenced by many variable factors, for example the development of the target cell cycle, patterns of gene expression activity, promoter activity, and activity of a particular cell type. The factors mentioned can influence cells to express the transfected protein. We found that the expression intensity of pmaxGFP differed significantly at each time point. The results obtained in Nucleofection, the number of cell death after 24 hours of transfection was higher compared to 7 hours and 17 hours after transfection. The observed cell death was characterized by the condition of the cells floating on the surface of the medium. Therefore, if cells are needed for further research, such as protein examination, RNA counting, etc., it is recommended that cells be incubated for 17 hours after Nucleofection. Research conducted by (Putri & Chen, 2018) explained that the expression of DNA material transfected into ZF4 cells was at the incubation time point for 17 hours post-Nucleofection (**Figure 1**). The success of transfection using Nucleofection is also influenced by the type of program used (Sherba *et al.,* 2020). There are many choices of programs on the nucleofector machine which means that each program has its own electrical pulse. In this study, we used the DS-134 program because from previous studies it was found that the best program that can transfect DNA into ZF4 cells is DS-134.

CONCLUSIONS AND SUGGESTION

Electrical-based transfection is able to transfer DNA genetic material into zebrafish cells which are known as cells that are difficult to transfect. The transfection efficiency produced by the Nucleofection method reaches 70%-75%. Transfection of DNA with other vectors into ZF4 cells using the Nucleofection method needs to be investigated to find out whether other vectors can be transfected into ZF4 cells as well as pmaxGFP.

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