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Identification of Transfection Efficiency Using Qualitative and Quantitative Analyses of Green Fluorescent Protein in CHO Cells

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Abstract: Transfection is a method that randomly introduce foreign DNA into cells either to produce genetically modified cells or to produce recombinant protein. However, efficient transfection is needed for expression of recombinant protein in mammalian cell. This study aim is to identify the transfection efficiency of novel expression vectors by qualitative and quantitative analyses. The novel expression vectors were named, pZAAGFP (without interested element), pZAAH1C (with integrated element) and pZAAM956 (with enhancer element). Chinese Hamster Ovary (CHO) cells were transfected with 3 different novel expression vectors by using Lipofectamine LTX. After 24 h, these cells were observed under confocal fluorescence microscope. The frequency of both transfected and non-transfected cells were determined. Then, the intensity of Green Fluorescent Protein (GFP) was quantified using fluorescence reader at emission wavelength 506 nm and excitation wavelength 500 nm. Qualitative analysis showed that all novel expression vectors were able to express GFP. More than 80% CHO cells had been successfully transfected with all of the novel expression vectors stated. Quantitative analysis also showed that GFP intensity of both novel expression vectors named, pZAAH1C and pZAAM956 were higher than commercial vector and the GFP intensity of pZAAGFP was about the same as commercial vector. This finding indicates that all novel expression vectors are able to express the GFP gene and both qualitative and quantitative analyses can be applied to determine transfection efficiency.

Key words: Transfection, green fluorescent protein, CHO cells

INTRODUCTION

Transfection is a method of introducing foreign nucleic acids into cells to produce genetically modified cells. Transfection is usually used by researchers to study about the gene function and regulation. Besides that, transfection also can be used in identifying protein function (Kim and Eberwine, 2010). Transfection can be classified into transient transfection and stable transfection. Transient transfection or transient gene expression in mammalian cells is a method that allows the production of recombinant protein in a shorter period as opposed to stable transfection which allows production of recombinant protein in longer period. This is because stable transfection involves the integration of DNA into

the cellular genome. Several approaches that can be used to transfect expression vectors into the mammalian cells are biological, chemical and physical approaches (Kim and Eberwine, 2010). Even though many efficient methods are available, modifications at molecular level are necessary in order to simultaneously increase the production of recombinant protein and the transfection efficiency by taking the approach of producing good expression vectors.

Transfection efficiency can be identified by using Green Fluorescent Protein (GFP) as a reporter gene. GFP was originally discovered back in the early 1960s when researchers studying the bioluminescent properties of the *Aequorea victoria* jellyfish isolated a blue-light-emitting bioluminescent protein called aequorin together with

another protein that was eventually named the green-fluorescent protein (Shimomura *et al.*, 1962). GFP can act as fusion protein when transfected into cells. The function and location of fusion protein was not disturb when they fusion with GFP. Other than that, GFP also can be used to localize and monitor protein translation when they move around the cell (Cody *et al.*, 1993). Some protein need external agents to make them fluoresce. But GFP is able to autocatalytically form a fluorophore (Chalfie *et al.*, 1994). This feature has made GFP one of the most widely used proteins as a marker of protein localization (T sien, 1998). Aim of this study is to identify the efficiency of three novel expression vectors that already constructed. Transfection analysis was analyzed using two analyses (qualitative and quantitative analyses).

MATERIALS AND METHODS

DNA preparation: All novel expression vectors named, pZAAGFP (without interested element), pZAAH1C (with integrated element) and pZAAM956 (with enhancer element) were transformed into *E. coli* TOP10 for plasmid propagation. The plasmids were extracted using QIAprep®Spin Miniprep, (Qiagen, Jerman). Concentration was taken using Biophotometer (Eppendorf, Germany).

Transfection: A day before transfection, cells were seeded at a density of 1.6×10^5 cell mL^{-1} in alpha minimal essential medium (AMEM) (Biowest, UK) with 5% fetal bovine serum (FBS) (Invitrogen, USA) in 35 mm petri dish. When the cells were 70-80% confluent (usually after 24 h), all the medium was removed and 0.6 mL serum-free medium, Opti-Mem® (Invitrogen, USA) was added into the culture. Two mixtures (A and B) need to be prepared separately for transfection purposes. Mixture A consisted of 2000 ng plasmid dissolved in 100 μL Opti-Mem® and 5 μL Plus Reagent and mixture B was made up of 3 μL Lipofectamine LTX and 100 μL Opti-Mem®. Mixture A was incubated for 15 min at room temperature before being added to mixture B. This cocktail was then incubated again at room temperature for another 15 min, allowing the DNA-reagen PLUS-Reagen Lipofectamine® complexes to form. These complexes were added into the culture. Petri dish was moved back and forward to make sure the complexes were evenly spread. The culture was then incubated for 3 h in CO_2 incubator. After 3 h, 1.2 mL of AMEM supplemented with 10% (v/v) FBS were added into the culture. Expression analysis was done after 24 h of transfection.

Expression analysis

Qualitative and quantitative analysis: Confocal fluorescence microscope, LSM 5 PASCAL (Laser Scanning Microscope-Ziess, Germany) was used to acquire the CHO cells images that had been successfully transfected with expression vectors where GFP was used in this study as a reporter gene. The images captured were used to count the number of transfected cells and non-transfected cells. Then, the percentage of transfected cells was calculated to determine the efficiency of expression vectors used. The images were taken 24 h following transfection. Other than imaging analysis, fluorescence reader was also used to identify the intensity of GFP based on the reading OD. The emission wavelength used is 506 nm and the excitation wavelength is 500 nm. The reading was expressed in Relative Fluorescence Unit (RFU).

Statistical analysis: Paired T-test was used to test the difference between the data of each vectors using SPSS version 15.0 (SPSS Inc., USA). Significant value is the data that showed p value less than 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

The novel expression vectors were named, pZAAGFP (without interested element), pZAAH1C (with integrated element) and pZAAM956 (with enhancer element). A commercial vector, phrGFP was used as a positive control. The transfection efficiency of all these modified expression vectors was compared to commercial vector; phrGFP. CHO cell was chosen to become a host because CHO cell is a preferred host for transient transfection (Baldi *et al.*, 2007).

Transfection was then carried out using Lipofectamine reagen which is categorized under cationic lipids group are positively charged at physiological pH and interact with the negatively charged DNA through electrostatic interactions. The lipid-DNA complexes, also called lipoplexes, were internalized through endocytosis and subsequently released the DNA in the cytoplasm (Dass, 2004). After 24 h of transfection, images of transfected cells were captured using confocal fluorescent microscope. Figure 1 showed the images of transfected CHO cells with all the novel expression vectors. The fluorescence cells represented the cells that had been successfully transfected and the GFP gene inside the vectors was expressed. Cells that did not emit any fluorescent light meanwhile, were non-transfected cells. Based on the fluorescence emitted by all of the

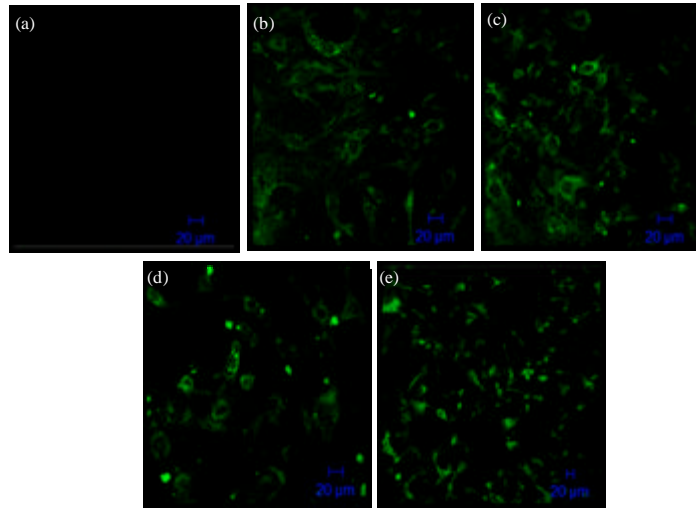


Fig. 1(a-e): Images of transfected CHO acquired by confocal fluorescence microscope at magnification 20X after 24 h of transfection. (a) Negative control, (b) Commercial vector, phrGFP, (c) pZAAGFP, (d) pZAAH1C and (e) pZAAM956

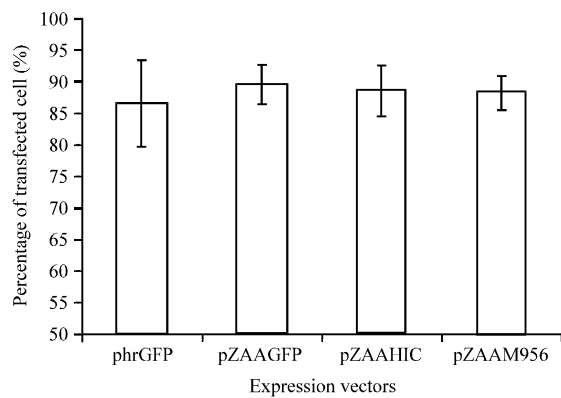


Fig. 2: Qualitative analysis was done by taking pictures using confocal fluorescent microscope. The percentage of transfected CHO cells (%) was obtained from the transfection of different expression vectors, phrGFP, pZAAGFP and pZAAM956. The control was commercial vector, phrGFP. Analysis was done 24 h after transfection. Statistical analysis using paired T-test showed there are no significant difference ($p > 0.05$) of all novel expression vectors to commercial vector, phrGFP

transfected cultures except for negative control's culture, this study concluded that all of the novel expression vectors expressed the GFP gene. This showed that optical imaging technique is important in imaging optical contrast agents and reporter molecules (Luker and Luker, 2008).

Numbers of transfected and non-transfected cells were identified and the percentage of transfected cells was calculated referring to the acquired images. The data showed that the percentage of transfected cells for all the vectors were more than 80% (Fig. 2). Percentages of transfected cells using pZAAH1C was 9% higher while pZAAM956 was 3% higher as compared to commercial vector. These showed that modified expression vectors efficiency is better than commercial vector, phrGFP. Paired T-test also showed that there are no significant different between commercial vector and novel expression vectors ($p > 0.05$).

Other method was done to validate the efficiency of novel expression vectors. Fluorescence reader was used to read the OD at emission wavelength 506 nm and excitation wavelength 500 nm. The cells were seeded in 96-well plate at a density of 1×10^5 cell mL^{-1} . The OD reading was taken and represented by Relative Fluorescence Unit (RFU). Figure 3 is the result of fluorescence intensity in RFU for all expression vectors. The graph for fluorescence intensity showed that the fluorescence intensity for all novel expression vectors was higher as compared to commercial vector (Fig. 3). Statistical analysis using paired T-test showed that there are significant differences ($p < 0.05$) between commercial vector, phrGFP and novel expression vector, pZAA M956. Fluorescence intensity for pZAA M956 was 0.6 fold higher than phrGFP. This result indicated that novel expression vector produced better GFP expression as compared to commercial vector, phrGFP.

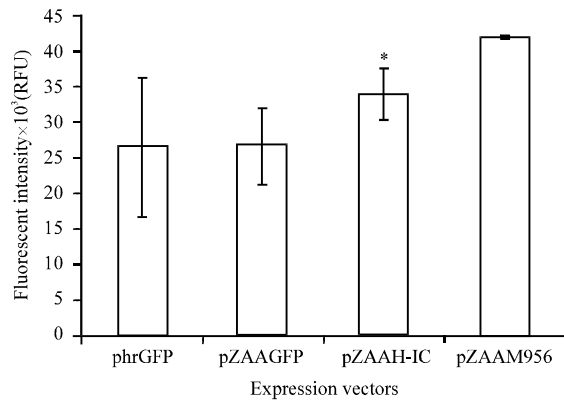


Fig. 3: Quantitative analysis was done using fluorescence reader. The OD was read at emission wavelength 506 nm and excitation wavelength 500 nm. The fluorescent intensity was obtained from the transfection of different expression vectors, pZAAGFP, pZAAH1C and pZAAM956. The control was commercial vector, phrGFP. Analysis was done 24 h after transfection. *corresponds to the significant difference ($p < 0.05$) of pZAAM956 to phrGFP

CONCLUSION

As a conclusion, qualitative and quantitative analyses can be used to identify the transfection efficiency. This study identified the transfection efficiency of all novel expression vectors by using these two analyses.

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