

## Research Article

# SELECTION OF pEGFP-c1-TRANSFECTED-CHO-K1 CELLS BY G418 DECREASED THE EXPRESSION OF GREEN FLUORESCENT PROTEIN

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## ABSTRACT

The most common proteins used for reporter protein is the green fluorescent protein (GFP). It is very convenient to detect the GFP fluorescent by fluorescent microscopy or flowcytometry to monitor the successful transfection. The *gfp* gene can be introduced into the cells by transfecting of two different plasmid vectors or one vector containing both *gfp* and the gene of our interest. In this current experiment, we used pEGFP-c1 plasmid to express *gfp* in CHO-K1 cells. We transfected the CHO-K1 cells by using cationic lipid Lipofectamin 2000. We used this study as a way for predicting our human erythropoietin gene expression study in the CHO-K1 cells. In this study, we showed that expression of GFP decreased after incubation of the cells in selection medium containing G418. Expression of GFP seemed to be stable after about three weeks incubation in selection medium. Recombinant erythropoietin was also detected in the day 20.

**Key words:** Cationic lipid, CHO-K1 cells, erythropoietin, G418, green fluorescent protein

## INTRODUCTION

In the field of pharmaceutical biotechnology, DNA recombinant technology is a very important tool for the production of protein based therapeutics (Leader *et al.*, 2008). DNA recombinant technology has been widely used for the expression of recombinant proteins, the study of protein regulations and signal transductions, and also reporter assay in mammalian cells (Backliwal *et al.*, 2008; Loignon *et al.*, 2008). Gene of interests, such as response elements, reporter genes or gene of the functional proteins, are introduced into the cells by plasmid or viral vectors. Introduction of the vectors into the cells can be done by transfection, electroporation, or transduction (Rauth and Kucherlarapati, 1984). Transfection by using plasmid vector has been commonly used for temporary expression of gene of interests (Bollin *et al.*, 2011; Mariati *et al.*, 2010).

There are several methods of transfection. The cheapest and simplest one is transfection by using calcium phosphate method. However, in some cases, this method performs low transfection efficiency (Girard *et al.*, 2001; Jordan *et al.*, 1996; Rauth and Kucherlarapati, 1984). Other ways to transfect the cells are by using cationic lipid, DEAE

dextran, PEI, activated dendrimer and magnetic nanoparticles (Hunt *et al.*, 2010). Recent methods show better performance and lower cytotoxicities (Jones *et al.*, 2010).

The most common protein which is used for reporter protein is the green fluorescent protein (GFP) (Cheng *et al.*, 1997; Gholamin *et al.*, 2010). The first isolated *gfp* gene is originally from jellyfish *Aequorea victoria* and has been sub cloned into vectors for mammalian expression (Tsien, 1998). It is very convenient to detect the GFP fluorescent by fluorescent microscopy or flowcytometry to monitor the successful of transfection (Misteli *et al.*, 1997; Stuchbury and Munch, 2010). The *gfp* gene can be introduced into the cells by transfection of two different plasmid vectors or one vector containing both *gfp* and gene of interest (Chen *et al.*, 1999). The expressed protein can be two separated protein or fusion of protein (Hanson and Ziegler, 2004; Suzuki *et al.*, 2006).

In our experiment, we used pEGFP-c1 plasmid to express *gfp* in CHO-K1 cells. We monitored the expression of GFP to predict the expression of our recombinant erythropoietin. We showed that expression of GFP was decreased after incubation of the cells

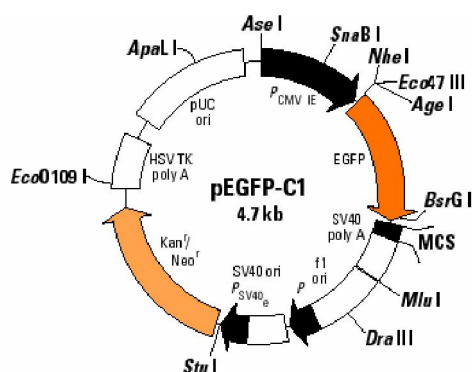


Figure 1. Map of pEGFP-c1 (Clontech).

in selection medium containing G418. Expression of GFP seemed to be stable after about three weeks incubation in selection medium. Recombinant erythropoietin was also detected in the day 20.

## METHODOLOGY

### Cell culture and reagents

CHO-K1 cells were obtained from Prof. Masashi Kawaichi, Nara Institute of Science and Technology (NAIST), Japan. The cells were grown in F-12 medium (Sigma) supplemented with 10% of fetal bovine serum (Sigma) and 100 U/ml of penicillin-100 ug/mL of streptomycin (Invitrogen) in CO<sub>2</sub> incubator with 5% CO<sub>2</sub> at 37°C. Plasmid pEGFP-c1 (Clontech) was obtained from Prof. Kawaichi and pJ-EPO was obtained from DNA 2.0 respectfully. Lipofectamin 2000 was from Invitrogen and antibiotic G418 was from Sigma.

### Transfection of CHO-K1 cells

CHO-K1 cells were grown in 25cm<sup>2</sup> flask in standard culture condition. After two passaging times, the cells were harvested by trypsinization and 200,000cells/mL were seeded into 6 well plate. The next day, cells were transfected with 1 or 2 µg of pEGFP-c1 or pJ-EPO DNA by Lipofectamin 2000 according to the manufacturer's protocol.

### Clones selection

One day after transfections, the expressions of GFP were detected by fluorescent microscopy. The transfected cells

were then passed with passing of ratios 1:5, 1:10, and 1:20 for mock control, GFP transfected and pJ-EPO transfected cells respectively. The following day, the medium was changed with selection medium which containing 2% of G418. The selection medium was refreshed every 2-3 days for 2-3 weeks.

## RESULTS AND DISCUSSION

GFP expression is very important tool for monitoring the transfection efficiency (Cheng *et al.*, 1997, Gholamin *et al.*, 2010). The presence of *gfp* gene in the pEGFP-c1 plasmid (Figure 1) resulted in the expression of GFP in the transfected cells. Transfected CHO-K1 cells expressing GFP fluorescence were observed under fluorescent microscopy. The use of 1 or 2 µg pEGFP-c1 DNA showed equal level of GFP detection one day after transfections (Figure 2).

To obtain stable transfection, the transfected cells have to be cloned in selection medium (Loignon *et al.*, 2008). The plasmid pEGFP-c1 provides selectable marker for eukaryotic expression system by the presence of *neomycin resistance (Neo<sup>r</sup>)* gene. The gene would express an enzyme which digests neomycin or commonly used antibiotic G418 (geneticin). Thus, the successful transformant will exist during the cells selection using selection medium containing G418.

Following the transfections, cells passages and selections decreased the expression level of GFP (Figure 3). Çeltikçi *et al.* (2010) reported that the expression of GFP was faded in the day 4.

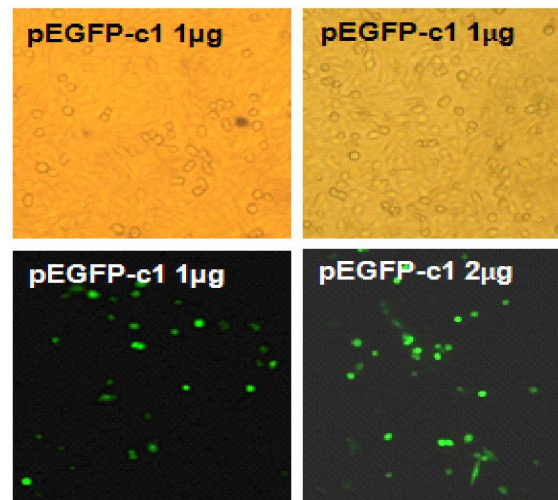


Figure 2. Expression of GFP one day after transfection.

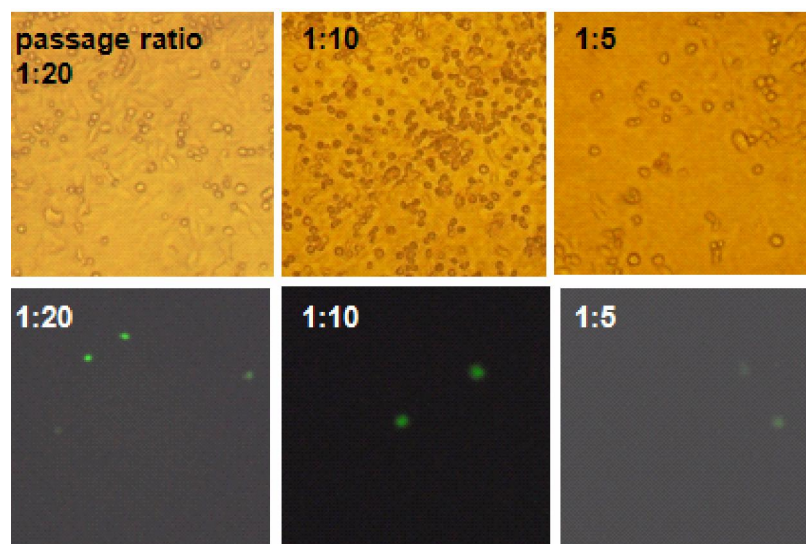


Figure 3. Expression of GFP after cell passage and selection with G418 7 days after transfection.

The sequence of circular pEGFP-c1 plasmid inserted randomly into chromosom by enzymatic digestion (Cherng *et al.*, 1999; Stuchbury and Munch, 2010). There is a possibility of inserted sequence with selectable marker but without *gfp* gene. Thus, the inserted sequence results in the survival of the transfected cells without expression of GFP. Trypsinization during cell harvesting also effects in the quality of the cells (Huang *et al.*, 2010). Moreover, the inserted sequence by

plasmid vector commonly does not guarantee the existence of *gfp* gene in the cell lineage (Stuchbury and Munch, 2010).

In the presence of G418 in the medium, mock control cells, pEGFP-c1 transfected cells, and pJ-EPO transfected cells seemed round and floating (Figure 4). Massive cells death were observed in mock control cells in the day 20. However, transfected CHO-K1 cells looked healthy. The cells grew well and reached confluency (Figure 5).

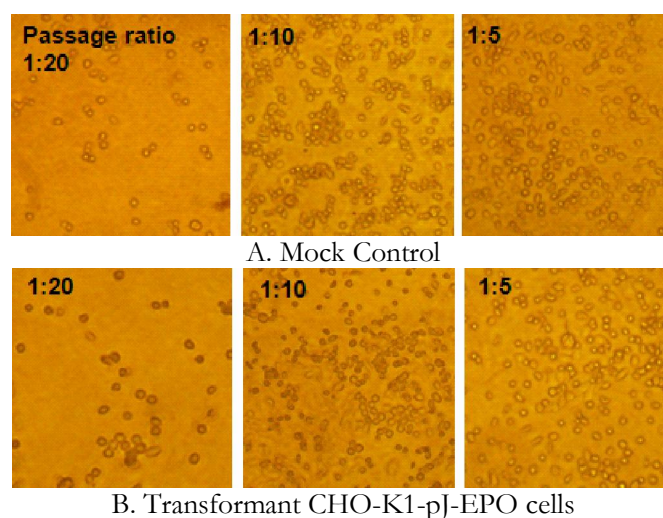


Figure 4. CHO-K1 cells after cell passage and selection with G418 7 days after transfection.

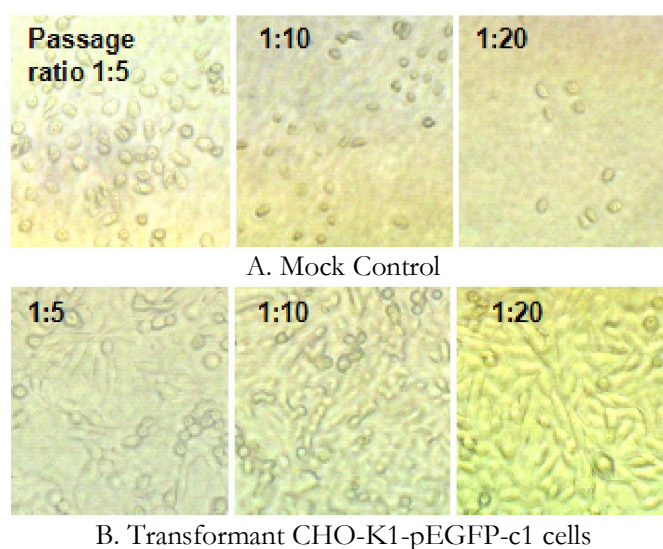


Figure 5. CHO-K1 cells after cell passage and selection with G418 20 days after transfection.

The results indicated the successful transfection of CHO-K1 cells. Without *neomycin resistance* gene, the cells would not survive. Moreover the expression levels of GFP were increased in the day 20 (Figure 6). The supernatants of pJ-EPO transfected cells were examined in day 20 due to the assumption of stable expression of rhEPO. The rhEPO was detected in supernatants of culture medium of clones 1, 2, and 3 (Figure 7).

The trends of decreased and increased expressions of GFP are very interesting phenomenons. After a few weeks, the GFP expressions were detected again in more pEGFP-c1 transfected cells. Consider to these trends, the assumption of successful transfection should be made later after stable transfection reached.

To improve the transfection efficiencies byusing GFP, the transfections were commonly



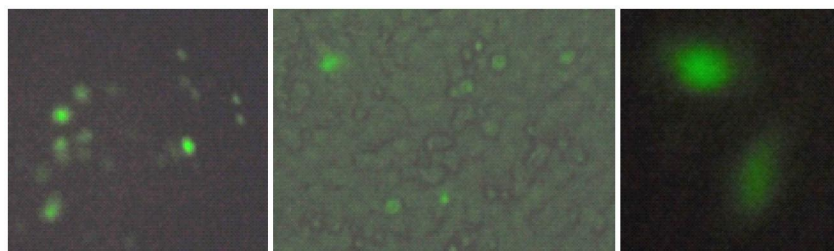


Figure 6. Expression of GFP after cell passage and selection with G418 20 days after transfection. CHO-K1 cells transfected with 2  $\mu$ g pEGFP-c1, passage ratio 1:5.

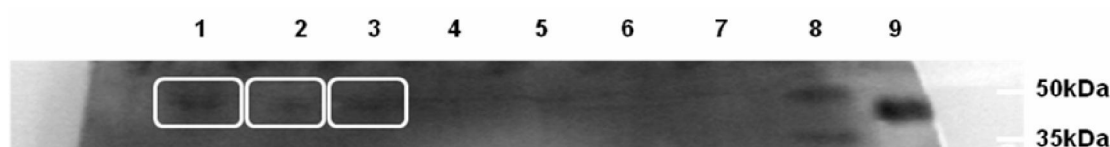


Figure 7. Detection of rhEPO. CHO-K1 cells were transfected with 2  $\mu$ g pJ-EPO DNA. Successful transformants were cloned by using selection medium containing G418. Supernatant of culture medium of transformant of CHO-K1-pJ-EPO were used for Western blot 20 days after transfections. Culture medium of CHO-K1 transfected with: 1-5= pJ-EPO, 6= pEGFP-c1, 7= mock control, 8= marker, 9= epoietin alfa.

done by using one plasmid which contains *gfp* gene and gene of interest. By this method, GFP expression represents the expression level of our protein of interest. This method also eases the process of isolation of single transfected cell or the separation of successful transformants (Suzuki *et al.*, 2006).

## CONCLUSION

Expression of GFP was decreased after cells passages and incubation of the cells in selection medium containing G418 7 days after transfections. Expression of GFP seemed to be stable after about three weeks incubation in selection medium.

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