Asian J. Med. Biol. Res. 2015, 1 (1), 121-129

Asian Journal of Medical and Biological Research
ISSN 2411-4472
www.ebupress.com/journal/ajmbr

Article

Transfection optimisation in HEK-293 cell line

Jay Prakash Sah[1], M. Salahuddin[2], M. Abu Sayed[3*], Abu Saim Al Salauddin[4], Md. Sayfullah[5], Mahjabin Rashid[6], Saidin Saclain[7], Mizanur Rahman Washim[8], Muhammad Aurang Zeb[9], Deluwer Hossain[10], Md. Rayhan Chowdhury[11] and M. A. Momin[4]

[1]Department of Medical Laboratory Science, School of Health and Allied Sciences, Pokhara University, Lekhnath- 12, Kaski, Nepal
[2]Department of Physiology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[3]Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh
[4]Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[5]College of Medicine, Shaheed Ziaur Rahman Medical College Hospital, Bogra, Bangladesh
[6]College of Medicine, Mymensingh Medical College and Hospital, Mymensingh, Bangladesh
[7]Department of Biotechnology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[8]Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh
[9]Department of Biochemistry, Hazara University Mansehra, Khyber Pakhtunkhwa, Islamic Republic of Pakistan
[10]Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[11]Department of Pharmacy, Stamford University Bangladesh, Dhaka 1217, Bangladesh

[*]Corresponding author: M. Abu Sayed, Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh. Email: sayed_bmb@yahoo.com

Received: 20 May 2015/Accepted: 22 June 2015/ Published: 30 June 2015

Abstract: Transfection is the most popular experiments among biological scientist to study the gene expression. But it is really tedious to find out the optimum conditions for protein expression for transfection while working with plasmid DNA. In our present study, the main objective was to setup suitable conditions to study the protein expression. From different conditions, protein expression was observed for 2µg of DNA. We tried different cell number and time frame and got 6.25×10^5 cell/ well of 6 well and 24 hour incubation were the best condition for maximum expression of protein. In our experiment 1:3 ratio of DNA: Lipfectamine was the best for optimum expression of protein in mammalian cell line.

Keywords: cell line; DNA; protein expression; transfection

1. Introduction

Transfection is the insertion of foreign DNA into mammalian or any types of cells. A number of physical, chemical and biological transfection techniques are used such as calcium phosphate precipitation, microinjection, electroporation, receptor-mediated gene transfer, particle guns, viral vectors, polyfection and lipofection (Rosalie *et al.,* 2010; Pfeifer and Verma, 2001; Hacein-Bey-Abina *et al.,* 2002;Woods *et al.,* 2003; Schenborn and Goiffon, 2000; Sheri *et al.,* 1995; Washbourne and McAllister, 2002; O'Brien and Lummis, 2006; Inoue and Krumlauf, 2001; Shirahata *et al.,* 2001;Yao *et al.,* 2008; Kim *et al.,* 1996; Dobson 2006). Calcium phosphate precipitation was the best approach than all other techniques though it is least used recently as because of several disadvantages which include large DNA sample, cellular toxicity and low amount of DNA intake. On the other hand, plasmid DNA transfection is noted as the most effective and superior approach for its

advantages which are: requirement of small DNA sample, minimal cytotoxicity and immunogenicity, cost effectiveness etc. However, the insertion of DNA-lipid complex into nucleus is yet to be fully understood wheareas DNA-lipid complex inevitably a clear pathway. In this well known pathway, the positively charged lipid formed complex with negative charged DNA, this DNA-Lipid complex is known as lipoplex. Lipoplex is slightly cationic which fused with the anionic cytoplasmic membrane of the cell and then enter into the cell cytoplasm through a process called endocytosis (Wagner *et al.*, 2005). Generally, there are two types of transfection stable transfection and transient transfection. Stable transfection requires several weeks to express large amount of protein and contrary transient transfection takes 1-3 days usually used to see the gene expression, development of molecular medicine, to study the signaling methods etc (Kaufman 1997; Colosimo *et al*., 2000; Durocher *et al.*, 2002; Girard *et al.*, 2002). HEK-293 cell line is mostly used cell line to study the transfection, gene expression and virus production, cell cycle research, metabolism, receptor binding, and different molecular biological techniques. HEK-293 choosen over other cell lines due to its merits which are: adaptability, growing capacity in both serum or serum free media, stability, high gene expression rate, yielding large amount of proteins, growing capacity in both adherence and suspension culture conditions etc. This cell line has several variants, such as, HEK-293, HEK 293-T (expressing SV 40 large T antigen), HEK-293 EBNA (expressing the nuclear antigen from Epstein-Barr virus EBNA), HEK293 F (Suspended type) etc. In this study, we have used HEK-293 cell line to setup the optimum conditions to study the best expression of gene and also to minimize the trouble shooting and trialing of our transfection approach (Garnier, *et al.*, 1994; Durocher *et al.*, 2002; Pham *et al.*, 2003; Wurm and Bernard 2001; Van Craenenbroeck *et al.*, 2000; Meissner *et al.*, 2001; Wright *et al.*, 2003; Kim *et al.*, 1997; Pick *et al.*, 2002).

2. Materials and Methods

2.1. Isolation of Plasmid DNA

Plasmid DNA of pFLAG-ptgs2 was prepared using Quiagen Midiprep Plasmid DNA isolation kit. Firstly, a single colony from a freshly streaked selective pFLAG-ptgs2 plate were collected and inoculated into a starter culture of 4 ml LB medium containing the ampicillin and incubated for approximately 8 hrs at 37˚C with vigorous shaking at 300rpm. Secondly, starter culture was diluted 1/1000 into selective 50mL LB medium and grown for 16 hrs .At the next day, bacterial cells were harvested by centrifugation and resuspended the bacterial pellet in 4 ml resuspension (P1) buffer. After that, cells were lysed by adding 4ml of Lysis buffer (P2), then mixed thoroughly by vigorously inverting the sealed tube 6 times, incubated at room temperature for 5 minutes. Next, bacterial cells were neutralized by adding 4 ml of chilled buffer P3 (Neutralising Buffer), following step was incubating on ice for 15 mins then centrifuged at 20000xg for 30 mins at 4˚C. After centrifugation, supernatant containing DNA promptly collected into a new tube and centrifuged again at 20000xg for 15 min at 4˚C. Next supernatant containing plasmid DNA promptly collected and simultaneously the QIAGEN-tip 100 was equilibrated by applying 4 ml buffer QBT. Then, Plasmid DNA containing solution filtered through the column and colum was washed by 10 ml buffer QC (Washing buffer) to remove the impurities. DNA was eluted by Elusion buffer (QF) and precipitated the purified DNA was done by isopropanol and 70% ethanol, last but not least , DNA pellet was dissolved in Trypsin-EDTA buffer. Finally the amount of DNA were measured by Nano-drum.

2.2. Measurement of DNA concentration

The amount of purified plasmid DNA was measured by Nano-drum software (NanoDrop 1000 v3.8.00). Firstly, the machine was blanked using DNAase free water, secondly a drop of DNA was loaded into the loading chamber finally the amount of DNA were measured by clicking measure button of the NanoDrop 1000 v3.8.00 software. The amount of plasmid DNA were more than 500 ng/µl.

2.3. Cell culture

HEK-293 cell were cultured in the DMEM media containing 10% serum and antibiotic (PEN-Strep).The day before transfection different number of cells ($5 \text{X} 10^5$, $5.25 \text{X} 10^5$, $5.75 \text{X} 10^5$, $6.25 \text{X} 10^5$ and $7 \text{X} 10^5$) were cultured in 2 ml of FDMEM media in each well of six well plate. In the morning of the day of transfection media were changed by the fresh media and cells were allowed to grown for different time point in the incubator at 37^{o}C with 5% CO_2.

2.4. Western blot

Western blot analysis was performed as described previously Felgner *et al*., 1994 (35). In brief, cells were washed in cold PBS, and lysed with lysis buffer containing 50mM Tris-HCl (pH-7.4), 150mM NaCl, 1mM DTT, 30mM NaF, 10mM Na3VO4, 0.5% NP40, and a protease inhibitor cocktail (Roche). Cell lysates were subjected to 12% SDS-PAGE and transferred to a PVDF membrane (Millipore). For immunobloting, membranes were blocked for 2 h at room temperature with 5% nonfat dry milk in 0.5% TBST. Membranes were washed four times in TBST and incubated overnight at 4^0C with primary antibodies in 5% nonfat dry milk. Following four additional washes in TBST, the membrane were further incubated for 1 h with HRP-conjugated secondary antibodies. Bounded antibodies were detected using chemiluminescent HRP substrate (Millipore, USA) and Chemiluminescent Imaging System (ChemiDoc^{TM}).

2.5. Transfection

HEK-293 cells were cultured in 6 well plate. The day before transfection cells were trypsinised using Trypsin-EDTA ,trypsinised cells allowed to grow overnight, in the next morning media changed with fresh FDMEM media before transfection. For transfection 2ug of DNA first added to 500 ul OPTIMEM and incubated 5 minutes, then added 6 µl of (DNA:LIPO=1:3) to the OptiMEM containing DNA, mixed well then incubated 30 minutes. After 30 minutes DNA-Lipo complex added to the cell drop by drop and incubated cell at different time frame (12-96 hours). In some experiment the tranfected media were changed intentionally after 6 hours to observe the effects of media change in tranfection.

3. Results and Discussion

There are lots of techniques available in science to study the transfection and gene expression but still transfection through plasmid is indeed unique and superior. We explored combination of several contributing factors to alter the transgene expression capacity in transfection. Firstly, the issue of using antibiotics has revealed from our experiment. Generally, penicillin and streptomycin are used in cell culture to prevent the cells from contamination. Although, the antibiotics are detrimental for cells as they reduced the rate of transfection. In our study, we have found that antibiotics have minimum or no effects on transgenic expression and it did not even interfere with the expression of protein. To study the effects of antibiotic on expression of PTGS2 gene in HEK-293 cell, we have used the both media with or without antibiotics. At first culture, the cell from the day before transfection with antibiotic, whereas, on the day of transfection, media of one sample changed without antibiotic and another with antibiotic then incubated the HEK-293 transfected cell for 48 hours more and we have not realized any significant difference between antibiotic and non antibiotic containing mediums (Figures 1A and 1B).

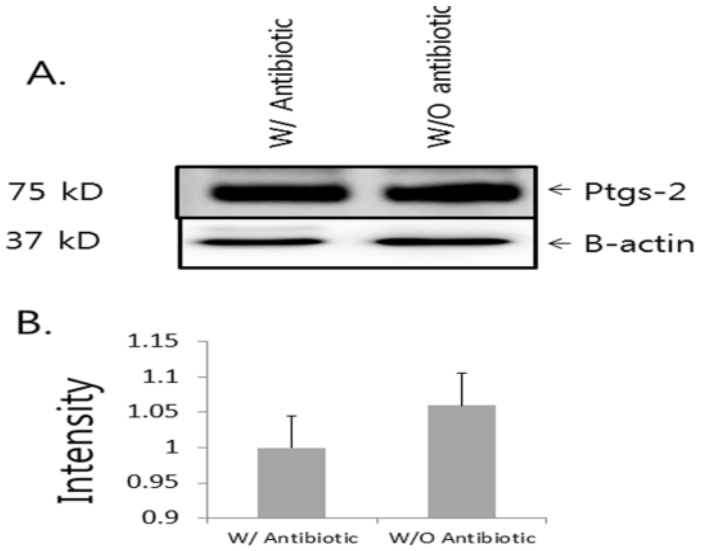


Figure 1. Effects of antibiotic in transfection. HEK-293 cells were cultured in DMEM media and Transfected with ptgs2. Then incubate for 48 hours in the presence and absence of antibiotic. A. Expression of ptgs by western blot. B. Analysis the intensity of the transfected protein analyse by ImageJ software.

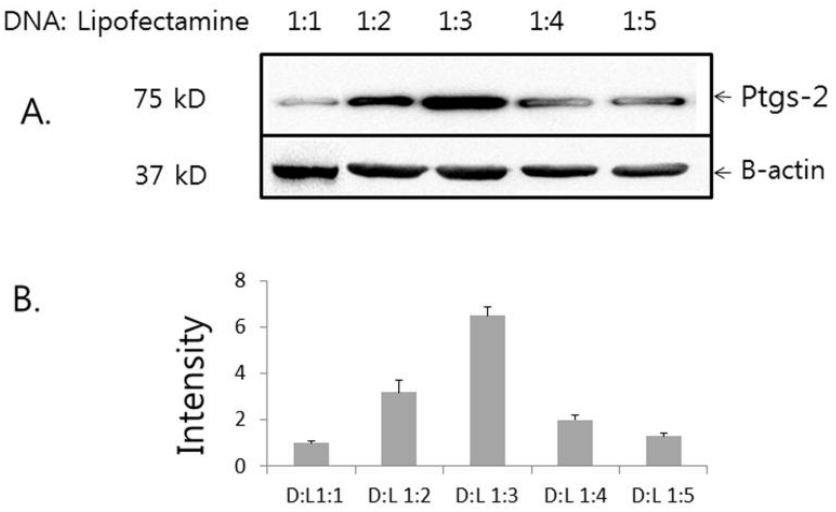
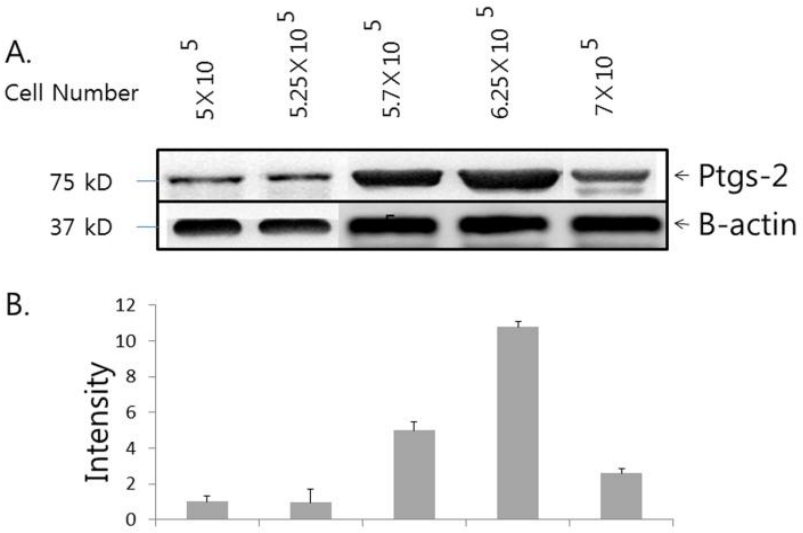


Figure 2. Effects of different ratio of DNA and Lifectamine in transfection. HEK-293 cells were cultured in DMEM media and Transfected with ptgs2. Then incubate for 24 hours in the presence of different ration of DNA and Lipofectamine (DNA:Lipo, 1:1, 1:2, 1:3, 1:4, 1:5). A. Expression of Ptgs2 by western blot. B. Intensity of the transfected protein to different ratio of DNA and lipoanalyse by ImageJ.



.

Figure 3. Effects of different cell number in transfection. HEK-293 cells were cultured in DMEM media and Transfected with ptgs2. Then incubate for 24 hours in the presence of different number of cell ($5X10^5$, $5.25X10^5$, $5.75X10^5$, $6.25X10^5$ and $7X10^5$). A. Expression of Ptgs2 by western blot. B. Intensity of the transfected protein in different number of cells.

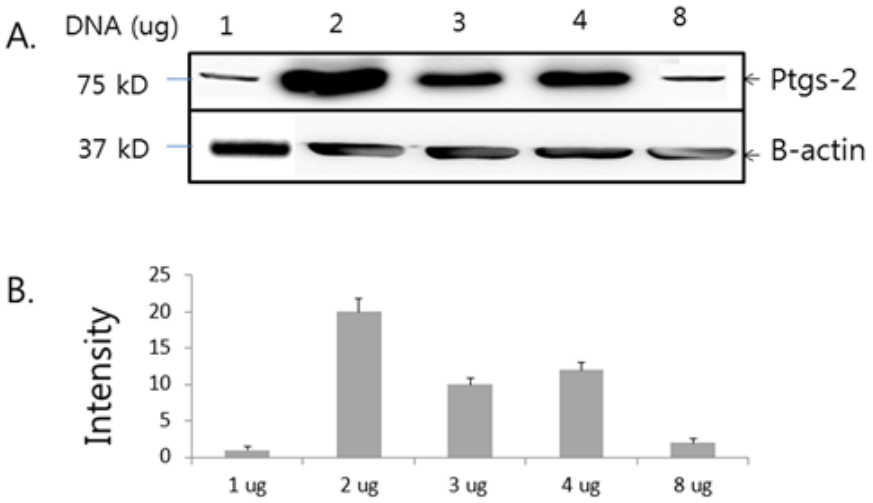


Figure 4. Effects of the amount of DNA in transgene expression. HEK-293 cells were cultured in DMEM media and Transfected with different amount of ptgs2 . Then incubate for 24 hours in the presence of 6.25×10^5 cells and DNA:Lipo 1:3. A. Expression of Ptgs2 at different amount of DNA by western blot. B. Intensity of the transfected protein in presence of various amount of DNA.

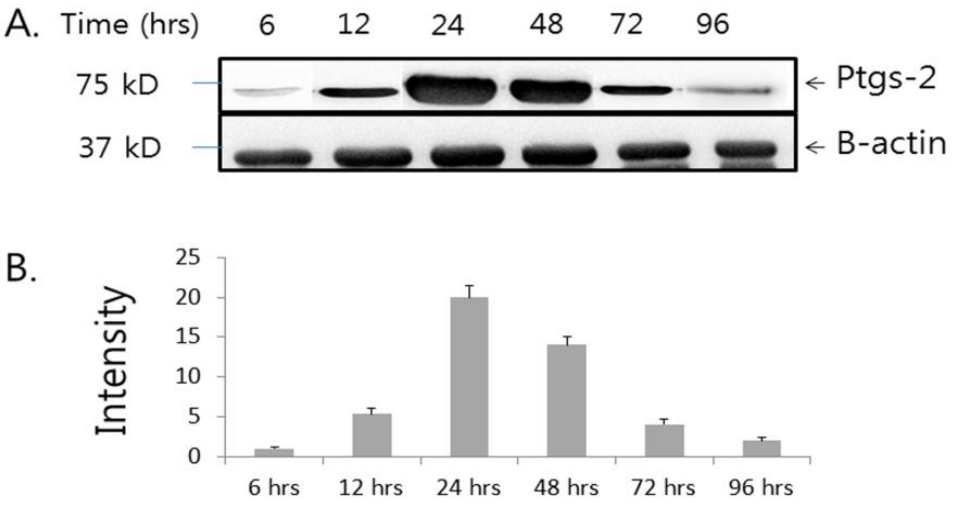


Figure 5. Effects of different time incubation in transfection and transgene expression. HEK-293 cells were cultured in DMEM media and Transfected with ptgs2. Then incubate for 6-96 hours in the presence of 6.25×10^5 cells. A. Expression of Ptgs2 at different time by western blot. B. Intensity of the transfected protein in different fashion.

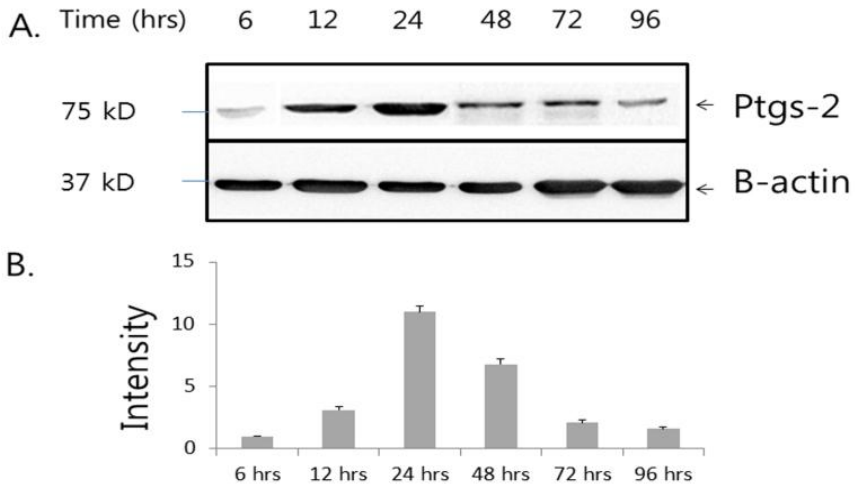


Figure 6. Effects of changing the tranfected media after 6 hrs post transfection in transgene expression. HEK-293 cells were cultured in DMEM media and Transfected with ptgs2. Then incubate for 6-96 hours in the presence of $6.25 \text{X} 10^5$ cells and DNA:Lipo 1:3.A. Expression of Ptgs2 at different time after changing of transfected media by western blot. B. Intensity of the transfected protein at different time fashion.

Moreover, in transient transfection process we incubated transfected cell for very short span of time (24-48hrs) so antibiotics were not essential as we strictly maintained the sterilization procedures. Later, we studied the effects of different ratio of DNA and lipofectamine in transgenic expression. The ratio of lipofectamine to DNA is significantly important to study the expression of transgene (Colosimo *et al.*, 2000; Felgner *et al.*, 1994) because excess amount of lipid complex leads to cytotoxicity of cell whereas the very low amount cannot carry the DNA to cytoplasm for expression of the protein. In several consecutive experiments, we found that low amount of lipofectamine (DNA:Lipo) is not sufficient to induce the expected level of ptgs2 protein expression in western blot experiment. In contrast, too much DNA: Lipo (>1:4) has also the negative effects on protein expression due to cellular toxicity (Figure 2A). The best expression we get DNA:lipofectamine is 1:3 ratio which ratio of DNA to lipo is around 2 times more than 1:2 and three to four times than 1:4 or 1:5 (Figure 2B). The excessive amount of lipofectamine higher than 1:5 is highly poisonous to the cell and severe cellular death occurred when DNA: Lipo 1:8 (data not shown). Number of the cell and confluency during transfection is one of the most considerable factor. Now, the expression efficiency of transfected gene greatly depends on the confluency of cell. If the cell numbers are too low, the high amount of DNA or lipo become toxic to the cell and if there are over confluent cells can also reduce the expression of target gene. According to the purpose of study scientists recommended different cell numbers for different types of cells i.e. usually 30-90% confluency at the time of transfection. In our experiment, we found that $6.25 \text{X} 10^5$ cell/2 mL/well of six well plate, when the confluency of cells becomes 60% at the time of transfection is the best for expression of our PTGS2 protein in HEK-293 cell. The expression of PTGS2 was around six times compared to $5 \text{X} 10^5$, $5.25 \text{X} 10^5$ cell/well, two times to $5.75 \text{X} 10^5$ and 3.5 times to $7 \text{X} 10^5$ (Figure 3A), we observed the confluency at the time of transfection was around 40-55% incase of $5 \text{X} 10^5$-$5.75 \text{X} 10^5$ cell, and 60-70% incase of $6.25 \text{X} 10^5$ cell/each well. And confluency of the $7 \text{X} 10^5$cell/well was more than 90% during transfection. We experienced at least three focus in each plate to confirm the confluency. Though it was great dilemma as over confluent cells showed lower expression than optimum number ($6.25 \text{X} 10^5$) of cell (Figures 3A and 3B). The speculation in this case possibly the competition between cells to uptake the nutrition and lipoplex from media which hinders the maximum entrance and excessive metabolites produces from the cells may also inhibit the uptake of lipoplex. It is possible assumption of yielding 3 times less expression of ptgs2 protein in HEK-293 transfected cell with more than 90% confluency compared to 60% confluent cell. In case of delivery and expression of gene, determination of the optimum mass of plasmid DNA is most important issue in transfection and transgene

expression study. The higher amount of DNA is not efficient like as lower amount of DNA (Figures 4A and 4B). So finding the exact amount is very important in transgene expression. In our repeated experiments we have found that 2 µg DNA is the optimum and sufficient to express the highest amount of PTGS2 protein in HEK-293 cell. Too much DNA greatly reduce the expression of the protein also too less DNA unable to produce the expected amount (Figure 4A). There are some report that DNA mass more than 4 is highly cytotoxic even 4 ug DNA can induce the cellular death at a high level whereas 1.5 ug DNA is sufficient to express the highest amount of protein (Galbraith *et al.,* 2006;Ye *et al.,* 2009; Carpentier *et al.,* 2007; Colosimo *et al.,* 2000). In our experiment, we understood that, 8 µg DNA induce huge cell death and reduced the expression of protein almost basal Level. The expression level of PTGS2 protein in 2 µg was 2-3 times higher than cell transfected with 3 or 4µg DNA (Figure 4B). Optimization of cell transfection typically includes determination of the optimum mass of plasmid to be transfected and the time point after transfection that provides the maximum expression. Also the change of original media after some hours of transfection with freshly supplied media reduced the expression of transfected gene (Figure 5A). The cells continued to proliferate in media and number of the cells increased after transfection in a time dependent manner. At the same time, the proliferative cell dilute the amount of transfected DNA for the expense of proliferation. As a result, determination of the optimum time point for maximum expression of protein is really important to effective study of the protein expression. In our consecutive experiments, we have explored that maximum expression of protein occurs within first 24 hours. After 24 hours the amount of expressed protein starts to decline and after 96 hours the amount of expressed protein are not present at considerable level (Figure 5A). The relative expression of protein at 24 hours is about 3 times compared to 12 hours and 1.5 times compared to 48 hours post-transfection (Figure 5B).

Expression of protein reduced to half when transfected media changed by fresh media after 6 hours of transfection (Figure 6A). It has observed that the expression level reduced to almost half in 24 and 48 hours post-transfection when tranfected media changed after 6 hours (Figure 6B). Many protocols suggest to change the media after some hours of post-transfection ,though we have found that it was not good to change the transfected media. Instead of post-transfection media replacement it was better to change the media at the morning before transfection to minimize or reduce the cellular metabolites and also to remove the dead cell debris from media. Because dead cells and cellular metabolites significantly hinders the uptake of DNA thereby expression of protein reduced.

4. Conclusions

Determination of optimum conditions for the transgene expression was really a challenging task which related with number of factors like cell number, presence or absence of antibiotics, number of cell, amount of DNA, DNA:Lipid reagent ratio, time of post-incubation, whether to replace transfected media after transfection or not etcetra. Protein expression levels could be different with the same cell line by using different genes and also by different genes in same cell line. However, considering all of these data we can easily conclude that the best conditions to get the maximum expression of protein in transfection of HEK-293 cell with 2 µg DNA, DNA: Lipo ratio 1:3 and 24 hour post-tranfection.

Acknowledgements

We are grateful to Dr. K. Yoo for his constant inspiration and technical support.

Conflict of interest

None to declare.

References

Carpentier E, S Paris, AA Kamen and Y Durocher, 2007. Limiting factors governing protein expression following polyethylenimine-mediated gene transfer in HEK293-EBNA1 cells. J Biotechnol., 128: 268–280.

Colosimo A, KK Goncz, AR Holmes, K Kunzelmann, G Novelli, RW Malone, MJ Bennett and DC Gruenert, 2000. Transfer and expression of foreign genes in mammalian cells. Biotechniques, 29:314-331.

Colosimo A, KK Goncz, G Novelli, B Dallapiccola and DC Gruenert, 2001. Targeted correction of a defective selectable marker gene in human epithelial cells by small DNA fragments. Mol. Ther., 3:178-185.

Dobson J, 2006. Gene therapy progress and prospects: magnetic nanoparticle-based gene delivery. Gene Ther., 13:283–287.

Durocher Y, S Perret and A Kamen, 2002. High-level and high-throughput recombinant protein production by transient transfection of suspension-growing human 293-EBNA1 cells. Nucleic Acids Res., 30:e9.

Felgner JH, R Kumar, CN Sridhar, CJ Wheeler, YJ Tsai, R Border, P Ramsey, M Martin and PL Felgner, 1994. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. J. Biol. Chem., 269:2550-256.

Galbraith DJ, AS Tait, AJ Racher, JR Birch and DC James, 2006. Control of culture environment for improved polyethylenimine-mediated transient production of recombinant monoclonal antibodies by CHO cells. Biotechnol. Prog., 22:753–762.

Garnier A, J Coté, I Nadeau, A Kamen and B Massie, 1994. Scale-up of the adenovirus expression system for the production of recombinant protein in human 293S cells. Cytotechnology, 15:145-155.

Girard P, M Derouazi, G Baumgartner, M Bourgeois, M Jordan, B Jacko and FM Wurm, 2002.100-liter transient transfection. Cytotechnology, 38:15-21.

Hacein-Bey-Abina S, FD Le, F Carlier, C Bouneaud, C Hue, JPV De, AJ Thrasher, N Wulffraat, R Sorensen, S Dupuis-Girod, A Fischer, EG Davies, W Kuis, L Leiva and M Cavazzana-Calvo, 2002. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. N. Engl. J. Med., 346:1185–1193.

Inoue T and R Krumlauf, 2001. An impulse to the brain-using in vivo electroporation: Nat. Neurosci., 4:1156–1158.

Kaufman RJ, 1997. DNA transfection to study translational control in mammalian cells. Methods, 11:361-370.

Kim CH, Y Oh and TH Lee, 1997. Codon optimization for high-level expression of human erythropoietin (EPO) in mammalian cells. Gene,199:293-301.

Kim HJ, JF Greenleaf, RR Kinnick, JT Bronk and ME Bolander, 1996. Ultrasound-mediated transfection of mammalian cells. Hum. Gene Ther., 7:1339–1346.

Meissner P, H Pick, A Kulangara, P Chatellard, K Friedrich and FM Wurm, 2001.Transient gene expression: recombinant protein production with suspension adapted HEK293-EBNA cells. Biotechnol. Bioeng., 2001, 75:197-203.

Muller N, M Derouazi, TF Van, S Wulhfard, DL Hacker, M Jordan, FM Wurm, 2007. Scalable transient gene expression in Chinese hamster ovary cells in instrumented and non-instrumented cultivation systems. Biotechnol. Lett., 29:703–711.

O'Brien JA and SC Lummis, 2006. Biolistic transfection of neuronal cultures using a hand-held gene gun. Nat. Protoc. 1:977–981.

Pfeifer A and Verma IM, 2001. Gene therapy: promises and problems. Annu. Rev. Genomics. Hum. Genet. 2:177–211.

Pham PL, S Perret, HC Doan, B Cass, G St-Laurent, A Kamen and Y Durocher, 2003. Large-scale transient transfection of serum-free suspension-growing HEK293 EBNA1 cells: peptone additives improve cell growth and transfection efficiency. Biotechnol. Bioeng., 84:332-342.

Pick HM, P Meissner, AK Preuss, P Tromba, H Vogel and FM Wurm, 2002. Balancing GFP reporter plasmid quantity in large-scale transient transfections for recombinant anti-human Rhesus-D IgG1 synthesis. Biotechnol. Bioeng., 79:595-601.

Rosalie M, DS David, E Hamid, B Babak, A Alireza, P Hooman and CG Dieter, 2010. Comparative transfection of DNA into primary and transformed mammalian cells from different lineages. BMC Biotechnology, 10:9

Schenborn ET and V Goiffon, 2000. DEAE-dextran transfection of mammalian cultured cells. Meth. Mol. Biol., 130:147–153.

Sheri L, MW Holmen, RR Vanbrocklin, SR Eversole and CG Stapleton Leonard, 1995. Efficient lipid-mediated transfection of DNA into primary rat hepatocytes. In Vitro Cell. Dev. Biol. Anim., 31:347–351.

Shirahata Y, N Ohkohchi, H Itagak and S Satomi, 2001. New technique for gene transfection using laser irradiation. J. Investig. Med., 49:184–190.

Van Craenenbroeck K, P Vanhoenacker and G Haegeman, 2000. Episomal vectors for gene expression in mammalian cells. Eur. J. Biochem. 267:5665-5678.

Wagner E, C Culmsee and S Boeckle, 2005. Targeting of polyplexes: toward synthetic virus vector systems. Adv. Genet., 53:333-354.

Washbourne P and AK McAllister, 2002.Techniques for gene transfer into neurons. Curr. Opin. Neurobiol., 12:566–573.

Woods NB, A Muessig, M Schmidt, J Flygare, K Olsson, P Salmon, D Trono, C von Kalle and S Karlsson, 2003. Lentiviral vector transduction of NOD/SCID repopulating cells results in multiple vector integrations per transduced cell: risk of insertional mutagenesis. Blood, 101:1284–1289.

Wright JL, M Jordan and FM Wurm, 2003.Transfection of partially purified plasmid DNA for high level transient protein expression in HEK293-EBNA cells. J. Biotechnol., 102:211-221.

Wurm FM and A Bernard, 2001.Transientgeneexpression from mammalian cells - a new chapter in animal cell technology? Cytotechnology, 35:155-156.

Yao CP, ZX Zhang, R Rahmanzadeh and G Huettmann, 2008. Laser-based gene transfection and gene therapy. IEEE Trans. Nanobioscience, 7:111–119.

Ye J, V Kober, M Tellers, Z Naji, P Salmon and JF Markusen, 2009. High-level protein expression in scalable CHO transient transfection. Biotechnol. Bioeng., 103: 542–551.