Original Article

Optimization of Gene Transfection in Murine Myeloma Cell Lines using Different Transfection Reagents

Mahdi Shabani [1,2], Sheyda Hemmati [2], Reza Hadavi [3], Zahra Amirghofran [1], Mahmood Jeddi-Tehrani [2,4], Hodjatallah Rabbani [2,4], and Fazel Shokri [2,5*]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
3. Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
4. Immune and Gene Therapy Lab, Cancer Center Karolinska, Karolinska Hospital, Karolinska Institutet, Stockholm, Sweden
5. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Purification and isolation of cellular target proteins for monoclonal antibody (MAb) production is a difficult and time-consuming process. Immunization of mice with murine cell lines stably transfected with genes coding for xenogenic target molecules is an alternative method for mouse immunization and MAb production. Here we present data on transfection efficiency of some commercial reagents used for transfection of murine myeloma cell lines. Little is known about transfectability of murine myeloma cell lines by different transfection reagents. Mouse myeloma cell lines (SP2/0, NS0, NS1, Ag8, and P3U1) were transfected with pEGFP-N1 vector using Lipofectamine 2000, jetPEI and LyoVec commercial transfection reagents in different combinations. The transfection permissible HEK293-FT cell line was used as a control in transfection procedure. Transfected cells, expressing the Enhanced Green Fluorescent Protein (EGFP), were analyzed by flow cytometry 48 *hrs* post transfection. Our results showed transfection efficiency of 71%, 57% and 22% for HEK293-FT, 5.5%, 3.4% and 1% for SP2/0, 55.7%, 21.1% and 9.3% for NS0, 8.2%, 6% and 5.5% for NS1, 22%, 49.2% and 5.5% for Ag8 and 6.3%, 21.5% and 4.6% for P3U1 cell lines after transfection with Lipofectamine 2000, jetPEI and LyoVec reagents, respectively. Our data indicate that NS0 and Ag8 are efficiently transfected by Lipofectamine 2000 and jetPEI reagents. Finally, we propose Ag8 and NS0 cell lines as suitable host cells for efficient expression of target genes which can be used for mouse immunization and MAb production.

Avicenna J Med Biotech 2010; 2(3): 123-130

Keywords: Enhanced green fluorescent protein, Flow cytometry, Monoclonal antibody, Myeloma cell lines, Transfection

*** Corresponding author:**
Fazel Shokri, Ph.D.,
Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran, P.O. Box: 19615-1177
Tel: +98 21 22432020
Fax: +98 21 22432021
E-mail: fazshok@yahoo.com
Received: 29 Jun 2010
Accepted: 21 Aug 2010

Introduction

Over the past decade, gene delivery systems have been increasingly used to study and control gene expression [(1)]. There are two main types of transfection strategies based on the use of non-viral (chemical and physical) and viral transfection agents. The term transfection refers to the process of deliberately introducing nucleic acids into eukaryotic cells used notably for non-viral methods. Chemical methods include the use of cationic liposomes (lipoplex), polymers (polyplex), combinations of the two (lipopolyplex), calcium phosphate,

and DEAE dextran. In almost all of these chemical methods, the reagents promote transfection by complexing with the DNA to neutralize the charge, condensing the DNA, mediating interaction and attachment to the cell membrane, and promoting entry into the cell, typically via endocytosis and subsequent endosomal escape [2].

In addition to the chemical methods, a number of physical methods exist that promote the direct entry of uncomplexed DNA into the cell. These methods can include microinjection of individual cells, hydroporation, electroporation, ultrasound, and biolistic delivery (*i.e.*, the gene gun) [2, 3]. Nucleic acid transfection has provided useful tools to study up-regulation or down-regulation of gene expression and transcriptional and post-transcriptional regulation of various genes and gene products [2, 4, 5]. Furthermore, the most interesting clinical use of DNA transfection is gene therapy which has brought new hope for treatment of several diseases like cystic fibrosis [6 - 8].

One of the potential applications of these methods is monoclonal antibody (MAb) production. In this regard, mouse immunization with murine cell lines stably transfected with genes coding for xenogenic target molecules would focus the mouse B cell response on the transfected molecules leaving the self molecules of the mouse myeloma cells unrecognized. This procedure overcomes the difficulty of purification and isolation of cellular target proteins used for immunization. Since myeloma cell lines are commonly used as syngenic cell types for fusion with mouse splenocytes for establishment of hybridomas, the same cell lines are suitable tools for delivery and presentation of target genes to the immune system of immunized mice.

To our knowledge, no detailed reports have so far been published regarding lipoplex and polyplex-mediated transfection of DNA into different myeloma cell lines. In this study we employed the EGFP expression system to compare the efficiency of three different gene transfection reagents (lipoplex and polyplex) in five commonly used mouse myeloma cell lines.

Materials and Methods

Cell lines

Mouse myeloma cell lines (SP2/0-Ag14 [SP2/0], NS0, NS1, P3X63Ag8.653 [Ag8], and P3U1) (National Cell Bank of Iran, Tehran, Iran) and HEK293-FT (Invitrogen, CA, USA) were cultivated in RPMI-1640 (Gibco, Invitrogen, USA) culture medium supplemented with 10% fetal bovine serum (Biochrom AG, Berlin, Germany), penicillin (100 *U/ml*) (ICN Biomedicals, Ohio, USA) and streptomycin (100 *μg/ml*) (Sigma, St Louis, USA). All cell lines were cultured at 37 *°C* in a humidified incubator supplied with 5% CO_2 atmosphere.

Plasmid preparation

The competent *Escherichia coli (E. coli)* JM109 (Promega, Wisconsin, USA) was transformed by pEGFP-N1 vector (Clontech, Palo Alto, USA) according to standard protocol. Briefly, 100 *ng* of the pEGFP-N1 was added to 50 *μl* of competent cells and kept for 25 *min* on ice. Then the mixture was incubated for 70 *sec* at 42 *°C* and moved immediately on ice for 2 *min*. In the following step, 200 *μl* LB medium was added to transformed cells and incubated at 37 *°C* for 1 *hr*. The transformed bacteria were selected on LB-kanamycin agar plate. The pEGFP-N1 vector was purified from cultured transformed bacteria using plasmid purification Maxi kit (QIAGEN, CA, USA) according to the manufacturer's protocol. The purified vector was visualized by ethidium bromide agarose gel electrophoresis using 1% gel.

Cell transfection

The expression vector pEGFP-N1 encoding Enhanced Green Fluorescent Protein (EGFP) was used for evaluating transfection efficiency of the murine cell lines. Three comercialized transfection reagents, Lipofectamine 2000 (lipoplex) (Invitrogen, CA, USA), jetPEI (polyplex) (Polyplus, Paris, France) and LyoVec (lipoplex) (Invivogen, CA, USA)

were used to transfect the cell lines based on the manufacturer's recommendation. Briefly, 2 *µl* of Lipofectamine2000 and 2 *µg* of pEGFP-N1 were diluted separately in 50 *µl* of Opti-MEM reduced serum medium (Invivogen, CA, USA) and mixed gently. After 5 *min* incubation at room temperature, the Lipofectamine2000 and pEGFP-N1 were combined and incubated for an additional 25 *min* at room temperature to allow the DNA-Lipofectamine2000 complexes to form. The complexes were added to cells grown in Opti-MEM medium without serum and antibiotic. After 6 *hr* the medium was replaced with RPMI-1640 supplemented with 10% fetal bovine serum.

Four *µl* of jetPEI and 2 *µg* of pEGFP-N1 (N/P=5) were diluted with 50 *µl* of 150 *mM* NaCl. The DNA solution was then added to the jetPEI solution, and after 20 *min* incubation at room temperature, 100 *µl* of the complexes were added to cells grown in serum containing medium.

Two *µg* of pEGFP-N1 was mixed with 100 *µl* of LyoVec and incubated at room temperature for 15 - 30 *min* to allow the formation of the complex. Subsequently 25 *µl* of the complex was added to cells grown in serum containing RPMI-1640 medium. Each one of these complexes prepared by three different commercial transfection reagents was later used to transfect different cell types cultured in 24-well plate. After 48 *hr*, green fluorescence was detected by fluorescence microscopy (Olympus BX51, London, UK) and flow cytometry (Partec, Nuremberg, Germany). Data analysis was performed using FloMax analysis software (Partec, Nuremberg, Germany). Untransfected cells were included to account for background fluorescence.

Results

To determine the optimum levels of transfection reagent and DNA required for transfection, different ratios were selected for each reagent based on the manufacturers' recommendation. Thus, 2 *µg* and 4 *µg* of DNA were mixed with 4 *µl* and 6 *µl* jetPEI, respectively, 2 *µg*, 2 *µg* and 3 *µg* of DNA

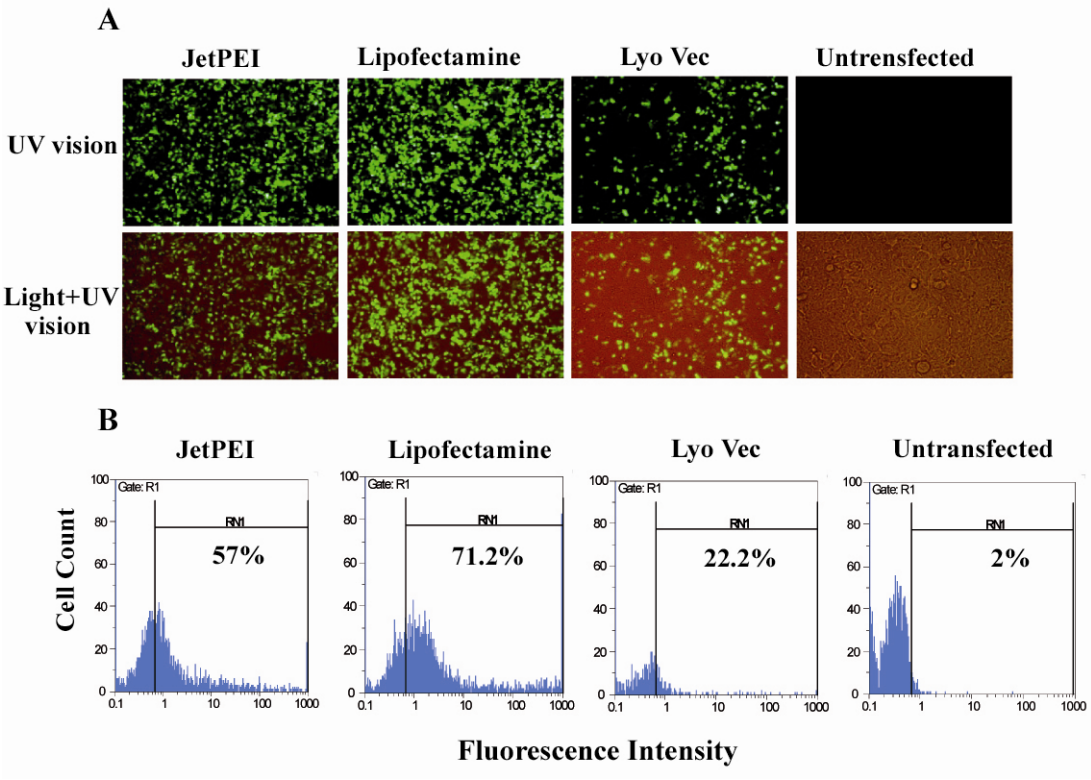

Figure 1. EGFP expression profile in HEK293-FT cell line transfected by different transfection reagents. A) fluorescence microscopy images (10x), B) flow cytometry plots, values presented in flow cytometry plots represent percent of EGFP expression

were mixed with 2 *µl*, 4 *µl* and 4 *µl* Lipofectamine2000, respectively and 0.5 *µg*, 0.75 *µg*, and 1.25 *µg* of DNA were mixed with a fixed volume of 25 *µl* of LyoVec. The optimum ratios of DNA and transfection reagents obtained for a number of cell lines, including two of the myeloma cell lines (SP2/0 and Ag8) were found to be 2 *µg*/ 4 *µl*, 2 *µg*/ 2*µl* and 0.5 *µg*/ 25 *µl* using jetPEI, Lipofectamine2000 and LyoVec, respectively (data not presented).

Taking the optimum ratio of DNA/transfection reagent obtained for each commercial reagent in these cell lines, transfection study was carried out in myeloma cell lines and HEK293-FT as a control. Flow cytometry analysis showed transfection efficiency of 71.2%, 57% and 22.2% for HEK293-FT, 5.5%, 3.4% and 1% for SP2/0, 55.7%, 21.1% and 9.3% for NS0, 8.2%, 6% and 5.5% for NS1, 22%, 49.2% and 5.5% for Ag8 and 6.3%, 21.5% and 4.6% for P3U1 cell lines after transfection with Lipofectamine2000, jetPEI and LyoVec reagents, respectively (Figures 1 and 2, Table 1). Our results indicate that SP2/0, NS1 and P3U1 myeloma cell lines were hardly transfected by transfection reagents. NS0 and Ag8, however,

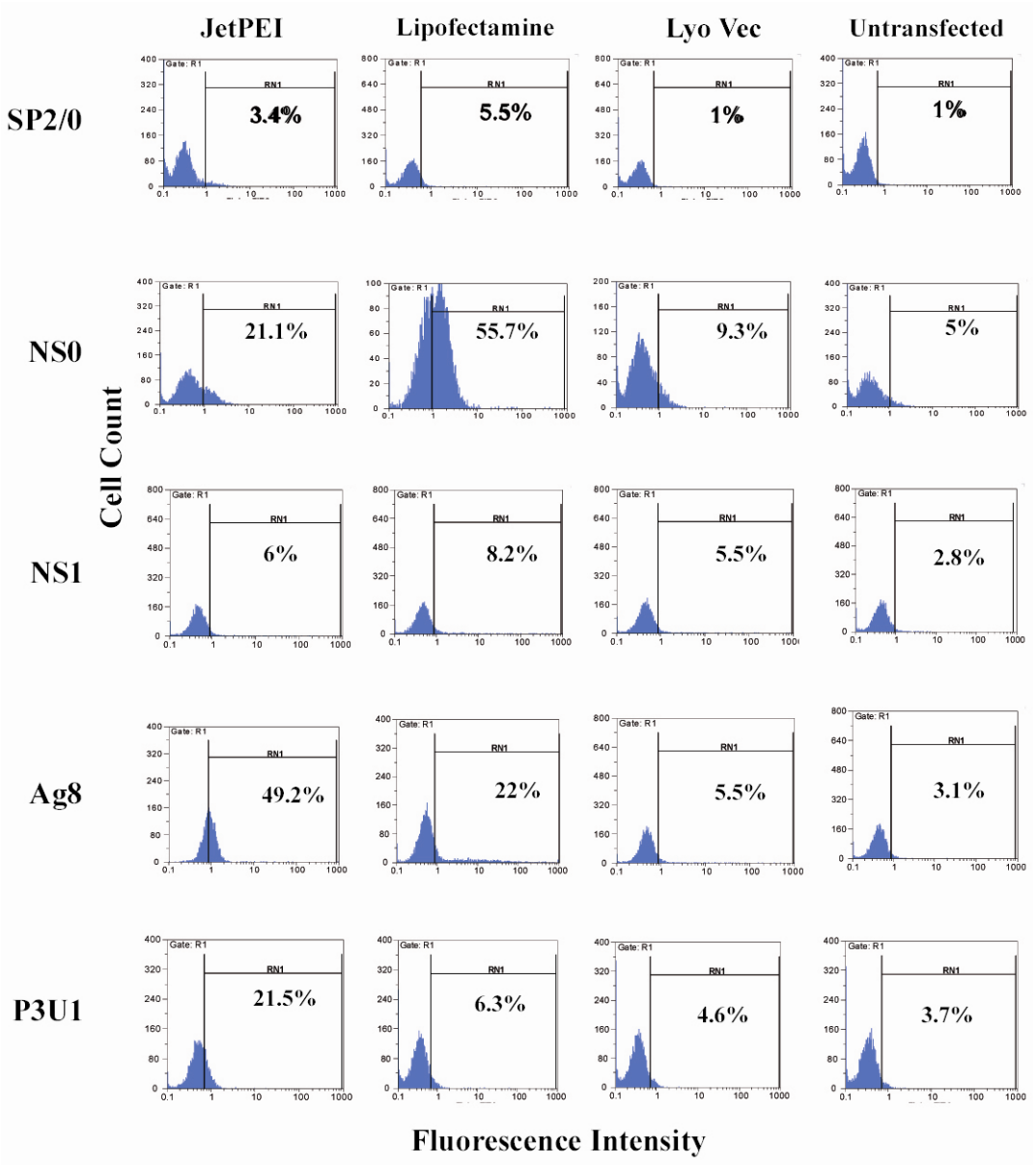

Figure 2. EGFP expression profile in myeloma cell lines transfected by different transfection reagents. Values presented in flow cytometry plots represent percent of EGFP expression

Table 1. The efficiency of pEGFP-N1 transfection in myeloma cell lines using different transfection reagents

Reagent / Cell	JetPEI (%)	Lipofectamine 2000 (%)	LyoVec (%)	Untransfected cells (%)
HEK293-FT	57	71.2	22.2	2
SP2/0	3.4	5.5	1	1
NS0	21.1	55.7	9.3	5
NS1	6	8.2	5.5	2.8
Ag8	49.2	22	5.5	3.1
P3U1	21.5	6.3	4.6	3.7

were efficiently transfected by Lipofectamine 2000 and jetPEI reagents.

Discussion

Development of MAb against the native form of membrane or cytoplasmic proteins is often difficult. Immunization with crude extract of target cells results in stimulation of a large number of B cells with specificity for a variety of cellular proteins including the target molecule, making it difficult to screen and select the desired MAb. Purification of the target molecule from a cellular lysate is also practically problematic [9]. In addition, recombinant proteins may not be good immunogens because they may lose their native configuration [9]. This limitation can be circumvented by using DNA immunization [10,11], phage display of antibody fragments [12] and peptide-based antibody production [13].

Immunization of mice with transfected murine myeloma cell lines is considered as an excellent alternative. In several studies SP2/0 myeloma cells transfected with different genes was used to express recombinant proteins, however, no data was reported regarding the transfection effeciency of the target DNA in this cell line [14 - 19].

Our preliminary efforts to transfect SP2/0 for use in MAb production was unsuccessful. We employed some physical and chemical gene transfer methods (electroporation, Lipofection and calcium phosphate) for SP2/0 transfection, but none was found to be efficient (data not presented). Similar findings have also been reported for SP2/0 transfection using a variety of commercial transfection reagents, reporting a maximum of 10 - 25% transfection efficiency [20 - 23].

Since the transfection efficiency is dependent on the cell type and transfection reagents, in the present study we evaluated the transfection efficiency of three commercial reagents using five commonly used mouse myeloma cell lines. The cationic lipoplexes and polyplex have become very popular transfection reagents due to their limited toxicity, simplicity of production and relative effectiveness *in vitro* [24]. However, their transfection efficiency is lower than that observed with viral transduction [24, 25]. The HEK293-FT cell line was selected as a standard permissive cell line for its high transfection rate [20, 24].

Our results indicated that 22-71% of HEK293-FT cell line were easily transfected by the three commercial reagents used in this study (Figure 1). However, among the five myeloma cell lines, SP2/0, NS1 and P3U1 were hardly transfected by these reagents. NS0 and Ag8 cell lines, on the other hand, were efficiently transfected by Lipofectamine 2000 and jetPEI reagents (Figure 2).

It has been shown that the rate of transfection depends on several parameters, including the type of expression vector promoter and enhancer, purity of the expression vectors as well as the transfer method [3]. These parameters, particularly the transfer methods, need to be optimized for individual cell type [3].

In the current study two lipoplex (Lipofectamine2000 and LyoVec) and one polyplex

(jetPEI) reagents were used for transfection study. It has been shown that lipoplexes are internalized by cells solely by means of clathrin-mediated endocytosis, while polyplexes which are composed mainly of inorganic polymers, such as polyethyleneimine, are internalized both by clathrin-mediated and by caveolae mediated endocytosis.

While lipoplexes internalized via the clathrin-mediated route are fully transfection effective, for the polyplexes only the caveolae-dependent route leads to effective transfection [26]. Thus, the clatherin and caveolin expression by cells could affect their transfection efficiency.

Kichler and co-workers reported a low level of luciferase expression in HepG2 cells transfected with polyplex due to lack of endogenous caveolins and demonstrated that in these cells most of the internalized DNA was degraded in intracellular compartments because of clathrin-mediated endocytosis [27].

Defect in endocytosis in myeloma cell lines may contribute to their low transfection efficiency. Some studies have demonstrated differential gene expression levels induced by special promoters in different cells because transgene expression by plasmid vectors benefits from the use of cellular transcriptional regulatory elements that permit high-level gene expression [28 - 30]. Viral-driven promoters were shown to have different expression levels in adherent compared to suspension cell lines [31]. Perhaps, this could partly be the reason for a higher EGFP expression in the adherent HEK293-FT cell line compared to the suspension myeloma cell lines observed in our study.

Another point which needs consideration is that the species and tissue origin of transfected cell lines are important factors in gene expression [22, 24]. HEK293-FT cell line has been originated from human embryonic kidney cells which are undifferentiated and immature, whereas the murine myeloma cell lines were originated from mouse plasma cells, the end stage differentiated B cells.

The efficiency of DNA uptake and transient or stable expression of the expression vectors are all cell line dependent [30]. In addition, DNA transfection might be too inefficient to establish stable transfection, particularly in lymphocytes [25, 32].

Conclusion

In summary, our results indicate that transfection reagents have different transfection efficiencies in different mouse myeloma cell lines. While NS0 and Ag8 are efficiently transfected by Lipofectamine2000 and jetPEI, SP2/0, NS1 and P3U1 cell lines are not permissive to DNA transfection. Thus, Ag8 and NS0 cell lines could be used as suitable host cells for efficient expression of target genes which can be used for mouse immunization and MAb production. Transfected myeloma cells might also be employed for tumor inoculation in normal syngenic mice to study immunotherapeutic interventions.

Acknowledgement

This work was partly supported by a grant from the Food and Drug Administration of the Ministry of Health, Treatment and Medical Education of Iran (grant number S87P/3/414).

References

1. Han SY, Gai W, Yancovitz M, Osman I, Di Como CJ, Polsky D. Nucleofection is a highly effective gene transfer technique for human melanoma cell lines. Exp Dermatol 2008;17(5):405-411.
2. Dean DA. Nonviral gene transfer to skeletal, smooth, and cardiac muscle in living animals. Am J Physiol Cell Physiol 2005;289(2):C233-245.
3. Colosimo A, Goncz KK, Holmes AR, Kunzelmann K, Novelli G, Malone RW, et al. Transfer and expression of foreign genes in mammalian cells. Biotechniques 2000;29(2):314-331.
4. Li XG, Wang YX, Zhou BQ, Qi CH. The effect of VEGF-ASODN transfection on expression of VEGF and growth in tongue squamous cancer cell line Tca8113. Shanghai Kou Qiang Yi Xue 2009; 18(5):509-514.
5. Villarroel Dorrego M, Whawell SA, Speight PM, Barrett AW. Transfection of CD40 in a human oral squamous cell carcinoma keratinocyte line upregu-

lates immune potency and costimulatory molecules. Br J Dermatol 2006;154(2):231-238.

6. Halama A, Kuliński M, Librowski T, Lochyński S. Polymer-based non-viral gene delivery as a concept for the treatment of cancer. Pharmacol Rep 2009;61 (6):993-999.
7. Zhang H, Wang YS, Han G, Shi Y. TIMP-3 gene transfection suppresses invasive and metastatic capacity of human hepatocarcinoma cell line HCC-7721. Hepatobiliary Pancreat Dis Int 2007;6(5): 487-491.
8. Griesenbach U, Alton EW. Cystic fibrosis gene therapy: successes, failures and hopes for the future. Expert Rev Respir Med 2009;3(4):363-371.
9. Nagata S, Salvatore G, Pastan I. DNA immunization followed by a single boost with cells: a protein-free immunization protocol for production of monoclonal antibodies against the native form of membrane proteins. J Immunol Methods 2003;280 (1-2):59-72.
10. Chowdhury PS, Pastan I. Analysis of cloned Fvs from a phage display library indicates that DNA immunization can mimic antibody response generated by cell immunizations. J Immunol Methods 1999;231(1-2):83-91.
11. Chowdhury PS, Viner JL, Beers R, Pastan I. Isolation of a high-affinity stable single-chain Fv specific for mesothelin from DNA-immunized mice by phage display and construction of a recombinant immunotoxin with anti-tumor activity. Proc Natl Acad Sci USA 1998;95(2):669-674.
12. Marget M, Sharma BB, Tesar M, Kretzschmar T, Jenisch S, Westphal E, et al. Bypassing hybridoma technology: HLA-C reactive human single-chain antibody fragments (scFv) derived from a synthetic phage display library (HuCAL) and their potential to discriminate HLA class I specificities. Tissue Antigens 2000;56(1):1-9.
13. Bard F, Barbour R, Cannon C, Carretto R, Fox M, Games D, et al. Epitope and isotype specificities of antibodies to beta-amyloid peptide for protection against Alzheimer's disease-like neuropathology. Proc Natl Acad Sci USA 2003;100(4):2023-2028.
14. Qin S, Tang H, Zhao LS, He F, Lin Y, Liu L, et al. Cloning of HBsAg-encoded genes in different vectors and their expression in eukaryotic cells. World J Gastroenterol 2003;9(5):1111-1113.
15. Geisse S, Gram H, Kleuser B, Kocher HP. Eukaryotic expression systems: a comparison. Protein Expr Purif 1996;8(3):271-282.
16. Dorai H, McCartney JE, Hudziak RM, Tai MS, Laminet AA, Houston LL, et al. Mammalian cell expression of single-chain Fv (sFv) antibody proteins and their C-terminal fusions with interleukin-2 and other effector domains. Nat Biotechnol 1994;12(9):890-897.
17. Qian F, Xiao CZ, Gao LH, Zhang ZG, Guo ZX, Yu WY. Expression of prourokinase in different mammalian cells. Sheng Wu Gong Cheng Xue Bao 2000;16(3):349-352.
18. Bestagno M, Sola I, Dallegno E, Sabella P, Poggianella M, Plana-Durán J, et al. Recombinant dimeric small immunoproteins neutralize transmissible gastroenteritis virus infectivity efficiently in vitro and confer passive immunity in vivo. J Gen Virol 2007;88(Pt 1):187-195.
19. Zuo L, Cullen CM, DeLay ML, Thornton S, Myers LK, Rosloniec EF, et al. A single-chain class II MHC-IgG3 fusion protein inhibits autoimmune arthritis by induction of antigen-specific hyporesponsiveness. J Immunol 2002;168(5):2554-2559.
20. Fermentas life sciences [Internet]. [unknown]: Thermo Fisher Scientific Inc. (NYSE: TMO); c2001-2010. TurboFect™ in vitro Transfection Reagent; [2009]; [cited 2010 Augusts 24]; [4 p.]. Available from: http://www.fermentas.com/templates/files/tiny_mce/media_pdf/broch_turbofect_P26.pdf.
21. Eppendorf [Internet]. Hamburg: Eppendorf; [unknown]. Multiporator®: Sp20-Ag14 transfection protocol; 2001 November [cited 2010 Augusts 24]; [2 p.]. Available from: http://eppendorf-can.com/utilities/download_pdf.asp?DR=downloads%2Fpdf%2Fapplications%2FBS%2Fecet-protocols&FN=4308915-026.pdf
22. OZ Biosciences: the art of delivery systems [Internet]. Marseille: OZ Biosciences; [unknown]. DreamFect ™ List of cells; 2010 May [cited 2010 Augusts 24]; [2 p.]. Available from: http://www.ozbiosciences.com/docman/dreamfect-cells/download-2.html.
23. Maurisse R, De Semir D, Emamekhoo H, Bedayat B, Abdolmohammadi A, Parsi H, et al. Comparative transfection of DNA into primary and transformed mammalian cells from different lineages. BMC Biotechnol 2010;10:9.
24. Simões S, Slepushkin V, Gaspar R, Pedroso de Lima MC, Düzgünes N. Successful transfection of lymphocytes by ternary lipoplexes. Biosci Rep 1999;19(6):601-609.
25. Bartlett RJ, Secore SL, Singer JT, Bodo M, Sharma K, Ricordi C. Long-term expression of a fluorescent reporter gene via direct injection of plasmid vector into mouse skeletal muscle: comparison of human creatine kinase and CMV promoter expression levels in vivo. Cell Transplant 1996;5(3):411-419.

26. Rejman J, Bragonzi A, Conese M. Role of clathrin- and caveolae-mediated endocytosis in gene transfer mediated by lipo- and polyplexes. Mol Ther 2005; 12(3):468-474.

27. Kichler A, Leborgne C, Coeytaux E, Danos O. Polyethylenimine-mediated gene delivery: a mechanistic study. J Gene Med 2001;3(2):135-144.

28. Xu ZL, Mizuguchi H, Ishii-Watabe A, Uchida E, Mayumi T, Hayakawa T. Optimization of transcriptional regulatory elements for constructing plasmid vectors. Gene 2001;272(1-2):149-156.

29. Lin JH, Wang M, Andrews WH, Wydro R, Morser J. Expression efficiency of the human thrombomodulin encoding gene in various vector and host systems. Gene 1994;147(2):287-292.

30. Feng G, Hicks P, Chang PL. Differential expression of mammalian or viral promoter-driven gene in adherent versus suspension cells. In Vitro Cell Dev Biol Anim 2003;39(10):420-423.

31. Thomas P, Smart TG. HEK293 cell line: a vehicle for the expression of recombinant proteins. J Pharmacol Toxicol Methods 2005;51(3):187-200.

32. Toyoda E, Kurosawa A, Kamekawa H, Adachi N. Topoisomerase IIalpha inhibition following DNA transfection greatly enhances random integration in a human pre-B lymphocyte cell line. Biochem Biophys Res Commun 2009;382(3):492-496.