MAE Vet Fak Derg, 7 (3): 188-194, 2022
DOI: 10.24880/maeuvfd.1126466

Research Article

An optimized protocol for the electroporation of NCI H929 multiple myeloma cells

Ayşe Kızılyer[1]

[1]Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye

Key Words:
electroporation
multiple myeloma
NCI H929
transfection

Received : 05.06.2022
Accepted : 09.08.2022
Published Online : 31.12.2022
Article Code : 1126466

Correspondence:
A KIZILYER
(akizilyer@mehmetakif.edu.tr)

ORCID
A. KIZILYER : 0000-0001-9895-2242

ABSTRACT

Multiple myeloma cell lines are difficult to transfect with non-viral nucleic acid delivery methods. While electroporation is the most efficient tool for the transfection of most hard-to-transfect cells, human multiple myeloma cells differ in their permissiveness and each cell type require different electroporation conditions for an efficient transgene delivery. In this study, various parameters for NCI H929 human multiple myeloma cells are tested to generate an optimized electroporation protocol. Findings from this paper showed that besides the voltage and capacitance settings, cell count, the cell cycle status of cells, the amount of nucleic acid and removal of death cells all impacted the electroporation efficiency and viable cell count. These results are expected to serve as a starting point and a guide for researchers.

INTRODUCTION

In vitro culture and genetic manipulation of animal and human cells are indispensable tools for understanding the function and behavior of cells under normal and pathological conditions. For this purpose, exogenous genetic materials are introduced to cells by various methods to induce or suppress the expression of a target gene. Successful delivery of the nucleic acids into the recipient cells is the most critical and limiting step of the genetic interventions. Various viral and non-viral delivery methods have been developed and employed by the researchers (Chong et al., 2021; Fus-Kujawa et al., 2021; Mizrahy et al., 2017). Despite the diversity of available methods, while some cell types are very permissive for the entry of foreign nucleic acids, others are very resistant and requires special optimization (Canoy et al., 2020; Shih et al., 2019).

Multiple myeloma (MM) cell lines with different genetic composition such as RPMI 8226, U266 and NCI H929 cells are widely used to study the molecular mechanism of the plasma cell malignancy (Hattori et al., 1995). These cells are often modified to examine the role of certain genes or pathways in the transformation of cells and their response to drug candidates (Brito et al., 2010; Sun et al., 2017). While viruses could be successfully used to derive stable changes in these cells, non-viral methods, such as coating nucleic acids with cationic lipid mediators or electroporation, are more convenient when transient modifications, such as small interfering RNA (siRNA)-mediated knockdown, are desired and permanent presence of foreign sequences, such as CRISPR plasmids expressing a DNA-cleaving endonuclease Cas9, are not intended (Brito et al., 2010). However, MM cell lines are not susceptible to most transient transfection methods and electroporation, the common means of gene delivery for these cells, also presents challenges (Steinbrunn et al., 2014). Studies in the literature and databases generated by electroporator manufacturers provide certain parameters such as voltage and capacitance, however crucial details including cell density, nucleic acid amount, the final transfection efficiency and viable cell counts, are oftentimes unreported, which hinders the reproduction of reported findings by other groups (Steinbrunn et al., 2014). The aim of this study is to develop an optimized electroporation protocol for NCI H929 MM cell line. Various parameters are sequentially optimized and incorporated to the protocol. Our findings will guide the researchers step by step in their transient transfection studies for achieving an improved efficiency.

MATERIAL and METHODS

Cell culture

NCI H929 cells are obtained from American Type Culture Collection (ATCC) and cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Pen-Strep) under 37 ºC temperature and 5% CO_2 conditions. Unless otherwise stated, cells were passaged at a 1:2 ratio and supplemented with fresh complete medium the day before the transfection to promote log-phase in the culture.

Bu makaleye atıfta bulunmak için/ To cite this article:
Kızılyer A. An optimized protocol for the electroporation of NCI H929 multiple myeloma cells. MAE Vet Fak Derg. 2022; 7 (3): 188-194.

Transfection

Cells were counted and centrifuged at 200xg for 5 min. Pelleted cells were then resuspended in 200 µl serum-free, RPMI 1640 medium. Next, green fluorescent protein (GFP)-expressing pGIPZ empty plasmid (a gift from Dr. Onur Tokgun from Pamukkale University, Turkiye) were mixed with the cells in the tube by gentle stirring with the pipette tip and the DNA-cell mixture was immediately transferred into a 4 mm cuvette (Biorad). The cells in the cuvette were subjected to a single pulse of exponential decay wave using Gene Pulser Xcell (Biorad, California, USA) and 500 µl of pre-warmed media containing only 20% FBS was added to the cuvette and cells were immediately harvested by a transfer pipet. Cells were then seeded and cultured in a 6-well plate with 2 ml prewarmed, 20% FBS-containing media per well. The next day, live-dead cell count was assessed by Trypan blue staining, and cells were spun at 100xg for 5 min to remove dead cells. For complete elimination of dead cells, density centrifugation by Ficoll-Paque Premium (1.084, Cytiva) was performed. For this, at 24 h post-electroporation, 2 ml of cell suspension was laid on 3 ml Ficoll and spun at 400xg for 30 min using a swinging bucket rotor, with brake off. Following centrifugation, live cells were collected with a transfer pipet from the layer between the culture medium and Ficoll solution. The harvested cells were washed twice with 6 ml phosphate-buffered saline (PBS) by spinning the cells at 400xg for 10 min with brake on. At the end, the pelleted cells were resuspended in complete media (RPMI 1640 with 10% FBS and 1% Pen-Strep) and cultured for another day.

Cell imaging and analysis of transfection efficiency

Cells were examined and images were captured at 48 h post-transfection using an inverted fluorescent microscope (Nikon Eclipse Ts2). Total and GFP positive (GFP+) cell counts were determined from the images taken at 20x and 40x magnification. Transfection efficiency was reported as a percentage of GFP+ cells. Overlay images were generated in Image J/Fiji (Rueden et al., 2017). For the statistical analysis, the mean of GFP+ cell percentages were compared using student's t test and p value < 0.05 was considered significant. All statistical analyses were performed using Graphpad Prism 9.

RESULTS

Voltage and Capacitance

First, based on the manufacturer's recommendations and previous literature regarding MM cell lines (Biorad.; Steinbrunn et al., 2014), 950 µF capacitance with a voltage range of 100 V-300 V was tested. Independent of DNA amount, 100 V voltage had the best viable cell count, but the transfection efficiency was below 1% (data not shown). On the other hand, no viable cells remained at 300 V. Compared to previous settings, pulsing cells at 200 V yielded an improved transfection efficiency. The live cell count at 200 V was lower than at 100 V but better than at 300 V. Therefore, 200 V voltage and 950 µF capacitance settings were preferred for the rest of the experiments.

Logarithmic Phase of Cell Culture

Passaging or supplementing cells with fresh media is known to induce cell division (Oyeleye et al., 2016). Moreover, the plasmid vector is taken up by the mitotically active cells and is transmitted to the progenies, which ultimately yields a higher number of GFP + cells (Brunner et al., 2000; Hsu & Uludag, 2012). For NCI H929 cells, feeding cells with fresh media 24 h prior to the transfection, as well as passaging cells at 1:2 ratio were examined. When 2.5 µg and 5 µg of plasmid DNA with the same electroporation settings (950 µF, 200 V, 10^6 cells/200 µl media) were applied to cells, a very low transfection (<%1) efficiency was observed. However, both passaging cells at 1:2 ratio and feeding them with fresh media, 24 h-pre-transfection, drastically improved the transfection efficiency (Figure 1) and viable cell count. Hereafter, this became a part of the optimized protocol for preparing cells for transfection.

The Amount of Nucleic Acid

After an improved efficiency with logarithmic phase cells was observed, increased DNA input was tested. A range of 2.5, 5, and 10 µg pGIPZ-GFP plasmid was used to transfect 10^6 cells in 200 µl serum-free medium. Cells pulsed with predetermined instrument settings (200 V, 950 µF, a single exponential decay pulse) had the highest yield (GFP+ cell count) with 10 µg plasmid (Figure 2). Scaling up or down the cell count, transfection volume and DNA amount also generated equivalent efficiency (data not shown).

Cell Density

The ratio of media volume to cell number is another determinant of transfection efficiency and cell survival. Here, the same number of cells were resuspended in either 100 µl or 200 µl medium volume and were mixed with 5 µg plasmid DNA. As result of electroporation, 10^6 cells in 200 µl volume generated a better live cell count (Figure 3).

By scaling up the DNA accordingly, cell density up to $4x10^6$ cells in 200 µl volume was used for electroporating NCI H929 cells. No compromise in transfection efficiency was observed (Figure 4).

Dead Cell Removal

Electroporation transiently permeates the cell membrane for the exogenous nucleic acid entry, then cells recovers and continue to their normal growth and division cycle (Batista Napotnik et al., 2021). However, depending on the amplitude and duration of the electric pulse, a portion of cells is always lost and remnants of dead cells affect the quality of live cells in culture (Rols, 2017). Here, we first tried low-speed centrifugation (100xg for 10 min) for coarse removal of dead cells. However, dead cells still remained in culture after centrifugation. Alternatively, we performed Ficoll gradient centrifugation and successfully recovered a pure live cell suspension (confirmed by tripan blue staining). Live cells isolated by Ficoll gradient seemed relatively healthier and morphologically normal (Figure 5). GFP+ cell percentage was also significantly higher in cell population isolated by Ficoll gradient.

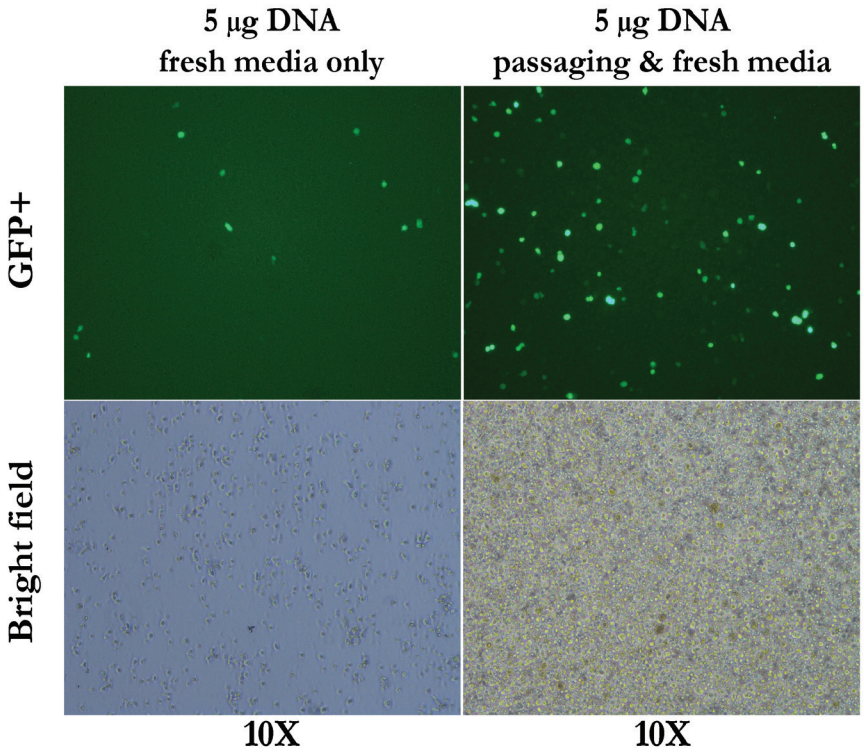

Figure 1. The effect of passaging and fresh media addition on transfection efficiency. Representative images from separate experiments show that 24 h prior to transfection, cells split at 1:2 ratio and fed with fresh media (right) contained higher population of GFP+ cells compared to only fresh media supplemented cells (left). Bright field and fluorescent images are captured 48 h post-transfection.

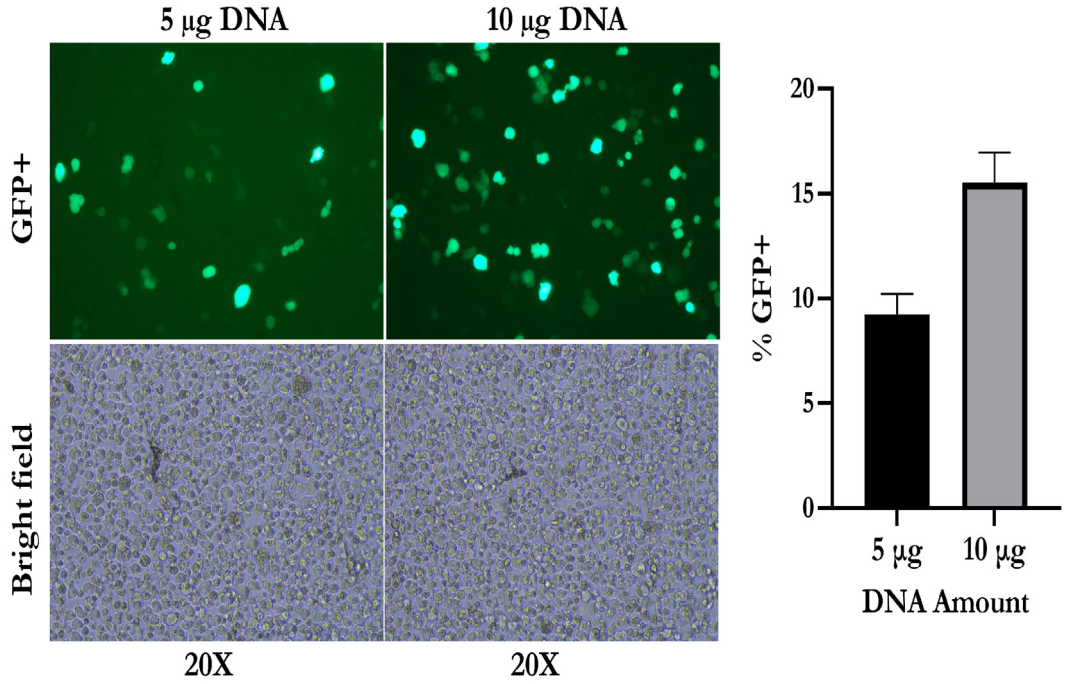

Figure 2. DNA input affects the transfection yield. 106 NCI H929 cells were taken from the culture that was split and fed with fresh media 24 h prior. Cells were pelleted and resuspended in 200 µl serum-free RPMI 1640 medium. Then, cells were mixed with DNA and electroporated (200 V, 950 µF, single exponential decay pulse). Each image is from separate experiments in which all the parameters except DNA amount were kept the same. Images (20x magnification) from at least two different fields were evaluated. The mean of GFP+ cell percentages was calculated and plotted with standard error of means.

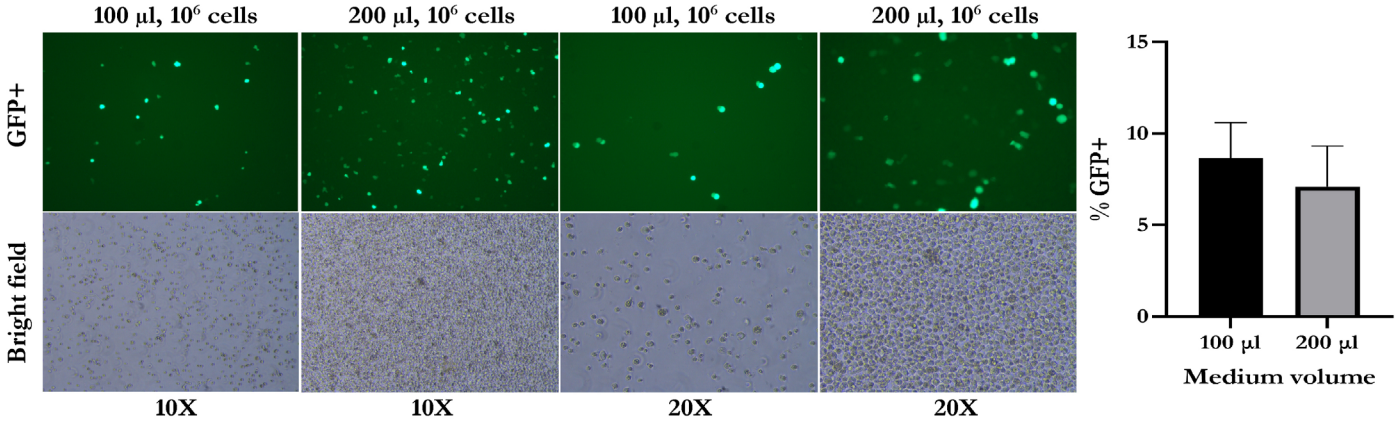

Figure 3. The effect of transfection solution volume on transfection efficiency. 10^6 NCI H929 cells were washed with serum-free RPMI 1640 medium once and resuspended either in 100 µl or 200 µl of the same medium. Cells were mixed with 5 µg pGIPZ plasmid DNA, electroporated (200 V, 950 µF) and seeded on a 6-well plate with a 2 ml prewarmed-media (RPMI 1640 with 20% FBS). At 24 h post-electroporation, cells were spun at 200xg for 5 min to eliminate cell debris. At 48 h post-electroporation, plates were monitored under the inverted fluorescent microscope and images were taken. Images represent the findings from the same experiments. GFP+ cell percentages were determined from at least two different fields. The mean of GFP+ cell percentages was calculated and plotted with standard error of means.

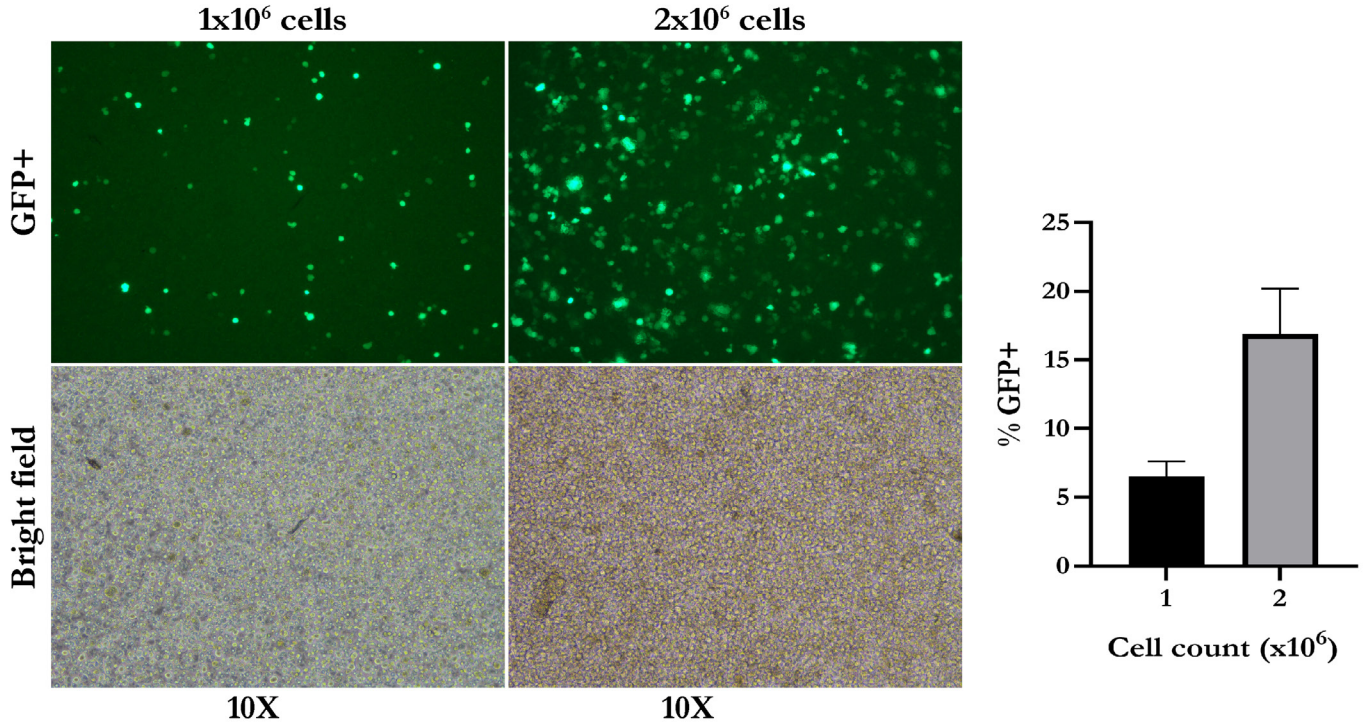

Figure 4. The effect of starting cell number on transfection efficiency. 1, 2 and 4 million NCI H929 cells were mixed in a tube with 10 µg of plasmid DNA and pulsed at 200 V, 950 µF with a single exponential decay wave. Cell images were taken 48 h post-transfection under fluorescent microscope. Compiled cell images in this figure panel reflect the transfection efficiency results from separate electroporation trials. GFP+ cell percentages were determined from at least two different fields. The mean of GFP+ cell percentages was calculated and plotted with standard error of means. unpaired student's t test analysis, p=0.09.

Overall Transfection Efficiency

Total 651 cells from four separate fields in the well were counted. 163 out of 651 cells were counted as GFP+ and the transfection efficiency was determined as 25%.

Final Protocol

• To promote logarithmic phase, cells are passaged and fed with fresh media 24 h prior to electroporation.

• The day of electroporation, one to four million cells per condition are resuspended in 200 µl serum-free RPMI 1640.

• 10 µg plasmid DNA is mixed with cells in a tube by gentle stirring with a pipette tip.

• Cell-DNA mixture is transferred to a 4 mm cuvette and

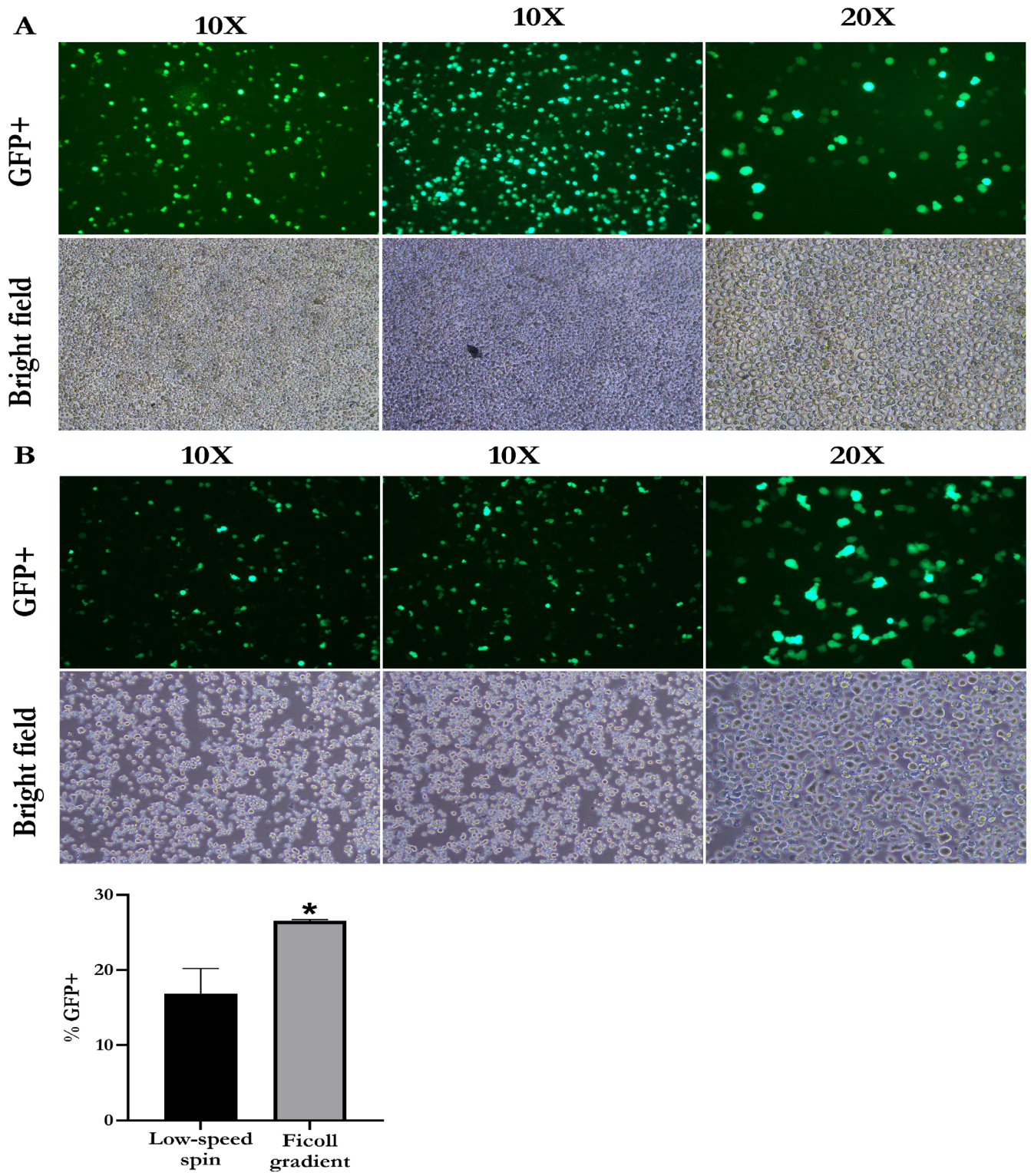

Figure 5. Cells transfected with optimized protocol and subjected either to low-speed centrifugation or Ficoll gradient centrifugation for dead cell removal. 1-4 x10^6 cells (split at 1:2 ratio and fed with fresh media the day before) are washed with and resuspended in 200 µl of serum-free RPMI 1640. 10 µg of pGIPZ plasmid was mixed with cell in a tube and transferred to a 4 mm cuvette. DNA-cell mixture was pulsed with a single exponential decay wave at 200 V, 950 µF. Electroporated cells were harvested after adding 500 µl of warm media (RPMI 1640 with 20% FBS, no antibiotics) into the cuvette and seeded in a 6-well plate containing 2 ml warm media. 24 h post-transfection, cells were either spun at low speed (100xg for 10 min) (A) or subjected to Ficoll-gradient centrifugation (B) to eliminate dead cells and seeded again in a new 6-well plate. Either two days (A) or four days (B) after electroporation, GFP-expressing cells were monitored under a fluorescent microscope and images were obtained. GFP+ cell percentages were determined from at least two different fields. The mean of GFP+ cell percentages was calculated and plotted with standard error of means. unpaired student's t test analysis, p=0.02.

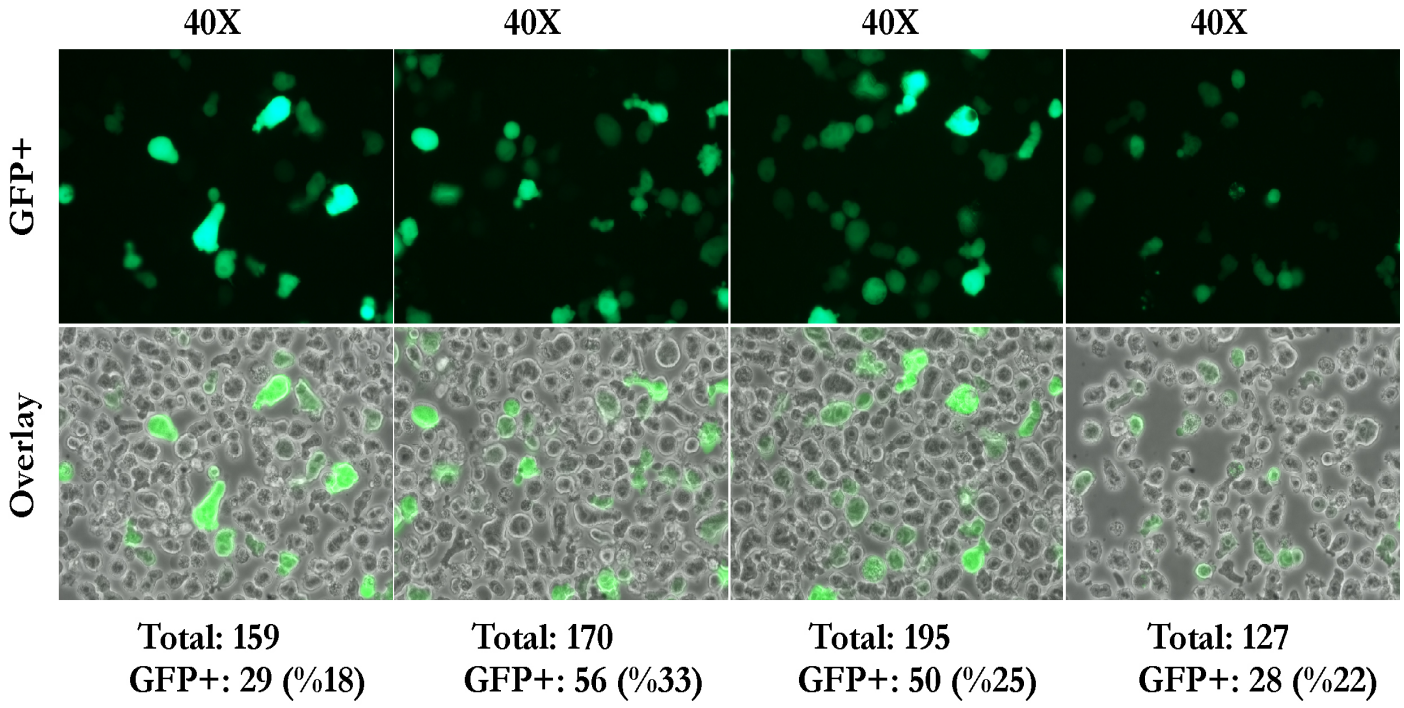

Figure 6. The efficiency of the optimized electroporation protocol. 4 x10^6 NCI H929 cells were mixed with 10 µg of pGIPZ plasmid in 200 µl serum-free medium. After 24 h, dead cells are eliminated by Ficoll gradient centrifugation and cell suspension was cultured in a 6-well plate up to 4 days after electroporation. Cell images were captured under fluorescent microscope. Images at 40x magnification were used to count the GFP+ cells in Image J software.

electroporated at 200 V, 950 µF with a single exponential decay wave.

• 500 µl of prewarmed media was added into the cuvette and cells are harvested by a P1000 pipette tip or a transfer pipet.

• Harvested cells are seeded into a 6-well plate containing 2 ml prewarmed 20% serum-containing, antibiotic free, RPMI 1640 media and cultured for additional 24 h.

• At 24 h post-electroporation, cells are examined and Ficoll gradient centrifugation is performed to eliminate dead cells.

• Isolated live cells are further cultured to expand or immediately used for experiments.

DISCUSSION

Multiple myeloma cell lines are common tools for experimental studies and electroporation of MM cells is a widely used method for allowing the entry of RNA mimics or antisense oligos to drive transient changes in gene expression. Despite the frequent use of electroporation method for these cells, the transfection efficiency is unreported and enrichment of transfectant cells by cell sorting methods are needed to acquire the necessary cell count to conduct an experiment. An ideal transfection protocol is expected to yield a good efficiency as well as cell survival rate. Both high efficiency and less cytotoxicity after transfection depends not only on cell density, transfection solution and electroporator settings but also on the type, size and content of the nucleic acid as well as culture conditions before and after electroporation. Initial settings for this study were based on a good comprehensive work from Steinbrunn et. al (2014). This group aimed to develop a standard procedure for the most common MM cells and suggested further optimization to achieve better outcome for a specific cell type. In contrast to the suggested voltages by Steinbrunn et. al (2014), any voltages above 200 V was not tolerable not only for NCI H929 but also RPMI 8226 and U266 cells in our hands. Moreover, voltages below 200 V (with 950 µF capacitance) increased the viable cell numbers but severely compromised the transfection efficiency. We examined various parameters for optimization and achieved an approximately 25 % electroporation efficiency for NCI H929 cells but could not improve the transfection efficiency for RPMI 8226 cells beyond one per cent (data not shown). The GFP-expressing pGIPZ construct used in this study is a relatively large (11.8 kilobase) lentiviral construct. Using either this construct or a CRISPR vector (~9.1 kb, plasmid #62988, Addgene), the number of recovered cells after 24 h post-transfection was about 1-2 x10^5 cells out of 10^6 cells (10-20%) under optimized conditions. This optimized protocol (950 µF, 200 V, 10 µg DNA, 4x10^6 cells in 200 µl serum-free culture medium) settings might generate different survival rate and transfection efficiency if the input DNA is a smaller plasmid construct or are short oligos such as siRNAs, which was not investigated in this study. Compared to current literature, this protocol provides an improved efficiency and could suffice the need of certain studies where the transformant cells are later enriched by additional methods such as flow cytometry-mediated cell sorting (FACS), magnetic cell sorting or antibiotic-mediated

selection.

DECLARATIONS

Conflict of Interest

The author declares that there is no conflict of interest.

Consent for Publication

I give my consent for this manuscript to be published in the Veterinary Journal of Mehmet Akif Ersoy University.

Author contribution

Idea, concept and design: AK

Data collection and analysis: AK

Drafting of the manuscript: AK

Critical review: AK

Data Availability

The data presented in this study contained within the article.

Acknowledgements

This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK), with the project, 118Z535. The author would like to thank Dr. Dilara Akcora Yıldız for the development of this project fund and thank to TUBITAK for the financial support.

REFERENCES

1.Batista Napotnik, T., Polajžer, T., & Miklavčič, D. (2021). Cell death due to electroporation - A review. Bioelectrochemistry (Amsterdam, Netherlands), 141, 107871. https://doi.org/10.1016/j.bioelechem.2021.107871

2. Biorad. (n.d.). Gene Pulser Xcell Electroporation System Instruction Manual (Issue 62). https://www.bio-rad.com/sites/default/files/2022-01/10000148530.pdf

3.Brito, J. L., Brown, N., & Morgan, G. J. (2010). Transfection of siRNAs in multiple myeloma cell lines. Methods Mol Biol, 623, 299–309. https://doi.org/10.1007/978-1-60761-588-0_19

4. Brunner, S., Sauer, T., Carotta, S., Cotten, M., Saltik, M., & Wagner, E. (2000). Cell cycle dependence of gene transfer by lipoplex, polyplex and recombinant adenovirus. Gene Therapy, 7(5), 401–407. https://doi.org/10.1038/sj.gt.3301102

5. Canoy, R. J., Andre, F., Shmakova, A., Wiels, J., Lipinski, M., Vassetzky, Y., & Germini, D. (2020). Easy and robust electrotransfection protocol for efficient ectopic gene expression and genome editing in human B cells. Gene Ther. https://doi.org/10.1038/s41434-020-00194-x

6. Chong, Z. X., Yeap, S. K., & Ho, W. Y. (2021). Transfection types, methods and strategies: a technical review. PeerJ, 9, e11165–e11165. https://doi.org/10.7717/peerj.11165

7. Fus-Kujawa, A., Prus, P., Bajdak-Rusinek, K., Teper, P., Gawron, K., Kowalczuk, A., & Sieron, A. L. (2021). An Overview of Methods and Tools for Transfection of Eukaryotic Cells in vitro. Frontiers in Bioengineering and Biotechnology, 9. https://www.frontiersin.org/article/10.3389/fbioe.2021.701031

8. Hattori, H., Tagawa, S., & Kitani, T. (1995). [Establishment and usage of human multiple myeloma cell line]. Nihon Rinsho, 53(3), 603–609. https://www.ncbi.nlm.nih.gov/pubmed/7699892

9. Hsu, C. Y., & Uludag, H. (2012). A simple and rapid nonviral approach to efficiently transfect primary tissue-derived cells using polyethylenimine. Nat Protoc, 7(5), 935–945. https://doi.org/10.1038/nprot.2012.038

10. Mizrahy, S., Hazan-Halevy, I., Dammes, N., Landesman-Milo, D., & Peer, D. (2017). Current Progress in Non-viral RNAi-Based Delivery Strategies to Lymphocytes. Molecular Therapy : The Journal of the American Society of Gene Therapy, 25(7), 1491–1500. https://doi.org/10.1016/j.ymthe.2017.03.001

11. Oyeleye, O., Ogundeji, S., Ola, S., & Omitogun, O. (2016). Basics of animal cell culture: Foundation for modern science. Biotechnology and Molecular Biology Reviews, 11, 6–16. https://doi.org/10.5897/BMBR2016.0261

12. Rols, M.-P. (2017). Parameters Affecting Cell Viability Following Electroporation In Vitro BT - Handbook of Electroporation (D. Miklavčič (ed.); pp. 1449–1465). Springer International Publishing. https://doi.org/10.1007/978-3-319-32886-7_149

13. Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics, 18(1), 529. https://doi.org/10.1186/s12859-017-1934-z

14. Shih, T., De, S., & Barnes, B. J. (2019). RNAi Transfection Optimized in Primary Naïve B Cells for the Targeted Analysis of Human Plasma Cell Differentiation. Frontiers in Immunology, 10. https://www.frontiersin.org/article/10.3389/fimmu.2019.01652

15. Steinbrunn, T., Chatterjee, M., Bargou, R. C., & Stühmer, T. (2014). Efficient transient transfection of human multiple myeloma cells by electroporation - An appraisal. PLoS ONE, 9(6). https://doi.org/10.1371/journal.pone.0097443

16. Sun, Y., Pan, J., Zhang, N., Wei, W., Yu, S., & Ai, L. (2017). Knockdown of long non-coding RNA H19 inhibits multiple myeloma cell growth via NF-κB pathway. Scientific Reports, 7(1). https://doi.org/10.1038/s41598-017-18056-9