

Transient Gene Expression following Single and Double Exponential Decay Electroporation Pulses

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Summary

A double pulse format for electroporation of mammalian tissue culture cells gave lower transient expression than a single exponential decay pulse. Moreover, separate administration of the two components of the double pulse revealed that transformation was due almost exclusively to the first, high voltage pulse and not to the second, long duration waveform.

Introduction

The Twin-pulse™ wave delivery is a novel electroporation waveform consisting of a short duration, exponential decay pulse followed by a second capacitor discharge yielding a long duration pulse. It is claimed that “the first high-voltage pulse creates pores, or openings in the cell walls due to the high voltage delivered... (and) the second low-voltage pulse delivers energy to those cells shocked by the first pulse, therefore favoring entry of exogenous molecules into the cells by means of creating an active electrophoresis field.”¹ While the transient formation of pores in the plasma membrane following delivery of appropriate electrical fields has been documented via electron microscopy,² the mechanism of entry and egress of materials through these pores is not known, and no evidence has been provided for the postulated Twin-pulse description.

De Chasseval and de Villartay described the use of double electroporation pulse format to introduce pairs of plasmids into human lymphoid cells.³ Their principal finding was that cotransfection of a plasmid encoding the SV40 large T antigen with a reporter plasmid bearing the SV40 origin of replication increased transient luciferase or CD2 expression several fold. The electroporation conditions were optimized for voltage, but not for capacitance, resistance, or timing between pulses. Furthermore, these researchers did not compare the results of single, exponential decay pulses to the expression produced by the double pulse procedure. De Chasseval and de Villartay did not claim that the use of the double pulse was essential for the success of these experiments.

In this report, the optimal Twin-pulse electroporation conditions from de Chasseval and de Villartay, as reported in the January, 1993 *EquiBio News*, were compared to single electroporation pulses generated by the Gene Pulser® II system for transient gene expression in COS cells.

Results and Discussion

The results in Table 1 and Figure 1 show the reporter gene activity obtained for optimal electroporation conditions of the two instruments. Maximum transient expression was obtained with both the Gene Pulser II system and the EasyJet Plus with a single pulse of 210V and 450 μF capacitance. The third treatment duplicates the optimum Twin-pulse conditions reported by EquiBio for mammalian cells – a 500 V, 25 μF pulse with a 99 ohm resistor in parallel, a 7 second inter-pulse delay, and then a 74 V, 2,100 μF pulse with a 99 ohm resistor in parallel.

Table 1. Comparison of Optimal Conditions with Different Electroporator.

Pulse Conditions Device / Volts Set / μF / Shunt Resistor	Luciferase RLU*/20μl mean ± S.D.	Galactosidase RLU*/20μl mean ± S.D.
1. Gene Pulser II / 210 / 450 -	392334 ±32835	105151 ±10535
2. EasyJet Plus / 210 / 450, -	360936 ±20676	93048 ±7087
3. EasyJet Plus / 500 / 25 / 99/7sec/74 / 2,100 / 99	182244 ±829	53127 ±3437
4. EasyJet Plus / 500 / 25 / 99	135155 ±20894	35941 ±4734
5. EasyJet Plus / 74 / 2,100 / 99	5915 ±931	6806 ±175

*Relative Light Units

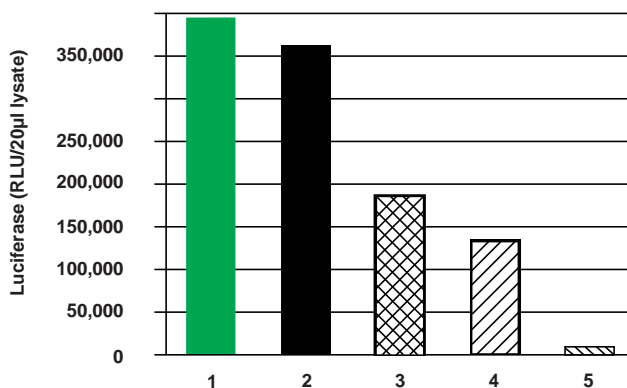


Fig. 1. Luciferase expression after single or double pulses.

It is clearly apparent that a single, optimized exponential decay pulse is more effective than this Twin-pulse form, generating about twice the level of transient gene expression. Moreover, when the two parts of the double pulse were delivered to cell samples separately in treatments 4 and 5, ca. 70% of the Twin-pulse expression was obtained when the first, high voltage component was administered alone. Most of the gene transfer occurring during a double pulse is therefore taking place during the brief initial, pore-formation phase, with very little enhancement in expression contributed by the second pulse element, which is purported to be the DNA movement phase. While the Twin-pulse mechanism seems plausible in theory, in practice its benefits were not observed, and published reports demonstrating its efficacy compared to single pulses could not be found.

Comparison of luciferase and β -galactosidase expression resulting from each of the electroporation treatments in Table 1 also shows that the two reporter enzymes varied in a parallel fashion. This indicates that these measurements of enzyme activity are an accurate representation of the plasmid uptake and gene expression in these cell samples.

Waveform optimization experiments using single pulses produced by the Gene Pulser II system were performed with either low voltage and high capacitance or high voltage and low capacitance. The results (not shown) indicate that the pulse parameters used in the first experiment are essentially the optimum combinations of voltage and capacitance, *i.e.* pulse energy.

Materials and Methods

Cells and Electroporation Conditions

COS-M7 cells were grown in DMEM containing 10% fetal calf serum so that cultures were ca. 70% confluent on the day of electroporation. Cells were trypsinized, collected by centrifugation, washed twice in PBS, and resuspended in DMEM without serum at a density of 1.5×10^6 cells per 200 μ l aliquot, the amount used for one electroporation. DNA in a total mass of 0.33 μ g as a mixture of equal amounts of two plasmids was placed in a 0.2 cm gap electroporation cuvette, then the cell suspension was added. Exponential decay electroporation pulses were administered as specified in Table 1 by a Gene Pulser II system with Capacitance Extender II or an EasyJect Plus electroporator (EquiBio) according to the manufacturers' instructions. The cells were then cultured in 4 ml DMEM containing 10% serum in hexwell plates in a humidified 37 °C, 5% CO₂ environment.

Plasmids and Reporter Enzyme Assays

The expression plasmids pCH110⁴ and pSVZALAA5⁵, which contain the SV40 early promoter, were used to produce *E. coli* β -galactosidase and firefly luciferase respectively. These reporter enzyme activities were assayed in cell extracts prepared 24 hours after electroporation. Adherent cells were washed twice with cold PBS and lysed in 300 μ l luciferase lysis buffer. Extracts were centrifuged briefly to remove cell debris. Luciferase was quantitated using luciferin (Analytical Luminescence Laboratory, San Diego) employed according to the manufacturer's protocol, collecting the light produced by 20 μ l of extract for 10 sec with an MGM Optocomp I luminometer. Galactosidase was determined in the same cell extracts using the chemiluminescent Galacto-Light™ assay (Tropix, Cambridge, Massachusetts) in the same luminometer.

Conclusion

A single, capacitor discharge electroporation pulse gave better transformation results than a Twin-pulse regime. A single pulse treatment is also much easier to optimize, since there are only two electrical parameters to set.

References

1. EasyJect™ Electroporator Instruction Manual, Stratagene, La Jolla, California (1994).
2. Chang, D. C. and Reese, T. S. Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy, *Biophys. J.*, **58**, 1-12 (1990).
3. De Chasseval, R. and de Villartay, J.-P. 1992. High level transient gene expression in human lymphoid cells by SV40 large T antigen boost. *Nucleic Acids Res.*, **20**, 245-250 (1992).
4. Hall, C. V., Jacob, P. E., Ringold, G. M. and Lee, F. Expression and regulation of *E. coli* lacZ gene fusions in mammalian cells. *J. Mol. Appl. Genet.*, **2**, 101-109 (1983).
5. DeWet, J. R., Wood, K. V., DeLuca, M., Helinski, D. R. and Subramani, S. Firefly luciferase gene: structure and expression in mammalian cells. *Mol. Cell. Biol.*, **7**, 725-737 (1987).

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