Gene electrotransfer with different pulse parameters L1

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Duration of the experiments: 60 min, 60 min the following day for results
Max. number of participants: 4
Location: Cell Culture Laboratory 3
Level: Basic

PREREQUISITES

Participants should be familiar with the Safety rules and Rules for sterile work in cell culture laboratory (following the recommendations of the Good laboratory practice). No other specific knowledge is required for this laboratory practice.

THEORETICAL BACKGROUND

Gene electrotransfer is a non-viral method used to transfer genes into living cells by means of high-voltage electric pulses. An exposure of a cell to an adequate amplitude and duration of electric pulses leads to a temporary increase in cell membrane permeability which allows various otherwise nonpermeant molecules, including DNA, to cross the membrane and enter the cell. The mechanisms of the process are not fully explained, however it was shown that three steps are crucial for gene electrotransfer: interaction of DNA molecules with the cell membrane, translocation and expression.

One of the most important parameters for successful DNA electrotransfer is pulse duration. Some studies hypothised that long, several millisecond pulses are necessary for transfection, while other showed that also shorter pulses with higher amplitude achieve efficient gene electrotransfer.

The aim of this practical exercise is to demonstrate how different electric pulses have influence on the efficiency of gene electrotransfer.

EXPERIMENT

We will transfect Chinese hamster ovary cells (CHO-K1) with plasmid DNA (pEGFP-N_1) that codes for GFP (green fluorescent protein) using two different pulse protocols. We will try to see the difference in the fluorescence intensity of transfected cells for both pulse protocols.

Protocol: CHO cells will be grown in multiwells as a monolayer culture in Ham's tissue culture medium for mammalian cells with 10% fetal bovine serum (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) at 37º C. Cells will be plated 24 hours before the experiment in concentration 5 x 10^5 cells per well.

Just before the experiment remove culture medium and replace it with 150 µl of electroporative buffer (10 mM phosphate buffer Na_2HPO_4/NaH_2PO_4, 1 mM $MgCl_2$, 250 mM sucrose; pH = 7.4) containing plasmid DNA with concentration 10 µg/ml. Incubate cells with plasmid for 2-3 minutes at room temperature. Then apply a train of pulses to each sample with Jouan electroporator. Monitor pulses on osciloscop (LeCroy 9310C).

Use two different pulse protocols:

a) 8 x 1 ms; 0.8 kV/cm; 1 Hz
b) 4 x 200 µs; 1.2 kV/cm; 1 Hz

Cells in the control are not exposed to electric pulses.
Immediately after exposure of cells to electric pulses add 37 µl of fetal calf serum (FCS-Sigma, USA). Incubate treated cells for 5 minutes at 37º C and then add 1 ml of culture medium.

After 24h incubation at 37º C observe the difference in the fluorescence intensity of transfected cells for both pulse protocols and control by fluorescent microscopy (Leica, Wetzlar, Germany).

View samples using a fluorescent microscope at 20x magnification using GFP filter with excitation at 488 nm.

FURTHER READING:

Faurie C., Reberšek M., Golzio M., Kandušer M., Escoffre J. M., Pavlin M., Teissie J., Miklavčič D., Rols M. P. Electro-mediated gene transfer and expression are controlled by the life-time of DNA/membrane complex formation. *J Gene Med* 12: 117-125, 2010

Haberl S., Pavlin M., Miklavčič D. Effect of Mg ions on efficiency of gene electrotransfer and on cell electropermeabilization. *Bioelectrochemistry* 79: 265-271, 2010

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Mir L. M. Nucleic acids electrotransfer-based gene therapy (electrogenetherapy): past, current and future. *Mol Biotechnol* 43: 167-176, 2009

Pavlin M., Flisar K., Kandušer M. The role of electrophoresis in gene electrotransfer. *J Membrane Biol* 236: 75-79, 2010

Pavlin M., Haberl S., Reberšek M., Miklavčič D., Kandušer M. Changing the direction and orientation of electric field during electric pulses application improves plasmid gene transfer in vitro. *J Vis Exp, in press*

Rols M.P., Teissie J. Electropermeabilization of mammalian cells to macromolecules: control by pulse duration. *Biophys J* 75: 1415-1423, 1998

NOTES & RESULTS