Abstract

Primary cells as well as certain cell-lines are known to be refractory to traditional chemical transfection methods. The physical technique of electroporation has emerged as a method of choice for nucleic acid delivery into these “hard to transfect” cells. Ingenio® Electroporation Solution is a broad spectrum reagent capable of supporting electroporation of plasmid DNA and siRNA into multiple mammalian cell lines. Ingenio is compatible with most standard electroporators including Lonza-amaxa® Nucleofector®, Bio-Rad Gene Pulser Xcell™ and Harvard-BTX® cell electroporators. Cells can be efficiently transfected using a simple protocol with Ingenio Electroporation Solution, that can be further optimized for either exponential decay or square wave pulse types offered on standard electroporators. Certain cell types transfect better with square wave pulses while others respond better to exponential decay, empirical testing is required for each cell type. Regardless of the type of pulse used, optimization of pulse strength is absolutely critical in ensuring high efficiency electroporation without loss of cell viability. Best electroporation results are achieved using Ingenio Electroporation Solution by titrating the pulse variables such as voltage and capacitance. Electrotransfection using optimized pulse parameters with Ingenio Electroporation Solution or kits affords increased gene expression or knockdown in primary cells and several different “hard to transfect” cell lines, with minimal cytotoxicity.

General Methods

Harvest cells

Centrifuge cells and resuspend at 2-10x10^6 cells/mL in Ingenio® Solution

Add DNA (20µg/µl) or siRNA (250nm) to 100µl or 250µl cells and add to 0.2 or 0.4cm cuvette

Electrotransfer with optimal pulse

Transfer electroporated cells to culture vessel, incubate and harvest

Optimize Ingenio® Electroporation

• Pulse Type: Exponential Decay, Square Wave
• Pulse Conditions: Voltage, Capacitance, Resistance, Time, Pulse Interval
• Nucleic Acid Concentration
• Cell Density

Delivery of Plasmid DNA and siRNA Using Ingenio™

Pulse Condition Optimization: Electroporations performed using (1A) exponential decay pulse in 5K-N-MC cells in 0.4 cm cuvettes on Gene Pulser™ Xcell Eukaryotic System (Bio-Rad) and (1B) square wave pulse in primary Mouse Embryonic Fibroblasts in 0.2 cm and 0.4 cm cuvettes using ECM 830 (BTX®). Exponential decay pulse titration was performed by varying voltage keeping capacitance constant and vice-versa. For square-wave optimization, voltage was varied at a fixed capacitance of 950µF. EGFP efficiency and viability assay of Propidium Iodide stained cells were performed using BD™ LSR III flow cytometer at 24 hours post-electroporation.

Results and Discussion

High Efficiency Plasmid Delivery into Hard to Transfect Cells

3A. Achieve Efficiencies Similar to amaxa® Using Ingenio®

3B. Efficient Delivery of Plasmid DNA into Primary Cells

2A. Plasmid Delivery: Cy3™-labeled control plasmid DNA
2B. Plasmid Expression: Cy3™-labeled plasmid expressing EYFP protein (yellow)
2C. siRNA Delivery: Cy3™-labeled noncoding control siRNA

Twenty-four hours post-electroporation with Ingenio, CHO-K1 cells were fixed and counterstained with TO-PRO®-3 and Alexa Fluor 488® phallloidin (Invitrogen). Confocal images were recorded using Zeiss LSM510 confocal microscope using lasers set at 488nm, 550nm and 633nm wavelengths. Images were analyzed by 2-stack AxioVision software (Zeiss) to determine intracellular localization.

Conclusions

• High efficiency delivery of plasmid DNA or siRNA into primary cells and hard to transfect cell-lines
• Multi-platform compatible with any electroporation instrument including Bio-Rad Gene Pulser Xcell™, amaxa® Nucleofector®II and BTX® ECM 630 & 830
• Simple and straight-forward protocol with optimization steps for delivering nucleic acids with exponential decay or square wave pulse type
• Cost effective and reliable method of delivery into primary cells and cell-lines

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