# Developing a Reliable and Efficient Method for Delivering Nucleic Acids into Hard to Transfect Cells in Culture


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## Abstract


Electroporation is a vital tool used for nucleic acid delivery because it can be applied to a wide variety of cell types including hard-to-transfect eukaryotic cells. Research scientists at Mirus Bio have developed an electroporation solution to facilitate highly efficient, easy and reliable delivery of nucleic acids to hard-to-transfect cells, such as immune and neuro-derived cell-lines, for which chemical mediated transfection is difficult. This electroporation solution is capable of supporting electroporation in multiple cell lines and is compatibile with most electroporators. Efficient nucleic acid delivery is achieved while minimizing toxicity and reducing harmful side effects. The high efficiency electroporation solution could potentially facilitate wider applications in the field of *in vitro* gene transfer, gene expression studies and a host of other related areas. Efforts in developing, characterizing and understanding the capabilities of this electroporation solution, as well as the possible mechanisms underlying the process of electroporation, shall be discussed.


## General Methods

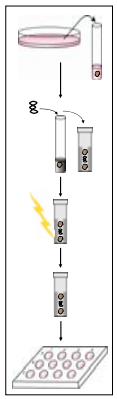
Harvest cells

Centrifuge cells and resuspend at ~$10^7$ cells/mLin Ingenio Solution

Add DNA (20µg/ml) to 250µl cells and add to 0.4cm cuvette

Electroporate with optimal pulse

Immediately transfer electroporated cells to culture vessel, incubate and harvest

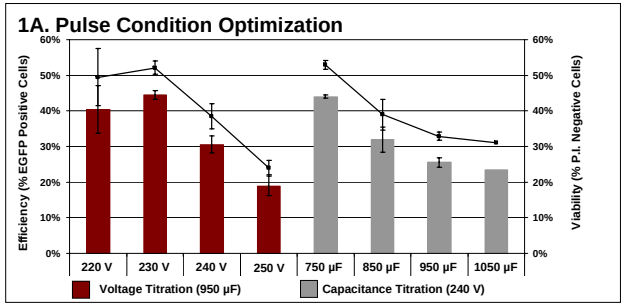


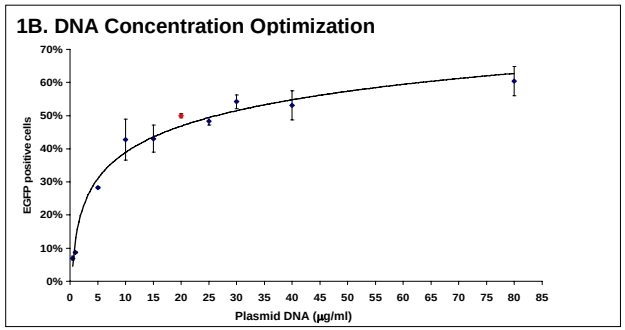
**Optimize Ingenio Electroporation**

- Pulse Conditions: Voltage, Capacitance, Resistance, Time, Pulse Interval
- Pulse type: Exponential Decay, Square Wave, Time constant
- Nucleic Acid Concentration
- Cell Density

## Results and Discussion

### Ingenio Protocol Optimization

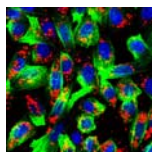


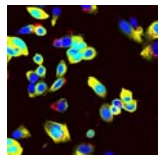

1A-B. Electroporations performed on Gene Pulser Xcell Eukaryotic System (BioRad) in 0.4 cm cuvettes. EGFP efficiency and viability assay performed using BD LSRII flow cytometer at 24 hours post electroporation.

### Localization of Plasmid DNA and siRNA Delivered by Ingenio

2A. Plasmid Delivery



2B. Plasmid Expression



2C. siRNA Delivery

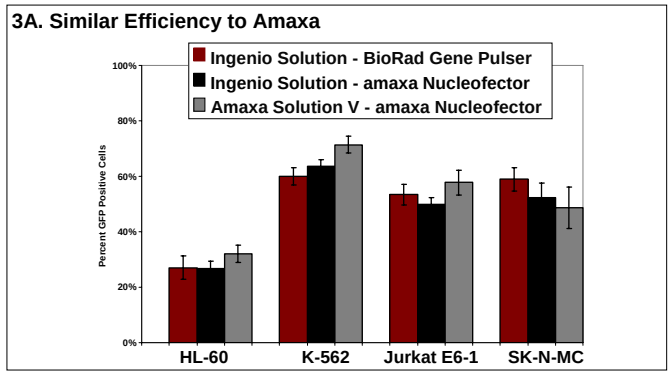
2A. Cy3-labeled control plasmid DNA

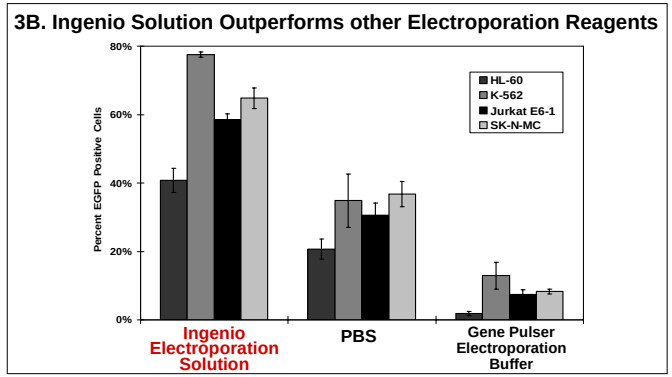
2B. Cy3-labeled plasmid expressing EYFP protein (yellow)

2C. Cy3-labeled noncoding control siRNA

Twenty-four hours post electroporation in Ingenio, cells were fixed and counterstained with TO-PRO-3 for nuclei (blue) and Phalloidin-A588 for actin (green). The image was acquired using a confocal microscope.

### High Efficiency Plasmid Delivery into Hard to Transfect Cells




3A. Cells were electroporated in triplicate on same day with same EGFP reporter. BioRad Gene Pulser Xcell was compared to amaxa Nucleofector II with Ingenio or amaxa Nucleofector Kit V. Cells were assayed at 24 hours by flow cytometry and reported as percentage of live cell population.
3B. Cells electroporated in either Ingenio, Phosphate Buffered Saline (PBS), or Gene Pulser Electroporation Buffer (BioRad) on same day in triplicate.

## Conclusions

**Ingenio Electroporation Solution**

- High efficiency delivery of plasmid DNA into hard to transfect cells without compromising cell health
- Compatible with any electroporation instrument including Gene Pulser Xcell, Amaxa Nucleofector, and BTX
- Simple and straight forward optimization steps
- One solution that delivers both plasmid DNA and siRNA