High Throughput Transfection of Stem Cells, Primary Cells and Difficult-to-Transfect Cell Lines: Jurkat, CHO, Human Skeletal Muscle Cells & Primary Neuronal Cell Transfection using a Scalable, Electroporation-Based Technology

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Scientific Poster

Abstract

Most high throughput, high content screening and profiling assays rely on exogenous gene expression of reporter genes, fusion proteins and artificially engineered proteins or overexpression of a target of interest. While stable cell lines are a convenient tool for producing the large number of cells required to perform a single screen, they are time-, labor- and cost-intensive to create. Transient transfection offers the ability to quickly develop working assays, however, many of these technologies have limitations on compatible cell types. Additionally, they can require multiple small-scale transfections or use costly transfection reagents to produce a large number of cells. MaxCyte® scalable electroporation offers a cost-effective, reproducible transfection of up to billions of cells in less than 30 minutes with broad cell type compatibility. In this poster we present the transient transfection of a variety of difficult-to-transfect cells and their use in downstream assays. Specifically, we demonstrate how large-scale electroporation of Jurkat, CHO, human skeletal muscle cells and primary neuronal cells produces large numbers of quality transfected cells and can ease the burden of stable cell line engineering.

MaxCyte® Transfection

MaxCyte STx® Scalable Transfection System. MaxCyte offers a proprietary, scalable electroporation technology to transfect a variety of cell types, including primary cells, with DNA, RNA, siRNA, proteins or other biomolecules of interest. MaxCyte instruments feature optimized electroporation (EP) protocols for a wide range of cell types, simplifying assay development while maximizing performance and reproducibility. For a wide range of cell types, transfection efficiencies exceed 85% and cell viability is better than 90%. The MaxCyte STx can perform small-scale transfections of $5x10^5$ cells in seconds for use in basic research and assay development, or in less than 30 minutes for bulk transfections of up to $1x10^{10}$ cells in full-scale screening and profiling experiments.

- Simple
- Rapid
- High efficiency
- Broad cell type compatibility
- Scalable

Current MaxCyte STx® Protocols

- Jurkat
- K562
- NIH 3T3
- Renca
- Vero
- PC12
- Hep G2
- CV-1
- THP-1
- Min-6
- Panc-1
- L5278Y OS
- SH-SY5Y
- COS-1
- A549
- PC-3
- BHK-21
- RBL
- Neuro2a
- NS0
- C6
- CaCo-2
- RLE
- COS-7
- LNCaP
- DLD-1
- C2C12
- CHO
- Hela
- HEK 293
- Huh-7
- Primary Fibroblasts
- Mesenchymal Stem Cells

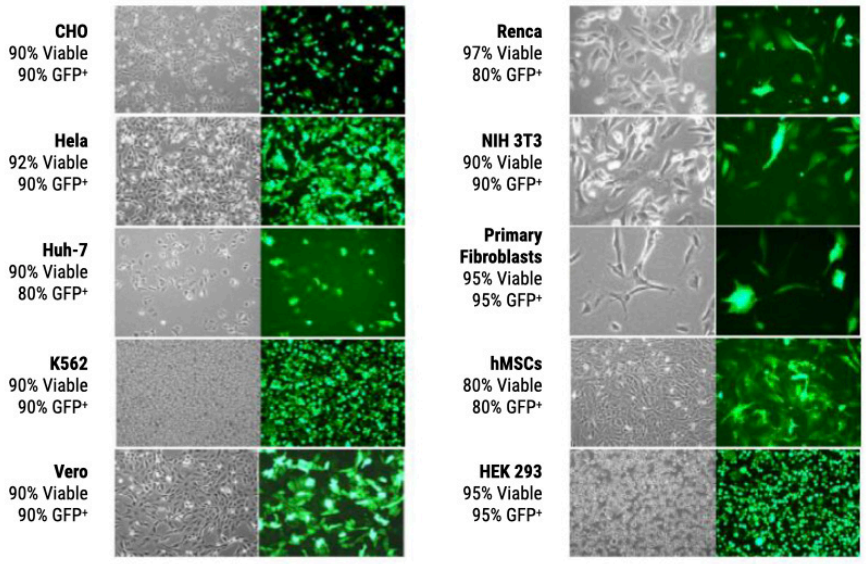

Figure 1: High Efficiency, High Viability Transfection. Ten different cell types were transfected with 200 µg/ml pGFP DNA using the appropriate pre-loaded protocol. 24 hrs post transfection cells were examined for cell viability (% cells excluding propidium iodide) and transfection efficiency (%GFP+ cells).

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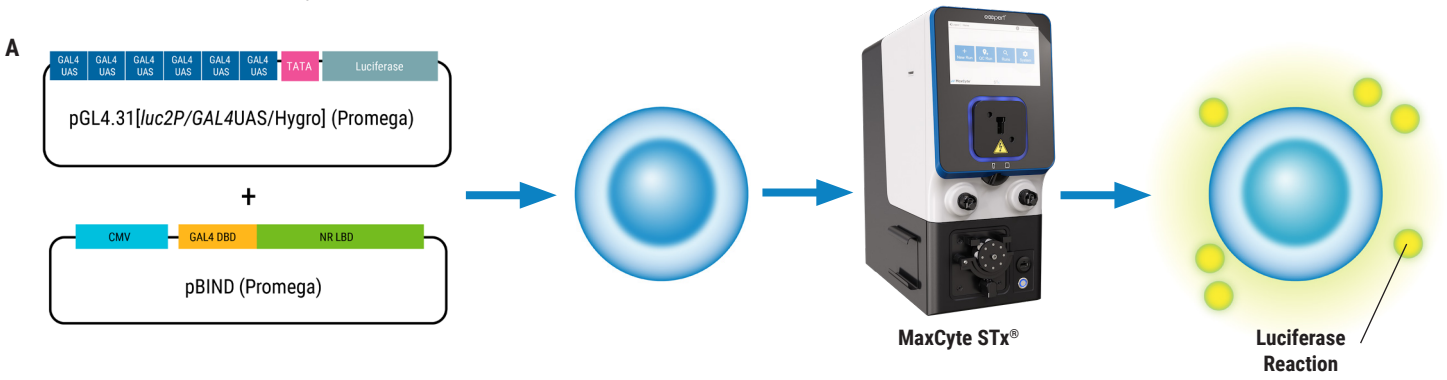
Jurkat Transfection

Nuclear Hormone Receptor Assay



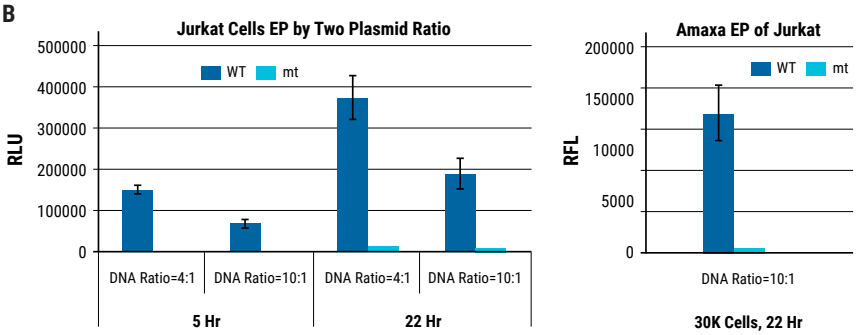
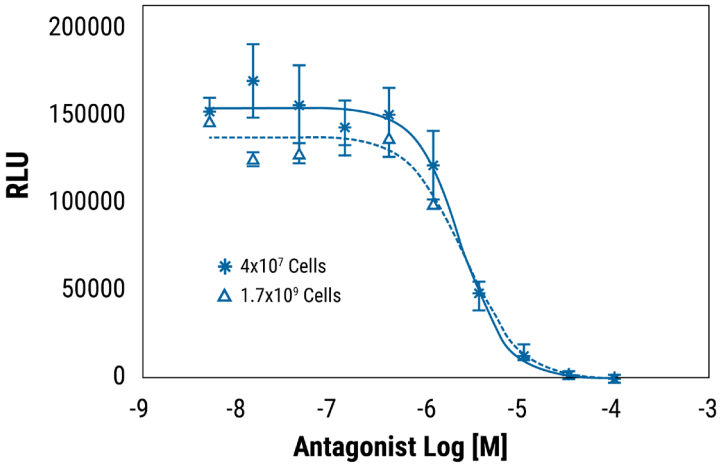


Figure 2: Electroporation Technology Comparison for a Nuclear Hormone Receptor Assay. A) Assay Schematic: Cells were co-transfected with luciferase reporter plasmid and activator plasmid B) Cells transfected using small scale MaxCyte STx electroporation at 2 plasmid ratios (4:1 or 10:1) or the Amaxa® electroporation system at a 10:1 plasmid ratio. Cells were seeded in 384-well plates and luciferase activity measured at 5 and 22 hrs post EP. N = 24 wells. The nuclear receptor assay sensitivity was found to be 10 times greater using the MaxCyte STx EP system.

Figure 3: Nuclear Hormone Receptor Assay Scale up & Cryopreservation. Jurkat cells were transfected with a 200 µg/mL plasmid mixture of reporter and wild-type activator plasmids using small scale and large scale MaxCyte EP. Cells were cryopreserved 30 minutes post EP. After cell thawing, cells were immediately treated with varying concentrations of inhibitor and luciferase activity measured. Data illustrate the scalability of MaxCyte's EP process and the ability of STx-transfected cells to be cryopreserved prior to use in functional assays.

MaxCyte Electroporation	EC 50	S/B
Small-Scale	2.39E - 06	635
Large-Scale	2.60 E - 06	604



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CHO Transfection

High Throughput Ion Channel Assay

Method	Method	Method	Method	Method	Method
Lipid Transfection (20 µg DNA + 60 µl lipid reagent)	Single Hole	77%	191±46 MΩ	4%	1.1±1.0 nA
MaxCyte Electroporation (150 µg/ml cDNA, 48 hr post-electroporation)	Single Hole	82%	248±87 MΩ	93%	2.8±1.4 nA
	Population Patch Clamp	100%	72±31 MΩ	98%	1.3±0.30 nA

Table 1: MaxCyte transfection vs lipid transfection: Cell Performance in an Automated Ion Channel Assay. CHO K1 cells were transfected with Kv1.5 α-subunit plasmid DNA using a commercial lipid-based transfection reagent or MaxCyte EP. Cells were assayed in the single hole and population patch clamp mode on the IonWorks® Quattro™ system. MaxCyte electroporation had far superior transfection efficiency. *Data courtesy of BioFocus.*

Antibody Production

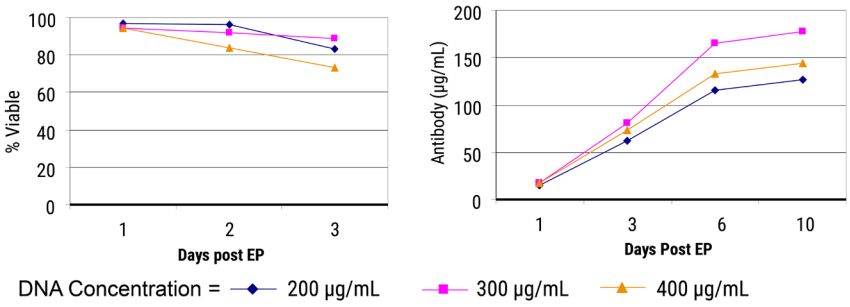

Figure 4: CHO Antibody Production. Cells were transfected with an equimolar mixture of heavy and light chain expression plasmids on day 0. Cell viability was measured on days 1, 2 & 3 following EP. Total IgG concentration in cell supernatant was measured using an ELISA on days 1, 3, 6 & 10. Transiently transfected cells exhibit a high level of viability for an extended period of time and produce a high titer of expressed antibody for greater than 10 days.

Primary Cell Transfection

Human Skeletal Muscle Cell Transfection

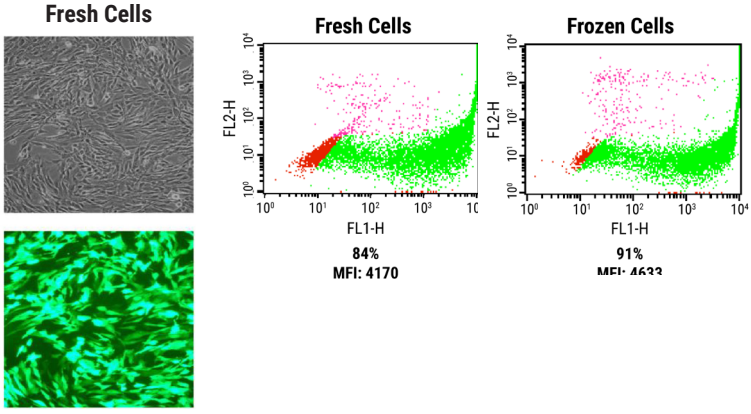

Figure 5: Highly Efficient Transfection of Primary Cells. Table: Results of processing primary cells with DNA plasmid encoding GFP. Efficiency expressed as % cells GFP+ at 24 hours post electroporation; viability as % cells excluding propidium iodide. Panels: Human Skeletal Muscle Cells (hSkMCs) were isolated from adult biopsy samples and transfected with 200 µg/mL of pGFP. Cells were either examined 1 day post EP (fresh) or cryopreserved post EP and examined 1 day following cell thawing (frozen). GFP expression was assessed via microscopy and FACS analysis. The data illustrate the high transfection efficiency and expression levels (84% GFP+; 4170 MFI) of primary cells using MaxCyte STx EP. Additionally, comparison to results of cryopreserved cells demonstrates the ability to cryopreserve transfected primary cells without sacrificing performance in subsequent cellular assays.

Cell Type	Efficiency	Viability
Human Fibroblasts	95%	95%
Human Myoblasts	90%	90%
Human Mesenchymal Stem Cells	80%	80%
Human Dendritic Cells	50%	80%
Human Lymphocytes - B Cells	85%	90%
Human Lymphocytes - T Cells	50%	70%
Human HSC (CD34+ Cells)	60%	60%
Human MCL	40%	50%
Human CCL	50%	70%
Human NK Cells	50%	70%

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Neuronal Cell Transfection

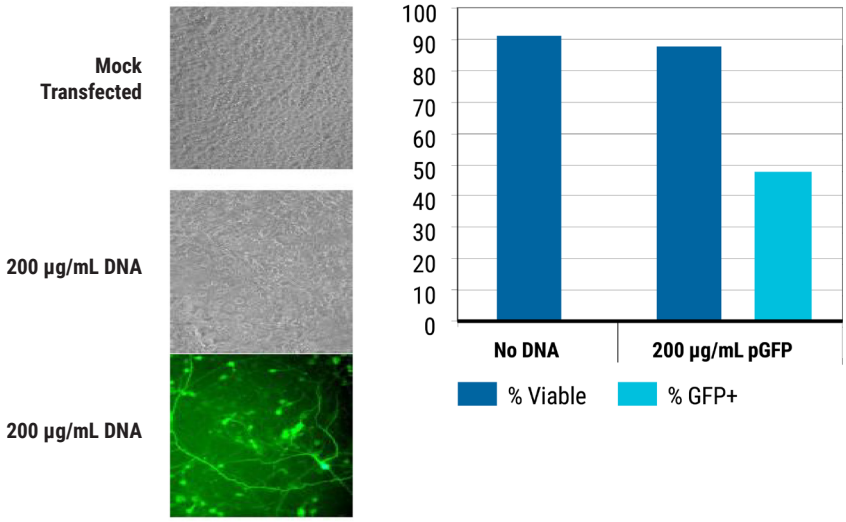

Figure 6: Exogenous Protein Expression in Primary Neuronal Cells. E18 rat hippocampal, cortical and ventricular neurons were electroporated with either 0 or 200 µg/mL pGFP. Cells were plated at $5x10^5$ cells/cm2 in multiwell plates. 5 days post EP cells were assayed for cell viability and GFP expression. Cell viability was greater than 85% with approximately 50% of cells positive for GFP expression. The mean fluorescence intensity was over 135 (data not shown).

Summary

- MaxCyte electroporation can be used to transiently (co)transfect a variety of primary cells, stem cells and historically difficult-to-transfect cell lines with high transfection efficiencies and cell viability.
- The MaxCyte STx transfection system is fully scalable, allowing researchers to rapidly transfect from $5x10^5$ to $1x10^{10}$ cells using the same protocol.
- MaxCyte electroporation can be used to express a variety of functional proteins including secreted proteins, membrane proteins and nuclear proteins.
- Cells transfected using the MaxCyte STx produce quality results when used in a variety of cellular assays and in antibody protein production.

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