

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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MaxCyte STX™
SCALABLE TRANSFECTION SYSTEM



Abstract

Most high throughput/ high content screening and profiling assays rely on exogenous gene expression such as reporter genes, expression of fusion proteins, artificially engineered proteins or over expression of a target of interest. While stable cell lines are convenient given 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 also require multiple small-scale transfections or use costly transfection reagents to produce a large number of cells. MaxCyte's scalable electroporation offers a cost-effective means to reproducibly transfect billions of cells in less than 30 minutes with the added benefit of broad cell type compatibility. We present data in this poster for the transient transfection of a variety of difficult-to-transfect cells and their use in downstream assays. Specifically, we will describe the transfection of Jurkat, CHO, human skeletal muscle cells and primary neuronal cells using MaxCyte's scalable electroporation technology and their performance in a variety of cell-based assays. These data demonstrate how large-scale electroporation can be used to eliminate the reliance on stable cell lines and costly transfection reagents, by producing large numbers of quality transfected cells for use in high throughput cellular screening and profiling.

Jurkat Transfection

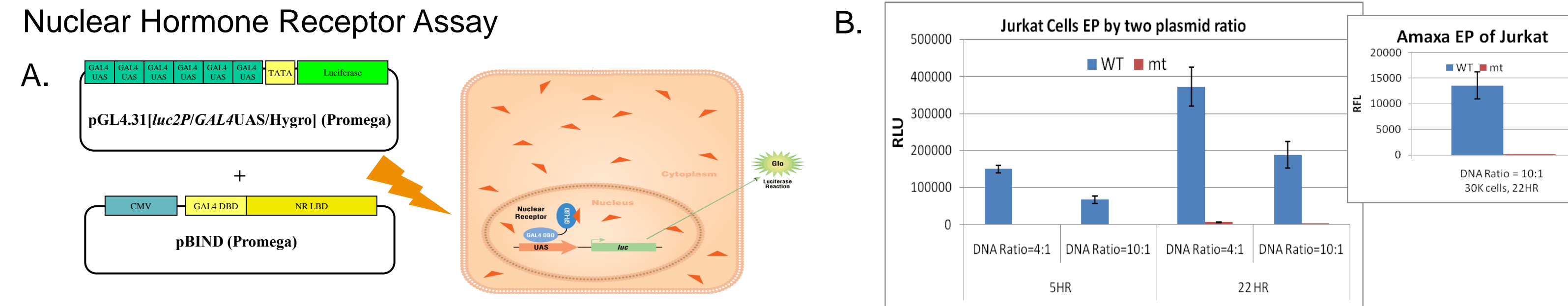


Figure 2. Electroporation System Comparison for a Nuclear Hormone Receptor Assay. A). Assay Schematic: Cells are cotransfected with a reporter plasmid (luciferase expressed from a minimal promoter containing multiple GAL4 UAS sequences) and an activator plasmid (constitutively expressed GAL4 DNA binding domains linked to either a wild-type, WT, or mutant, MT, nuclear receptor ligand binding domain). B). Cells were transfected using small scale MaxCyte STX EP at 2 plasmid ratios (4:1 or 10:1) or using the Amaxa EP 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.

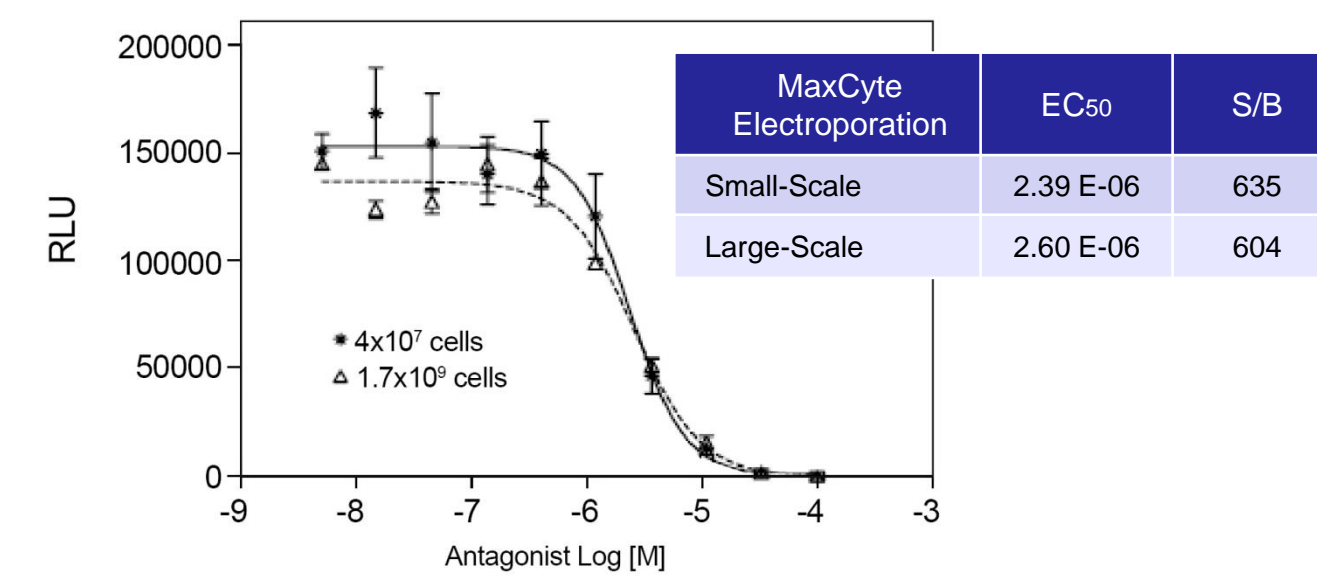


Figure 3. Nuclear Hormone Receptor Assay Scale up & Cryopreservation. Jurkat cells were transfected with a 200 µg/mL plasmid mixture of the reporter and wild-type activator plasmids described in Figure 2 using small scale and large scale MaxCyte EP. Cells were cryopreserved 30 minutes post EP. Cells were immediately treated with varying concentrations of inhibitor after cell thawing and luciferase activity measured. These data illustrate the scalability of MaxCyte's EP process and demonstrate the ability of STX transfected cells to be cryopreserved prior to use in functional assays.

CHO Transfection

High Throughput Ion Channel Assay

Method	PatchPlate	% Seals (>100MΩ)	Seal Resistance (mean±SD)	Expression (>0.5nA)	Current Amplitude (mean±SD)
Lipid Transfection (20µg DNA + 60µl 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 Population Patch Clamp	82% 100%	248±87 MΩ 72±31 MΩ	93% 98%	2.8±1.4 nA 1.3±0.30 nA

Table 1. MaxCyte transfection superior to lipid transfection: Cell Performance in an Automated Ion Channel Assay. CHO K1 cells were transfected with K_v1.5 α-subunit plasmid DNA using a commercial lipid-based transfection reagent or with 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

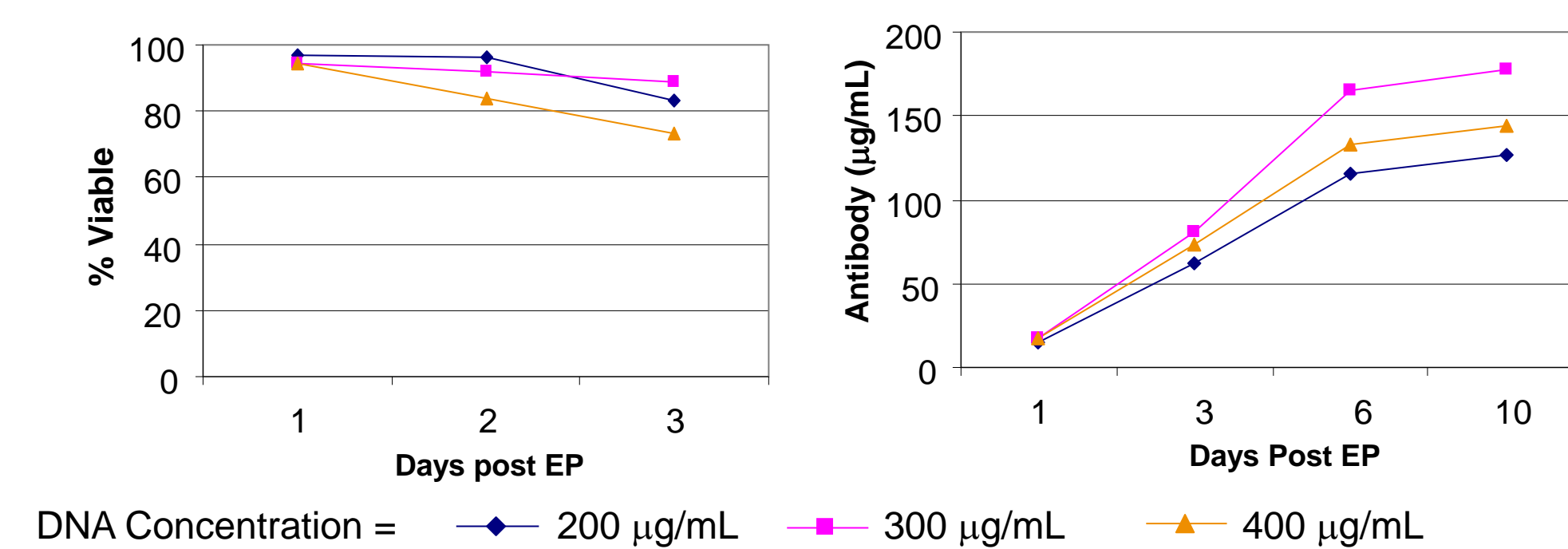


Figure 4. CHO Antibody Production. Cells were transfected with an equimolar mixture of heavy and light chain expression plasmids on day 0. Cell viability was measured on days 1, 2 & 3 following EP. Total IgG concentration in cell supernatant was measured using an ELISA on days 1, 3, 6 & 10. Transiently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

Primary Cells Transfection

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 CLL	50%	70%
Human NK Cells	50%	60%

Human Skeletal Muscle Cell Transfection

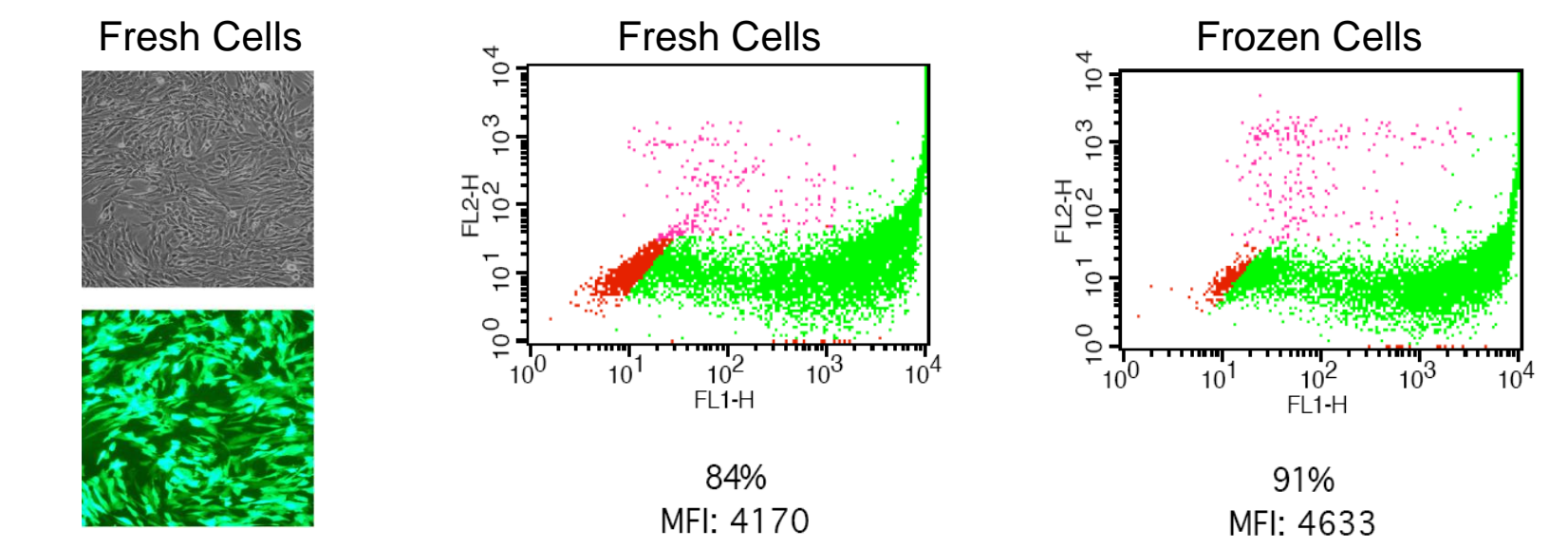


Figure 5. Highly Efficient Transfection of Primary Cells. Table: Results of processing primary cells with DNA plasmid encoding GFP. Efficiency expressed as % cells GFP+ at 24 hours post electroporation; viability as % cells excluding propidium iodide. Panels: Human Skeletal Muscle Cells (hSkMCs) were 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.

Neuronal Cell Transfection

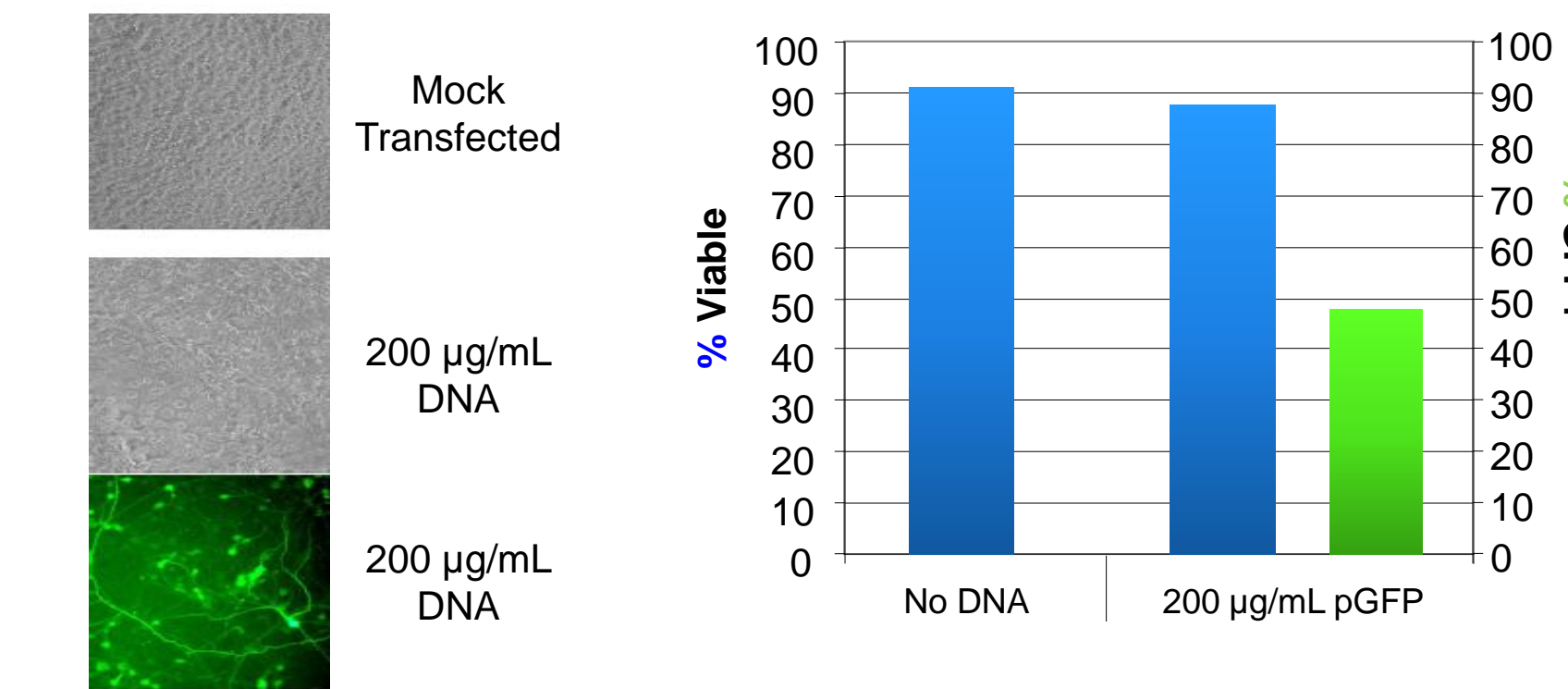


Figure 6. Exogenous Protein Expression in Primary Neuronal Cells. E18 rat hippocampal, cortical and ventricular neurons were electroporated with either 0 or 200 µg/mL pGFP. Cells were plated at 5x10⁵ cells/cm² in multiwell plates. 5 days post EP cells were assayed for cell viability and GFP expression. 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).

MaxCyte Transfection



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

MaxCyte® STX™ Scalable Transfection System. The MaxCyte STX uses a proprietary, scalable electroporation technology to (co)transfect a variety of cell types, including primary cells, with DNA, RNA, siRNA, proteins or other biomolecules of interest. The MaxCyte STX comes preloaded with optimized electroporation (EP) protocols for a wide range of cell types, thus simplifying assay development while maximizing performance and reproducibility. Transfection efficiencies are routinely greater than 85% and cell viability greater than 90%. The MaxCyte STX can perform small-scale transfections of 5x10⁵ cells in seconds for use in basic research and assay development or perform bulk transfections of up to 1x10¹⁰ cells in less than 30 minutes for use in full-scale, screening and profiling.

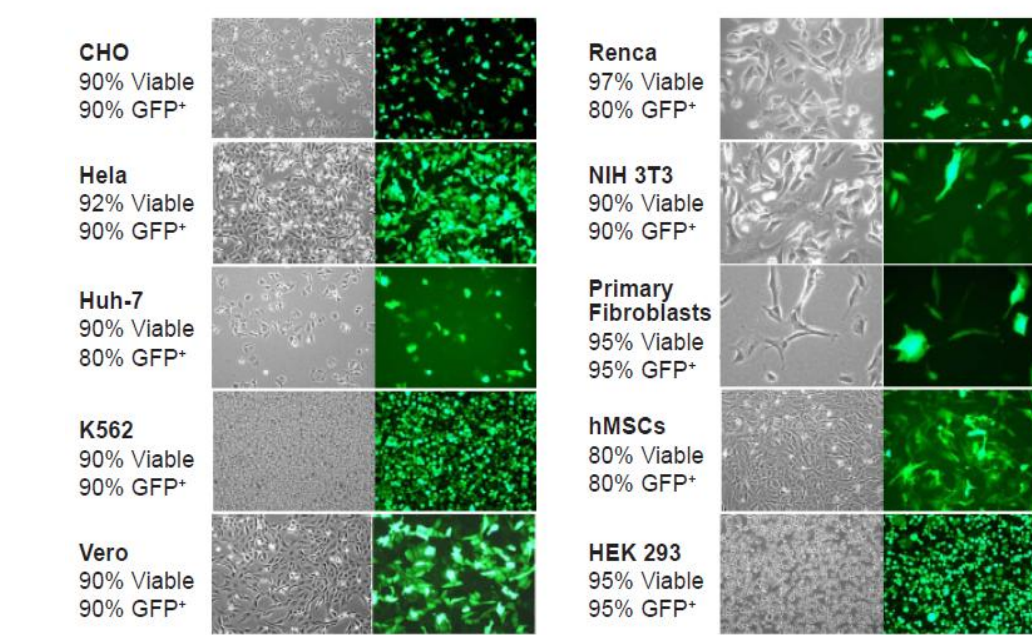


Figure 1. High Efficiency, High Viability Transfection. Ten different cell types were transfected with 200 µg/ml pGFP DNA using the appropriate pre-loaded protocol. 24 hrs post transfection cells were examined for cell viability (% cells excluding propidium iodide) and transfection efficiency (% GFP+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

Current MaxCyte STX Protocols

- > CHO
- > HeLa
- > HEK 293
- > Huh-7
- > K562
- > NIH 3T3
- > Primary Fibroblasts
- > Mesenchymal Stem Cells
- > Jurkat
- > K562
- > NIH 3T3
- > Renca
- > Vero
- > PC12
- > Hep G2
- > CV-1
- > THP-1
- > Min-6
- > Panc-1
- > L5278Y
- > U2OS
- > SH-SY5Y
- > COS-1
- > A549
- > PC-3
- > BHK-21
- > RBL
- > Neuro2a
- > NS0
- > C6
- > CaCo-2
- > RLE
- > COS-7
- > LNCaP
- > DL-1
- > C2C12

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 5x10⁵ to 1x10¹⁰ 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 for use in antibody protein production.

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