Using the Right Cell for the Right Application: Expanding the Role of Transient Transfection

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Abstract

Pharmaceutical, biopharmaceutical, and biotechnology companies must efficiently identify, develop, and quickly bring to market candidates -- whether small molecule drugs, therapeutic proteins, or vaccines -- with the highest level of efficacy at the lowest cost. These needs drive companies to seek more 'relevant' host cells, which will have the greatest predictability to de-risk development. There is no single, ideal cellular system with native physiology that provides a cost- and time-efficient means of fulfilling all aspects biotherapeutic and drug development. Therefore, scientists must identify what they deem as the 'right cell' for the right application. This poster provides project-specific data generated with the MaxCyte STX® Scalable Transfection System using the most appropriate cell line for a given application: primary neurons for Alzheimer's research, human-derided cells for cystic fibrosis drug development, a human tumor cell line for siRNA screening, jurkat cells for a nuclear receptor assay, CHO cells for gram-scale antibody production, and insect cells for vaccine production.

MaxCyte Transient Transfection Platform

MaxCyte STX®

5E5 Cells in Seconds
Up to 1E10 Cells in <30 Min.

MaxCyte VLX®

Up to 2E11 Cells in <30 Min

The MaxCyte STX® and MaxCyte VLX® Transient Transfection Systems use fully scalable flow electroporation for rapid, highly efficient transfection with very high cell viability post transfection. Transfected cells support gram scale production of antibodies and proteins for efficient biotherapeutic development.

- Rapid & simple to use
- High efficiency & high cell viability
- CHO, HEK, NS0, Vero & Insect cell compatibility
- Streamlined scalability requiring no re-optimization

Summary

- MaxCyte electroporation can be used to transiently (co)transfect a variety of primary cells, stem cells, and difficult-to-transfect cell lines with high transfection efficiencies and cell viability.
- MaxCyte transient transfection can be used to develop a more physiological screening system based on human cells.
- MaxCyte electroporation offers seamless scalability and minimal impact on cell health for assay production.
- MaxCyte electroporation allows assay sensitivity to be tightly controlled by varying the DNA concentration.
- siRNA transfection using MaxCyte electroporation is a controlled process that has proven to enable target specific protein knockdown.
- MaxCyte electroporation can produce antibody titers in CHO cells >1.2 g/L with optimized conditions.
- Using MaxCyte electroporation in insect cells, it is possible to go from gene to plasmid in as few as 3 days.

Benefits

- MaxCyte electroporation enables assays to be developed in a biologically relevant cell, minimizing risk of irrelevant candidates being put forward.
- MaxCyte electroporation allows for the transfection of difficult-to-transfect cells, including primary cells and stem cells.
- Electroporation and scale-up protocols are optimized and computer controlled, with reproducible results from day to day and operator to operator. There is no need for reoptimization of reagents or upstream and downstream scale changes, thereby saving time and improving productivity by allowing more campaigns with the same amount of resources.

Human-derived Cell Lines

Strong GFP Expression in Transfected Primary Neurons

High Levels of Transfection Efficiency and Cell Viability

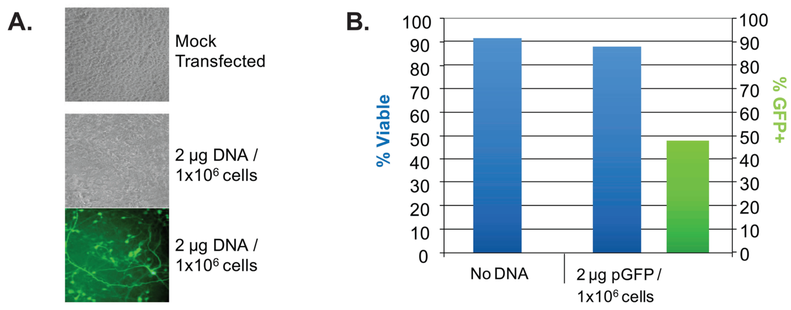


Figure 1: ***High Cell Viability and GFP Expression Following Electroporation of Primary Neurons.*** *Embryonic rat hippocampal, ventricular, and cortical neurons were electroporated with 0 or 2 µg GFP expression plasmid /1E6 cells using the MaxCyte STX. Cells were plated at 5E5 cells/cm² in 96-well plates. Cells were assayed for cell viability and GFP expression five days post-electroporation.* ***A.*** *Mock transfected and transfected or cells were imaged using bright field and fluorescence microscopy.* ***B.*** *Cell viability (blue bars) was determined by trypan blue staining and GFP expression (green bar) via FACS analysis.*

High Throughput Screening Using CFBE Cells

Relevant Human-derived Model Enables Rapid Screening of over 1,000,000 Compounds

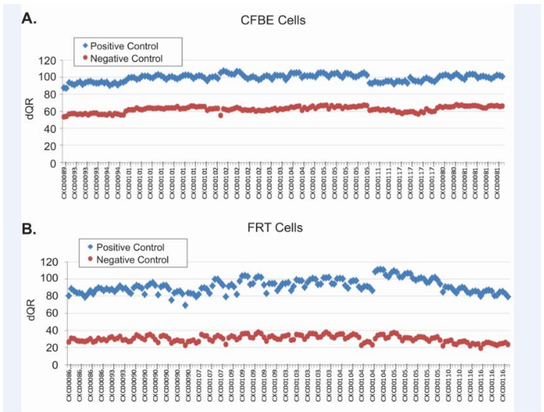


Figure 2: Validation of CFBE Co-transfected Cells for Primary Screening. Iodide flux assays were performed in 384-well microplates using either CFBE cells **A.** co-transfected with YFP and CFTR-dF508 plasmids or FRT cells **B.** stably expressing CFTR-dF508. A known positive and negative CFTR corrector was used. dQR = change is the YFP quenching rate. The data represent >600 assay plates from 7 screening experiments run over the course of 3 days. Assay plate IDs are shown on the x-axis. Each blue and red dot represents an averaged reading from a single assay plate for positive and negative control wells, respectively.

Straightforward Development of a Reporter Gene Assay

MaxCyte Electroporation Enables Tight Control of the Assay Window

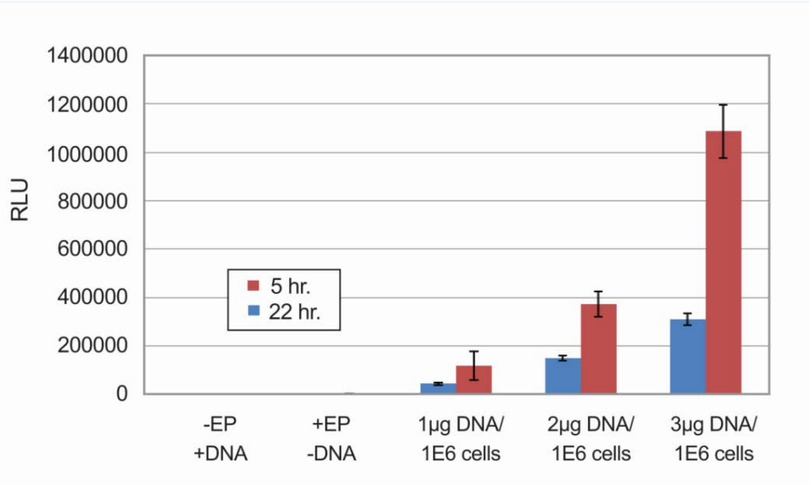


Figure 3: ***Nuclear Receptor Assay Sensitivity Directly Correlates with DNA Concentration.*** *Jurkat cells were co-transfected using small-scale MaxCyte electroporation with various concentrations of a plasmid mixture (4:1 receptor to activator). The reporter plasmid expressed luciferase from a minimal promoter containing multiple GAL4 UAS sequences. The activator plasmid constitutively expressed a fusion protein containing a GAL4 DNA binding domain linked to a nuclear receptor ligand binding domain. Transfected cells were plated in multi-well plates with approximately 15,000 cells/well and luciferase activity measured 5 or 22 hours post transfection.*

Reduction in Protein Expression With siRNA Transfection

MaxCyte Outperforms Liposome siRNA Cell Delivery

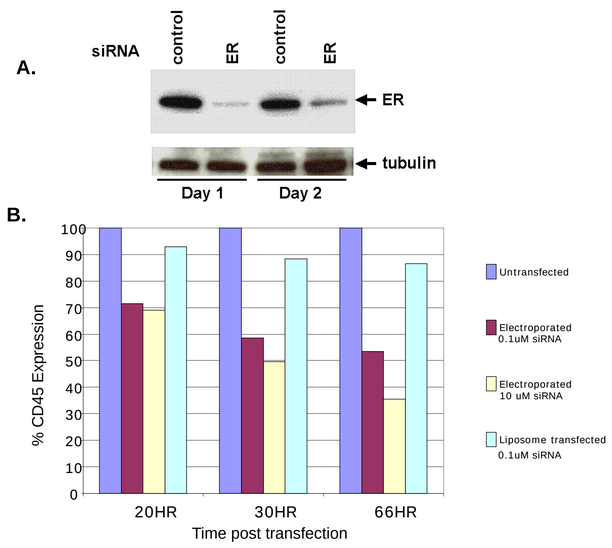
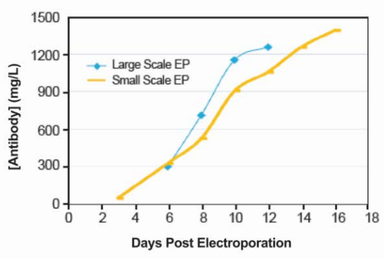


Figure 4. Efficient Delivery of siRNA and Expression Knockdown. A. MCF-7 cells were transfected with siRNA specific for estrogen receptor using small-scale MaxCyte electroporation. Expression of estrogen receptor and tubulin in transfected and untransfected cells were assessed using Western blot analysis. **B.** Primary CD45+ cells were transfected with siRNA specific for CD45 using MaxCyte electroporation (0.1 µM and 10 µM siRNA) or via liposome-mediated transfection (0.1 µM siRNA). CD45 expression was assessed via FACS analysis at 20, 30, 66 hours post transfection.

Protein Production in Manufacturing Cells

Rapid, Gram Scale Antibody Production

>1.2 g/L Antibody Titers with Optimized Feed



	Culture Volume	EP Volume	# of Cells	[IgG]	Total IgG Produced
Small Scale	20mL	0.4mL	8E7	**1.40 g/L**	28mg
Large Scale	2.8L	50mL	1E10	**1.22 g/L**	**3.42 g**

Figure 5: ***High-Titer Antibody Production from CHO Cells.*** *CHO-S cells were transfected with an antibody expression plasmid (2 µg DNA/1E6 cells) via small-scale (8E7 cells) or large-scale (1E10 cells) MaxCyte STX electroporation. Cells were seeded at 6E6 cells/mL post electroporation. 1 mM sodium butyrate was added to cultures and the temperature lowered to 32°C 24 hours post electroporation. Cultures were fed daily with a media optimized for antibody production. Secreted IgG titers were measured via ELISA on various days post transfection and total IgG production calculated.*

High Efficiency & Viability Transfection of Sf9 Insect Cells

Rapid, High-level Protein Production

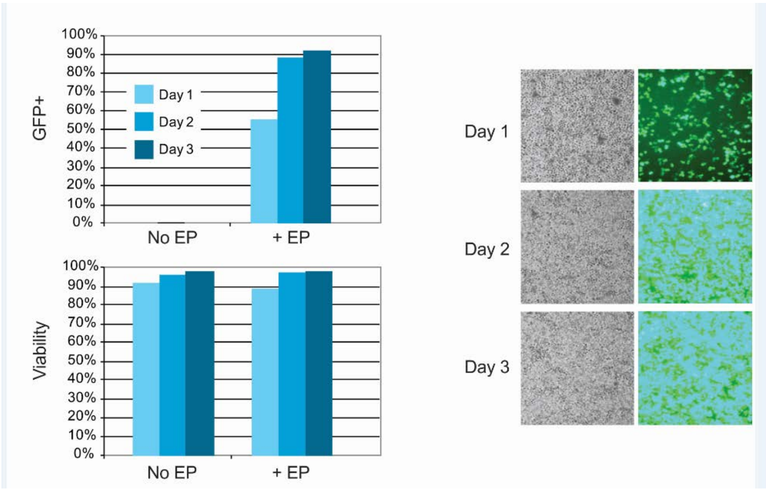


Figure 6: ***Sf9 VLP Production Using MaxCyte Electroporation. Plasmid to Protein in 2 to 4 Days.*** *Sf9 cells were transfected via small-scale electroporation with a single plasmid encoding 3 antigens that co-assemble into VLPs. Culture media was collected at various times from cells post EP or following baculovirus infection and analyzed using SDS-PAGE.*

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