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FLOW TRANSFECTION SYSTEMS™

Accelerating Discovery and Development: Reducing Risk with High Performance Transfection of the Right Cell for the Right Application

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To remain competitive in today's world, pharmaceutical, biopharmaceutical, and biotechnology companies must quickly identify, develop, and bring candidates to market with high efficiency at the lowest cost. The need for speed and improved patient care drives companies to conduct their discovery and development programs in ways that will provide the greatest predictability and likelihood of success. Whether small molecule drugs, therapeutic proteins, or vaccines, a commonality in the development and manufacturing of all candidates is the need for exogenous protein expression, be it for applications in basic research, high throughput screening, or protein production. MaxCyte electroporation represents a high performance, highly flexible transient transfection platform that allows for scalable transfection of a large variety of cell types, including primary and difficult-to-transfect cells, to generate proteins and modified cells for discovery, development, and production. This technical note presents case studies in which companies sought to develop biologically relevant systems using MaxCyte flow electroporation to accelerate discovery and development and improve the quality of their therapeutic candidates.

Today's pharmaceutical and biotechnology companies strive to bring highly efficacious drugs and biologics to market in a more time- and cost-effective manner. To accomplish this, researchers must minimize the risk of putting extraneous candidates forward by better predicting how a candidate will behave *in vivo*. Ideally, this means using a biologically relevant host, which has not always been possible. Whether developing small molecules, large molecules, or biologics, 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.

Considerations During Small Molecule Development

For successful identification of high quality hits through screening, the host cellular system must be biologically relevant, frequently defined as coming as close to native biology as possible. Primary cells and human-derived cell lines provide the most predictive biology; historically, however, these cells have been more difficult to modify than other cell lines.

The MaxCyte electroporation platform, originally designed for the functional modification of patient cells for cell therapy, represents a solution to the transfection challenges normally seen for primary cells. Case studies 1-4 describe the use of MaxCyte electroporation within disease-specific research and/or high throughput screening to increase the biological relevancy of cell-based assays by using primary cells or human-derived cell lines to identify candidates.

Considerations during Biotherapeutic Development & Manufacturing

Recombinant protein production is an inherent step in the development and ultimate manufacturing of biotherapeutic proteins and vaccines. Mammalian cells, insect cells, yeast, and bacteria are all used for protein production. Choosing a relevant host system for expression, whether for production of preclinical research materials or clinical biomanufacturing, has large implications. For each host system, scientists must consider not only the quantity of protein needed but also the desired characteristics of the protein, such as post-translational modifications, and the current regulatory environment.

Research continues to support the relationship between post-translational modifications such as glycosylation and a variety of protein properties including therapeutic efficacy.[1,2] The types and patterns of post-translational modifications are cell-type specific. This suggests that data generated during preclinical development may not be indicative of the final therapeutic product if the cellular background is changed when activities migrate down the pipeline from discovery through development and to large-scale production. Using different host cell lines during different stages of development may result in the progression of irrelevant candidates or the omission of promising candidates, both of which negatively impact the time and cost of biotherapeutic development and ultimate patient care.

Case studies 5 and 6 demonstrate the use of MaxCyte electroporation for the rapid expression of biotherapeutic

antibodies and vaccines during screening, characterization, and development stages in host cell lines relevant to manufacturing.

Maxcyte Transfection & Instrumentation

MaxCyte flow electroporation efficiently (co)transfects a variety of cell types with DNA, RNA, siRNA, proteins, and cell lysates without requiring specialized constructs, engineered cells, media additives, or chemical reagents. MaxCyte offers two benchtop, flow electroporation systems designed to support the needs of applications as diverse as small molecule screening, biotherapeutic development, protein production, and vaccine manufacturing: the MaxCyte STX® Scalable Transfection System and the MaxCyte VLX® Large Scale Transfection System. The MaxCyte STX can transfect from 5E5 cells within seconds up to 2E10 cells in <30 minutes. The MaxCyte VLX is ideal for extremely large-scale transfections with the capacity to transiently transfect up to 2E11 cells in less than 30 minutes.

Both MaxCyte instruments are supplied with a pre-loaded library of cell type-specific electroporation protocols optimized for a wide variety of cell types, simplifying process development while maximizing performance and reproducibility. These systems are computer-controlled, "plug-and-play" instruments that use single-use processing assemblies. Both instruments are ISO certified and cGMP-compliant with a Master File on record with the USFDA and Health Canada. In addition, the same chemically defined buffer, which maintains high levels of cell viability while maximizing conductivity, is used for all cell types.

MaxCyte Cell Compatibility

MaxCyte flow electroporation delivers proven high-quality transfection of a wide range of cell types including historically difficult-to-transfect cells, such as primary cells, as well as cell lines commonly used in screening and development of biologics (Figure 1). This broad level of cell compatibility is critical for enabling the migration to more biologically relevant cellular systems and the development of biologics in the cell background anticipated for use in biomanufacturing.

For a summary of transfection performance for commonly used cells please visit: http://www.maxcyte.com/technology/transfection-performance.

Primary Cell Transfection

Biologically relevant assays provide the greatest predictability of the *in vivo* activity of drug candidates. Incorporating primary cells within these campaigns has been limited due to the low transfection efficiencies of these cell populations. With MaxCyte electroporation, this obstacle has been removed. MaxCyte established its electroporation and transfection expertise by modifying patient cells for *ex vivo* cell therapy applications where the stringent requirements for efficiency, cell viability, reproducibility, and large cell numbers readily translate to benefits for biologically relevant cell-based assays and high throughput screening.

MaxCyte scientists have developed electroporation protocols optimized for a variety of primary cells that produce transfection efficiencies and viability rates (Table 1) that far exceed other transfection technologies. The same quality results are produced when transfecting primary cells in bulk, facilitating their use in screening assays. The current diversity and continual expansion of cell-specific MaxCyte electroporation protocols allow for future development of more physiological assays and migration of current assays from cell line models to primary cells.

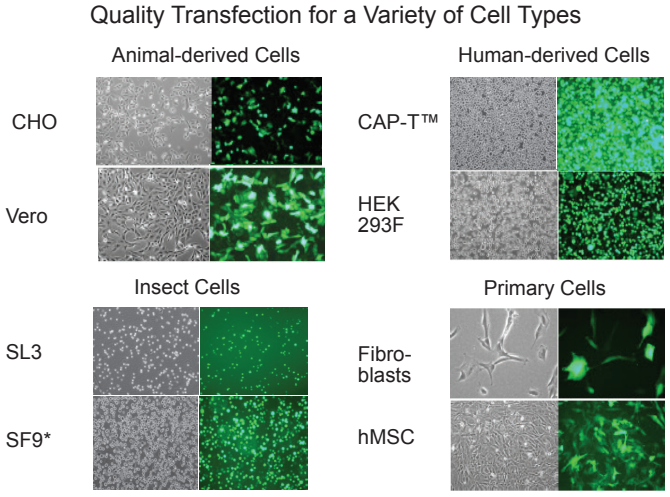


Figure 1: High Efficiency Transfection of Cell Types Commonly Used for Protein Production. *Various cells were transfected with 2 µg/1E6 cells of pGFP DNA using the appropriate MaxCyte STX protocol. Cells were examined for GFP expression using fluorescence microscopy 24 hr post electroporation. *SF9 cells examined at 72 hr post electroporation.*

Human Primary Cell Transient 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%

Table 1: High-Performance Transfection of a Variety of Human Primary Cells. *Primary cells were isolated from patient samples and transfected with pGFP DNA using MaxCyte electroporation. Efficiency expressed as % cells GFP+ at 24 hours post electroporation; viability as % cells excluding propidium iodide.*

Case Study 1: Overexpression of Proteins in Primary Neurons for Use in Alzheimer's Research

Alzheimer's disease (AD) is a chronic neurodegenerative disease that was estimated to affect 5.2 million individual in the United States alone in 2014.[3] The incidence of new cases of AD is staggering – 1 new case every 67 seconds in the U.S., due to the dramatic rise in the number of people over 65 years of age.[4] Five medications are currently approved by the U.S. Food and Drug Administration to treat Alzheimer's, although there is no medication that has been clearly shown to delay or halt the progression of the disease.[4]

Research conducted to date has identified AD as a protein misfolding disease, with extracellular amyloid beta (Aβ) deposits as the fundamental etiology. Due to neuronal involvement in the pathology of AD, primary neurons represent a highly biologically relevant model system. In this case study, scientists at a large pharmaceutical company overexpressed two proteins of interest in primary rat neurons via MaxCyte electroporation to establish a cellular model for small molecule screening.

Initial feasibility was assessed using transfection of primary rat embryonic hippocampal, ventricular, and cortical neurons using a GFP expression construct. Cell viability and transfection efficiency were assessed (Figure 2A) five days after MaxCyte electroporation. Mock-transfected neurons (electroporated with no DNA) and neurons electroporated with 2 µg GFP plasmid/1E6 cells had greater than 85% cell viability, demonstrating the low level of cellular toxicity from electroporation. Approximately 50% of neurons were positive for GFP expression.

Further studies using co-transfection of cDNAs encoding two proteins of interest demonstrated robust, DNA

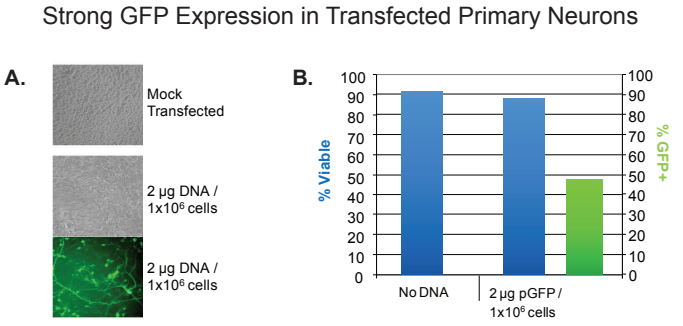


Figure 2: High Cell Viability and GFP Expression Following Electroporation of Primary Neurons. Embryonic rat hippocampal, ventricular, and cortical neurons were *electroporated with 0 or 2 µg GFP expression plasmid /1E6 cells using the MaxCyte STX. Cells were plated at 5E5 cells/cm² in 96-well plates. 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.*

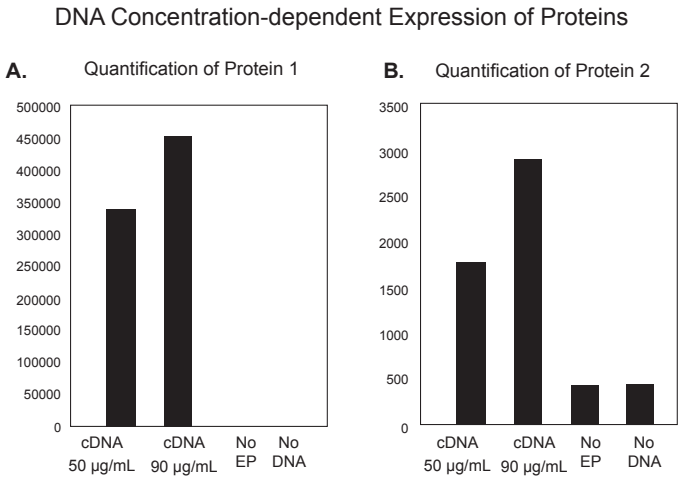


Figure 3: Quality Co-Transfection of Primary Neurons Provides for Robust Expression of Proteins of Interest. *E18 rat hippocampal, ventricular, and cortical neurons co-transfected with total cDNA quantities of 0, 0.5, or 0.9 µg/1E6 cells. A. Protein-specific detection assay for protein of interest 1. B. Protein-specific detection assay for protein of interest 2.*

concentration-dependent expression of the both proteins (Figure 3). These data demonstrate the ability of MaxCyte electroporation to transfect even difficult-to-transfect primary cells with high levels of transfection efficiency and cell viability for use in biologically relevant, disease-specific assays.

Case Study 2: Primary Screening Using Human-derived Cells in Cystic Fibrosis Drug Development

Cystic fibrosis (CF) is one of the most widespread life-shortening genetic diseases, affecting approximately 30,000 individuals in the United States.[5] CF is caused by mutations within the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is expressed in epithelial cells and is primarily responsible for controlling the efflux of halogens and for facilitating the movement of chloride within sweat glands. Over 1,000 mutations have been reported in the CFTR gene, but >70% of CF sufferers carry deletions of phenylalanine (F) at position 508 (dF508).[6] The CFTR-dF508 mutation causes deficiencies in both the trafficking of CFTR to the plasma membrane as well as altering channel-gating activities related to chloride (Cl^-) secretion.

While currently there is no cure for CF, many companies are developing small molecule drugs aimed at treating the disease at the level of CFTR. Traditional iodide flux assays used to screen for CFTR modulators frequently use the Fisher rat thyroid epithelial (FRT) cell line, which stably expresses mutated CFTR-dF508 and yellow fluorescent protein (YFP). CFTR activity causes iodide influx and quenching of the YFP signal. Hits identified using FRT cells, however, have shown to have sub-optimal correlation with compound activity in primary human

High Performance Transfection of Human-derived Cell Line

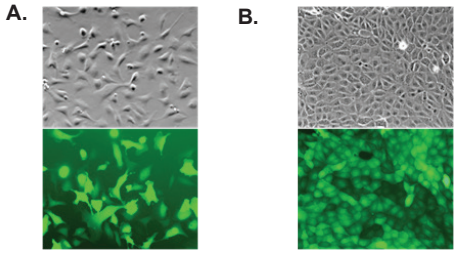


Figure 4: Strong YFP Expression upon Co-transfection of CFBE Cells. *Images of either CFBE cells co-transfected with an* ***(A)*** *YFP expression plasmid and CFTR-dF508 plasmid or* ***(B)*** *FRT cells stably expressing YFP and CFTR-dF508 via phase-contrast or fluorescence microscopy.*

Comparable Results of CFBE Cells in Functional CFTR Assay

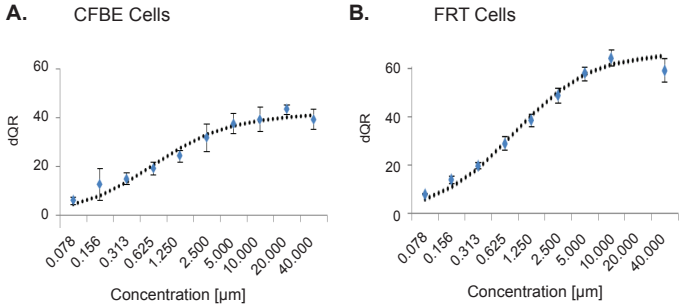


Figure 5: Side-by-side Comparison of Iodide Flux Assays Using CFBE Co-transfected or FRT Stable Cells. *dQR = change is the YFP quenching rate. Standard iodide flux assays were conducted using CFBE (**A.**) cells co-transfected with YFP and CFTR-dF508 plasmids or FRT cells (**B.**) stably expressing CFTR-dF508. Various concentrations of C-18, a known corrector, were used. Data are reported as mean±std, n=5.*

cells. Flatley Discovery Lab, a not-for-profit biotechnology company that focuses on discovering small molecule CFTR modulators, sought a more physiological screening system based on human cells.

CFBE cells, an immortalized bronchial epithelial cell line derived from a CF patient, are a potential human-derived model but are not available as a stable cell line expressing CFTR-dF508 and YFP. To develop a better screening model, CFBE cells were co-transfected with CFTR-dF508 and YFP expression plasmids using MaxCyte electroporation.

Co-transfection of CFBE cells resulted in a transfection efficiency of >85% and cell viability of >95% (Figure 4 and data not shown). A functional iodide screening assay using the co-transfected CFBE cells was developed and compared to the established assay using FRT stably transfected cells (Figure 5). Assays using both cellular systems showed comparable dose-response curves for a known CFTR small molecule corrector, C18, and were subsequently validated in a screening format (Figure 6).

Figure 6 summarized the results for seven experiments conducted over a 3-day period using FRT and CFBE cells.

High Throughput Screening Using CFBE Cells

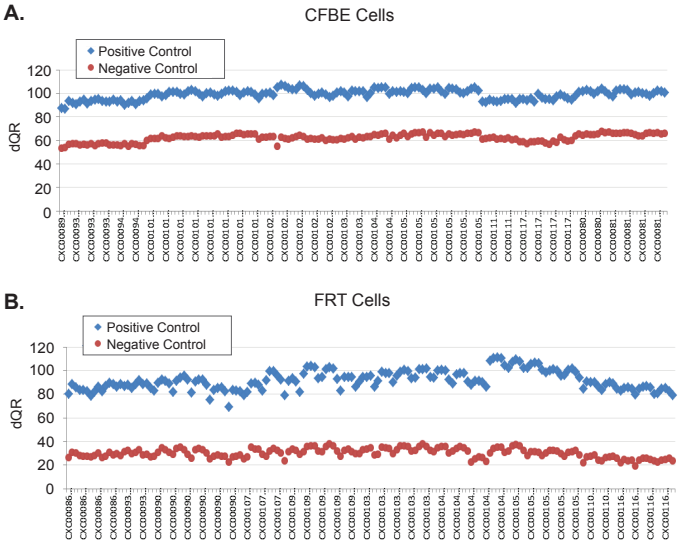


Figure 6: Validation of CFBE Co-transfected Cells for Primary Screening. *Iodide flux assays were performed in 384-well microplates using either CFBE cells (**A.**) co-transfected with YFP and CFTR-dF508 plasmids or FRT cells (**B.**) stably expressing CFTR-dF508. A known positive and negative CFTR corrector 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.*

Iodide flux assays were conducted on greater than 600 384-well plates using positive and negative controls. Despite CFBE cells being more difficult to culture than FRT cells, z' factors were consistently >0.5, supporting the feasibility of using the CFBE-based assay cells within the screening pipeline at Flatley.

CFBE co-transfected cells are currently used as the model system for primary screening at Flatley Discovery Lab using the general work flow described. Three to five times a week, CFBE electroporations are run using 1–2E9 CFBE cells per transfection. Cells are frozen immediately following a 30-minute recovery period and stored at -80°C for up to 6 months. Cells are thawed on day 1 of screening and seeded in 384-well plates. To screen for CFTR correctors, compounds are added and cells incubated for 24 hours at 37°C. Forskolin is added to stimulate CFTR transport of iodide on day 2 of screening and assay plates read following a 60-minute incubation. To date, the development of a highly relevant human-derived cell model using the MaxCyte transfection platform has enabled rapid screening over 1,000,000 compounds with several leads advanced for further development.

Case Study 3: siRNA Screening in a Human Tumor Cell Line for the Study of Breast Cancer

Breast cancer is characterized by a number of genetic alterations, many of which are involved in signal

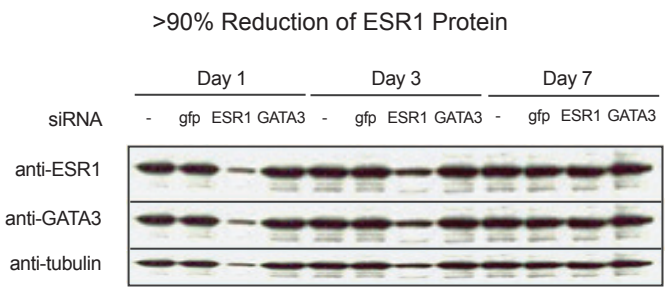


Figure 7: ESR1 Expression Inhibited by Transfected of ESR1 siRNA. *MCF-7 cells were collected on 1 and 3 days post electroporation with ESR1, GATA3-, or control GFP-specific siRNAs. Total proteins were isolated and probed with anti-ESR1, anti-GATA3, and anti-tubulin antibodies. Data courtesy of National Human Genome Research Institute.*

transduction pathways including hormone-dependent and growth factor-dependent signaling. Determining the regulation and relative importance of specific proteins to the causality and pathology of breast cancer can be difficult to assess. In this case study, researchers looked to develop a method of synthetic short interfering RNA (siRNA) transfection to silence the expression of estrogen receptor alpha (ESR1 gene in humans) and the trans-acting T-cell-specific transcription factor GATA-3 in an effort to assess the role these proteins play in breast cancer.

MCF-7 is a human-derived breast cancer cell line that has a luminal epithelial phenotype, contains estrogen receptors, and proliferates in response to estrogens. During initial feasibility studies, MCF-7 cells expressing GFP (MCF-7/GFP) were electroporated via the MaxCyte STX with FITC-labeled siRNA. The levels of GFP and FITC were measured via FACS analysis. More than 80% of the MCF-7/GFP cells were positive for FITC-labeled siRNA, illustrating the high level of MCF-7 transfection. FITC-positive cells exhibited corresponding decreases in GFP expression (data not shown), suggesting that GFP expression was suppressed by transfected siRNA.

ESR1 and GATA3-specific siRNAs were subsequently introduced into MCF-7 cells using MaxCyte electroporation, and protein expression was assessed via western blot analysis. Figure 7 illustrates the efficient silencing of the ESR1 or GATA3 genes in a siRNA-specific manner on day 1 and day 3 post electroporation. A control siRNA targeting GFP did not impact expression of either the ESR1 or GATA3 targets. In contrast, tubulin protein levels were unaffected by electroporation with either of these siRNA.

Monitoring of ESR1 and GATA3 siRNA transfected cells via microscopy showed changes to cellular morphology, unlike mock transfected cells or cells transfected with GFP siRNA. This demonstrates the utility of MaxCyte electroporation not only for the transfection of human-derived cells but also for highly efficient, target-specific siRNA silencing for disease-specific research in physiologically relevant systems.

Case Study 4: Streamlined Antagonist Screening in Jurkat Cells Using Cryopreserved Cells

Jurkat is a human-derived T lymphocytic cell line used in a variety of primary screening assays. In this case study, a pharmaceutical company needed to develop a nuclear receptor assay with sufficient signal-to-background (S/B) values for large-scale antagonist screening using assay-ready, cryopreserved cells.

Jurkat cells were co-transfected with two plasmids, a nuclear receptor and a luciferase reporter, using various concentrations of total DNA via small-scale MaxCyte electroporation during assay development. Cells were seeded in 96-well assay plates immediately after electroporation and luciferase activity measured at 5 and 22 hours post transfection (Figure 8). Luciferase activity was DNA concentration-dependent, which allowed the scientists to balance DNA usage with assay sensitivity. While overnight culture of transfected cells (22 hours) resulted in a more robust assay, expression of both constructs was sufficient to perform the assay as soon as 5 hours post electroporation. This enabled screening to be conducted on the same day as transfection if desired.

Studies to assess the feasibility of cryopreserving transfected cells prior to use in the reporter gene assay produced comparable IC_{50} values for a known antagonist, 1.3 µM and 1.4 µM, in fresh and frozen cells, respectively (data not shown). Thus, it was established that co-transfected cells

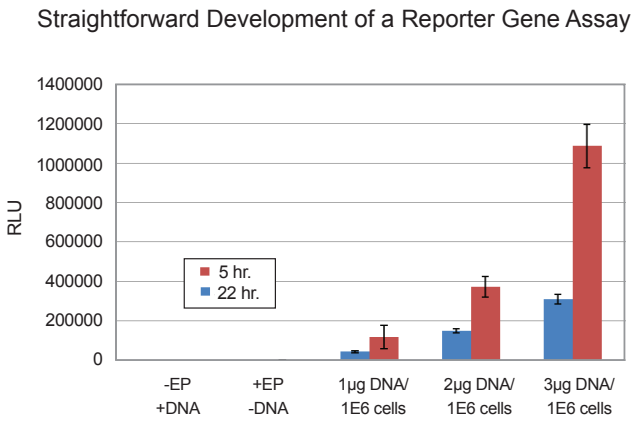


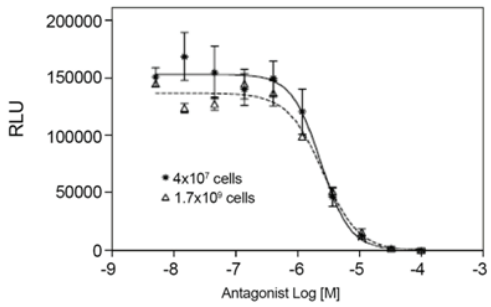
Figure 8: Nuclear Receptor Assay Sensitivity Directly Correlates with DNA Concentration. *Jurkat cells were co-transfected using small-scale MaxCyte electroporation with various concentrations of a plasmid mixture (4:1 receptor to activator). The reporter plasmid expressed luciferase from a minimal promoter 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 384-well plates with approximately 15,000 cells/well and luciferase activity measured 5 or 22 hours post transfection.*

could be cryopreserved to create a stock of assay-ready cells for increased work flow flexibility and improved control of experiments.

As a final validation prior to assay implementation, scale-up of MaxCyte electroporation was performed. Jurkat cells were aliquotted and cryopreserved following a 30-minute recovery period post small- or large-scale co-transfection of the two nuclear receptor assay plasmids using the same electroporation protocol. Cells were immediately treated with antagonist upon thawing and luciferase activity measured after an overnight incubation. Small- and large-scale electroporation produced nearly identical EC_{50} and S/B values as well as z' factors sufficient for screening (Figure 9). These data show that transfection quality of even difficult-to-transfect, biologically relevant cells and their performance in downstream functional assays are unaffected by scale-up and do not require protocol or assay reoptimization. Using the MaxCyte electroporation platform enables rapid assay development, validation, and implementation within a biologically relevant screening environment.

As an additional benefit, the high transfection efficiency and cell viability of MaxCyte electroporation allowed cryopreservation of transfected cells, which translated into further work flow flexibility. A large quantity of transfected assay-ready relevant cells enables multiple projects from the same batch of cells, improving experimental control and consistency and saving time.

Jurkat-based Nuclear Hormone Receptor Screening



MaxCyte Electroporation	EC_{50}	S/B	z'
Small-Scale	2.39 E-06	635	0.63
Large-Scale	2.60 E-06	604	0.86

Figure 9: Simple Assay Scale-up Using Cryopreserved Cells. *4E7 or 1.7E9 Jurkat cells, were co-transfected with a mixture of the reporter and activator plasmids (total DNA = 2 µg /1E6 cells) using small-scale or large-scale MaxCyte electroporation, respectively. Cells were cryopreserved 30 minutes post electroporation. After thawing, cells were immediately treated with varying concentrations of a known inhibitor and luciferase activity measured. Error bars denote standard deviations in 3 replicate wells. EC50, S/B, and z' values were calculated.*

Case Study 5: mAb Biotherapeutic Development—Gram-Scale CHO Antibody Production

Therapeutic monoclonal antibodies (mAbs) represent the predominant component of biotherapeutic development pipelines for a broad range of clinical indications. Production of stable Chinese hamster ovary (CHO)-derived cell lines remains the most commonly used system for biomanufacturing of clinical-grade antibodies.

Stable cell lines offer consistent product quality, regulatory familiarity, and lower costs for large-scale manufacturing. However, stable cell line generation is an extended process that takes many months even with improved cloning and expression technologies and is costly in terms of labor and materials. Industry has looked to transient gene expression (TGE) rather than developing stable cell lines as a means to rapidly screen large numbers of antibodies or antibody-like molecules early in the development process to identify promising candidates for further evaluation.

Initial CHO-based TGE activities were limited by poor transfection efficiencies and production of inadequate quantities of antibodies. This led to the use of transient systems based on human embryonic kidney (HEK) cells, which are easily cultured in suspension and generally have higher transfection efficiencies. Multiple studies, however, have reported differences in the glycosylation patterns of proteins and antibodies when produced in CHO versus HEK cells.[7-9] This suggests that preclinical development in HEK cells increases the risk of late-stage developmental failures due to potential alterations in biophysical properties upon migration to CHO-based stable expression. Thus, if CHO cells are the intended means of biomanufacturing, the most relevant candidates are identified using CHO cell-based protein production during early development.

High Performance CHO Cell Transfection

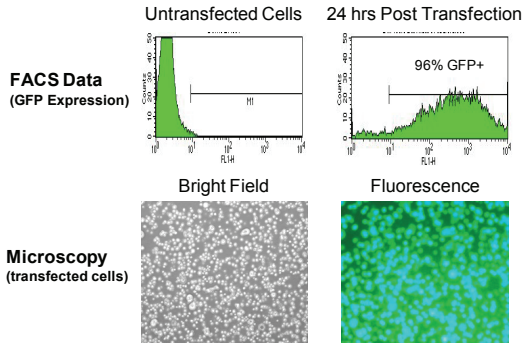
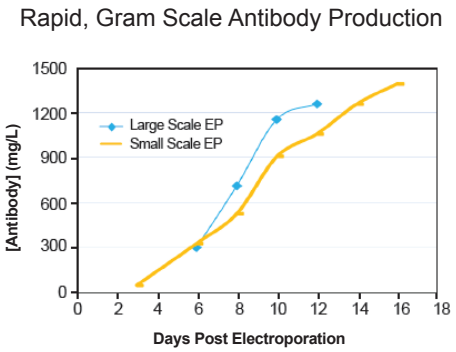


Figure 10: Greater than 95% CHO Cell Transfection Efficiency and Cell Viability Using MaxCyte Transient Transfection. *Non-engineered CHO-S cells were transfected with a plasmid encoding green fluorescent protein (2 µg DNA/1E6 cells) using small-scale, static electroporation on the MaxCyte STX. GFP expression and viability were measured by flow cytometry (FACS) 24 hours post electroporation.*



	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 11: 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.*

This case study demonstrates the high levels of transfection efficiency and cell viability following CHO cell transfection using MaxCyte electroporation. Additionally, data are presented for the application of CHO cell transfection for the gram-scale production of a monoclonal antibody for use in early- and mid-stage biotherapeutic development.

Non-engineered CHO-S cells transiently transfected via the MaxCyte platform with a GFP plasmid display greater than 95% transfection efficiency and cell viability (Figure 10). These high levels of transfection efficiency and cell viability routinely results in secreted antibody titers >400 mg/L, which can exceed >2 g/L with optimization of post transfection culture conditions, such as cell density, feeding conditions, media additives, and culture temperature.[10]

Figure 11 summarizes data in which post transfection process development optimization resulted in antibody titers greater than 1.2 g/L, thereby producing over 3.4 grams of antibody from a CHO culture of less than 3 liters 12 days post transfection. Comparable antibody titers were produced using identical electroporation parameters on the MaxCyte STX from both small- and large-scale runs, demonstrating that assay reoptimization is not required for scale-up (Figure 11). Further scale-up to biomanufacturing using the MaxCyte VLX allows for transfection of up to 2E11 cells, which, based on the above titers, would equate to the production of over 50 grams of antibody within 2 weeks of a single transient transfection.

These data illustrate that MaxCyte flow electroporation has the high-level performance and scalability to produce gram-scale quantities of protein from CHO cells. This enables scientists to not only postpone the generation of stable cell lines, but more importantly to conduct early-stage development in the anticipated manufacturing cell type, decreasing late-stage failure due to changes in host cell backgrounds.

Case Study 6: Vaccine Production via Insect Cell Transfection

Virus-like particles (VLPs) are a promising avenue of vaccination with an inherently higher level of safety compared to inactivated or live attenuated viral vaccines. VLPs consist of one or more recombinantly expressed viral structural proteins that self-assemble into complexes that closely mimic the three-dimensional structure of the native virus. VLPs are manufactured using a variety of cells including insect cells, which are advantageous as they post-translationally modify proteins in a manner similar to that of mammalian cells, yet are easy to culture with simplified cell growth that is readily adapted to high-density suspension. While both transient transfection and recombinant baculovirus platforms are commonly used methods for insect cell protein expression,[11,12] MaxCyte electroporation offers a more rapid and straightforward means of VLP production.

Baculovirus-mediated protein production remains an extended, multi-stage process, commonly taking 6- to 8 weeks, despite development of specialized media and baculovirus vectors aimed at simplifying gene cloning and virus stock production. In contrast, MaxCyte electroporation directly

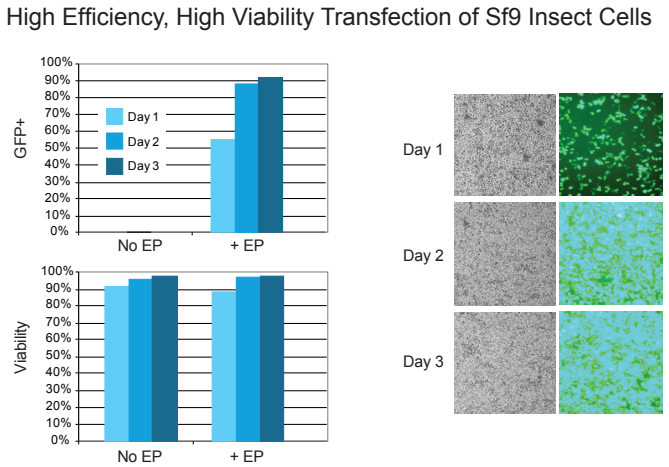


Figure 12: High Efficiency, High Viability Transfection of Sf9 Cells. *Sf9 cells were transfected with a GFP plasmid (baculovirus-based vector) at 2 µg/1E6 cells. GFP expression was assessed using FACS analysis, bright field microscopy, and fluorescence microscopy at days 1, 2, and 3 following electroporation.*

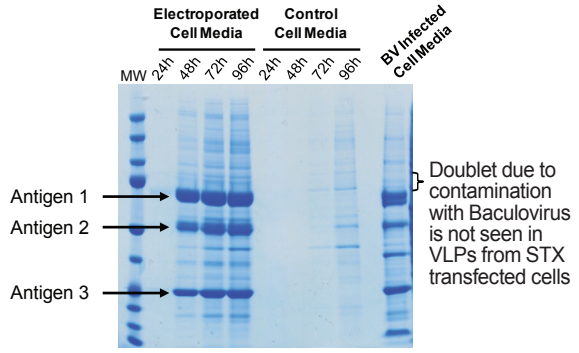


Figure 13: Sf9 VLP Production Using MaxCyte Electroporation. *Plasmid to Protein in 2 to 4 Days. Sf9 cells were transfected via small-scale electroporation with a single plasmid encoding 3 antigens that co-assemble into VLPs. Culture media was collected at various times from cells post EP or following baculovirus infection and analyzed using SDS-PAGE.*

transfects insect cells with the expression construct(s) of interest, allowing for strong protein expression within days of transfection.

This case study demonstrates the application of MaxCyte electroporation for the production of a VLP using Sf9 cells, a commonly used insect cell line. Initial studies of Sf9 cells electroporated with a GFP expression plasmid led to >90% transfection efficiency by day 3 post transfection (Figure 12). Cell viability was >90% at all time points with no differences in cell viability levels between electroporated and control, non-electroporated cells.

Sf9 cells were then electroporated with an expression construct encoding three VLP antigens, which resulted in significant secretion of the VLP within 48 hours post transfection (Figure 13). In tandem, a baculovirus expression system was used to produce VLPs containing the identical three antigens. SDS-PAGE analysis of cell supernatants showed the presence of the three VLP antigens in all samples; however, baculovirus protein contaminants were also present in supernatants from baculovirus-infected cells. The need to remove contaminating baculovirus proteins increases the time and cost of VLP production while reducing the overall yield.

These data demonstrate the extremely rapid and high-quality nature of direct insect cell transfection using MaxCyte electroporation, allowing the progression from plasmid to protein in as few as 3 days, eliminating the need for baculovirus usage and improving final yield.

Conclusions

To compete in today's market, pharmaceutical and biotechnology companies must make decisions regarding a candidate's potential quickly. Whether working in small molecule drug discovery or biotherapeutic research, scientists need to be able to transfect the right host cells, for the right application, at the right scale to make the best decisions on the most promising candidates. In each case study presented in this paper and many more, MaxCyte electroporation has proven to be a high performance means of transient transfection for a wide range of cell types and applications. This ability to transfect cell lines commonly used in screening and development of biologics as well as human-derived cells and primary cells facilitates the development of better qualified candidates, derisking development, and getting to the market and patients faster.

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