Flow Electroporation Capabilities and Case Studies: Rapid GPCR Screening and Functional Ion Channel Assays.

Contributing Authors: James Brady, Ph.D., Karen Donato, Ph.D. and Krista Steger, Ph.D.

Abstract

MaxCyte's proprietary flow electroporation technology has been successfully applied in *ex vivo* cell therapy (1) and drug discovery pipelines where reproducibility, efficiency and the need for increased cell numbers are critical. This technical paper discusses the merits of transient transfection using flow electroporation versus other recombinant expression approaches and its application in hit identification and lead optimization programs. Data are presented demonstrating the key features of the MaxCyte® STX™ Scalable Transfection System, including broad applicability, scalability, and the production of functionally relevant cells that enable its streamlined integration within cell-based assay methodologies. Case studies are presented for large-scale cellular GPCR screening and the functional characterization of multi-subunit voltage-gated ion channels.

Introduction

Most high throughput/high content cell-based screening assays rely on exogenous gene expression such as reporter genes, expression of fusion proteins, artificially engineered proteins or overexpression of a target of interest. Gene expression for these assays has historically been approached by either creating stable cell lines or using transient transfection. Stable cell lines have the advantage of long term protein expression; however, their creation is a costly, time consuming and labor intensive process that requires multiple rounds of selection and clonal isolation. Whether constructed internally or outsourced, the average time to create a stable cell line is approximately 10 to 15 weeks. This prolonged time between assay conceptualization and conducting a functional screen is eliminated using transiently transfected cells. Additional considerations of stable cell line usage include the complexity of expressing multiple targets or multi-subunit protein complexes, as well as potentially toxic targets. Due to time and budgetary constraints, many pharmaceutical companies have streamlined their compound screening and profiling initiatives by incorporating transient transfection systems within their drug discovery processes.

Transient transfection technologies have evolved from simple chemical carriers such as DEAE-dextran and calcium phosphate into sophisticated, highly engineered methodologies such as lipid-based reagents, viral-mediated delivery and high throughput electroporation (2). Newer technologies provide superior performance and broader applicability allowing introduction of DNA, RNA, siRNA and proteins into mammalian cells. A variety of transient transfection options are available, each having specific strengths and limitations. Researchers are tasked with matching their needs to the strengths of a specific technology. Factors to consider range from scientific performance such as cell viability, transfection efficiency, reproducibility and induction of off-target effects to more practical considerations such as ease of use, system flexibility and cost.

Additional factors must be taken into consideration for any assay technology to be successfully integrated within a screening environment. Most importantly, they must have high throughput capacities and reproducible results, while remaining easy to use and affordable. Chemical carriers, while affordable, are prone to high levels of variability and are thus not generally used in higher throughput applications. In contrast, lipid-mediated transfection, virus delivery and electroporation are more amenable to higher throughput settings. Second generation lipid-based technologies have relatively high transfection efficiencies and the ability to transfect a range of cells. Lipid transfections, however, require re-optimization of transfection conditions for higher throughputs and can become cost prohibitive.

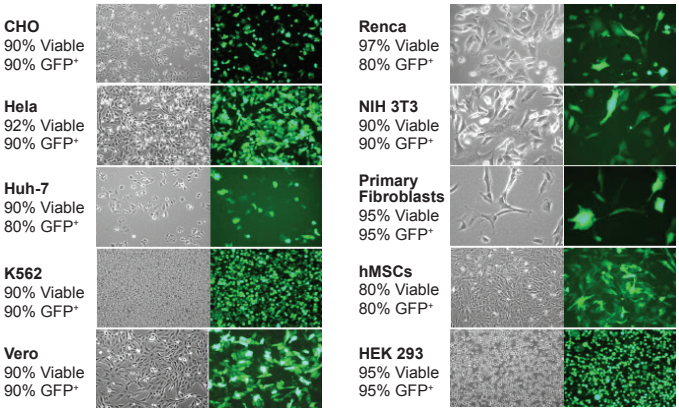


Figure 1: *The MaxCyte STX comes preloaded with optimized electroporation protocols for a variety of cell types. Ten different cell types were transfected with 2 µg/1E6 cells of pGFP DNA using the appropriate MaxCyte STX protocol. 24 hours post transfection cells were examined for cell viability (% cells excluding propidium iodide, PI) and transfection efficiency (% cells GFP+).*

While higher throughput viral delivery methods can achieve very high levels of transfection efficiency, vector type can limit cell compatibility. Additionally, creation of viral vectors and production of viral stocks can invoke biosafety considerations, often require a high level of user knowledge and are labor and time intensive.

MaxCyte's flow electroporation technology enables transfection of a large number of cells in a very short period of time. This technology combines broad applicability, ease of use and superior performance with the capacity to transfect 2 x 10^{10} cells in 20-30 minutes. This platform provides a cost effective solution that uniquely fulfills the demands of early phase drug development activities.

Maxcyte STX Capabilities

The MaxCyte STX is a highly versatile, benchtop transfection system based on MaxCyte's patented flow electroporation that overcomes many of the limitations previously associated with transient transfection. The MaxCyte STX can be used with a large number of cell types, including historically difficult to transfect cells such as primary cells, and can be used to transfect DNA, RNA, siRNA, proteins or other biomolecules of interest (3). This flexibility is augmented by its ability to perform small-scale transfections for assay development or in bulk for full-scale, high throughput screening.

MaxCyte STX transfection efficiencies are routinely greater than 85% and cell viability greater than 90% (Figure 1). MaxCyte technology causes minimal off-target perturbations of gene expression, resulting in assays with higher fidelity and improved efficiency (1). Additionally, MaxCyte scientists have developed electroporation protocols optimized for a variety of cell types, simplifying assay development while maximizing performance and reproducibility across a broad range of biological systems. The flexibility, scalability and ease of use of the MaxCyte STX translate into shorter times to screen, higher quality assays and improved physiologic relevance.

Primary Cell Compatibility

The demand for biologically relevant assays within early phase drug discovery continues to grow. Incorporating primary cells and stem cells within these campaigns has been limited due to the low transfection efficiencies of these cell populations. Thus, many high throughput and high content screening campaigns have had to be performed using cell lines.

MaxCyte has developed transient transfection protocols for a variety of primary cells that produce transfection efficiencies and viability rates that far exceed other transfection technologies (Table 1). 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 STX protocols allow for future development of more physiological assays and migration of current assays from cell line models to primary cells.

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: *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.*

Seamless Small- to Large-Scale Transfection

High content and high throughput campaigns require a large number of cells to perform a single screen. Other transfection technologies require multiple small-scale transfections, re-optimization of transfection protocols and/or bulk usage of costly transfection agents. The MaxCyte STX has the unique scalability to transfect as few as 5 x 10^5 cells within seconds for assay development and lead optimization or as many as 2 x 10^{10} cells in less than 30 minutes for library screening and protein production. Transfection quality and performance in downstream functional assays are unaffected by scale-up (1,3,4). Additionally, migration from small-scale to bulk transfection is seamless, requiring no further assay optimization.

To demonstrate the scalability of the MaxCyte STX, a high content PI3 kinase assay was performed on cells transiently transfected with a plasmid expressing the PI3P binding

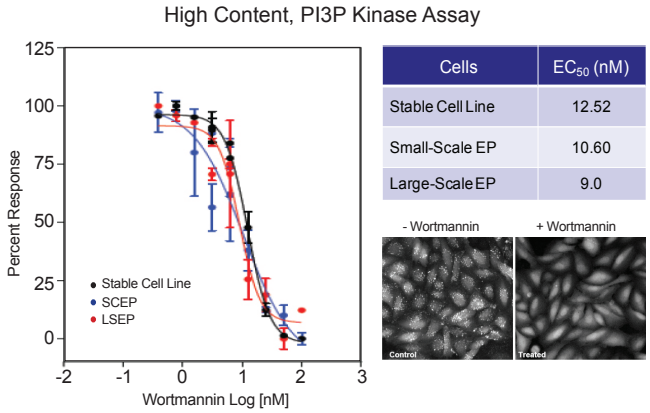


Figure 2: *Cells were transfected with a plasmid encoding eGFP expressed as a fusion protein with tandom PI3P binding domains (2XFYVE). Active PI3 kinase leads to localization of GFP to endosomes and visualized as granules. PI3K inhibition leads to redistribution of fluorescence throughout the cytoplasm. Transiently transfected cells or cells stably expressing eGPF-2XFYVE were incubated for 30 min with various concentrations of wortmannin. PI3K activity was assessed using high content screening to visualize granule localization.*

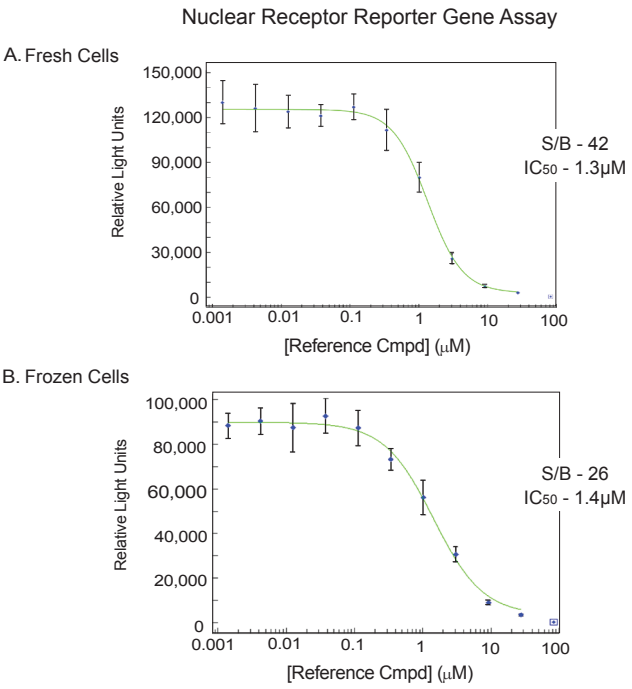


Figure 3: *Nuclear Receptor Assay. Jurkat cells were co-transfected with a luciferase reporter and a plasmid encoding a constitutively expressed fusion protein (GAL4 DNA binding domain linked to a nuclear receptor ligand binding domain). Transfected cells were plated A). immediately after electroporation (fresh cells) or B). cryopreserved after electroporation and plated after thawing (frozen cells). Cells were treated with varying concentrations of a nuclear receptor inhibitor immediately after plating, and luciferase activity measured 5 hours later.*

protein eGFP-2XFYVE using small-scale (SCEP) and large-scale electroporation (LSEP). In addition, a stable cell line expressing the identical reporter protein was tested. No significant differences in IC_{50} values following exposure to wortmannin, a PI3 kinase inhibitor, were observed between the three cell populations (Figure 2). These results highlight the capacity of the MaxCyte STX to produce functionally relevant cells via transient transfection at the multi-billion cell scale required for HTS and HCS.

Post-Transfection Cell Cryopreservation

The MaxCyte STX enables bulk transfection of billions of cells in less than 30 minutes using preprogrammed electroporation protocols. Transfected cells can be used in a wide range of assays immediately following electroporation. If more suitable to assay scheduling, transfected cells can also be aliquoted and cryopreserved for future use. MaxCyte has developed several cryopreservation protocols that enable cell archiving while maximizing cell viability and target expression upon thawing (4).

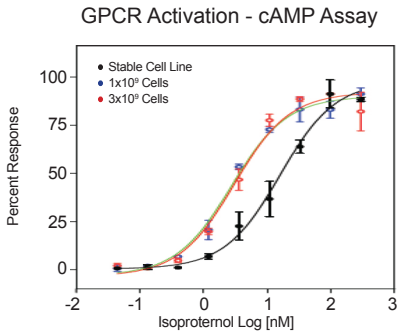
Figure 3 summarizes the results from a luciferase reporter gene assay that measured nuclear receptor activity in fresh versus cryopreserved transfected cells. Calculated IC_{50} values for a reference nuclear receptor inhibitor were comparable for both cell populations. These data demonstrate that downstream assay performance is not impacted by cell cryopreservation.

Case Study 1: GPCRs
Large-Scale Transfection vs. Stable Lines

GPCRs continue to be a leading target class in drug discovery programs. There are any number of functional and biochemical assay methods for assessing GPCR activation, receptor inhibition and signaling pathway usage. Many of these methods require cellular engineering such as overexpression of targets, artificial coupling to specific G alpha subunits or expression of fusion proteins.

Researchers must weigh the substantial time and resource commitment of creating stable cell lines against their value when developing new GPCR assays. An additional consideration is the divergence of stable cell lines from the 'normal' biology of cells, including receptor activation and signaling, as evidenced by changes in the performance of stable cell lines in functional assays. In contrast, transient transfection, and more specifically MaxCyte STX flow electroporation, quickly and reproducibly transfects cells with minimal off target effects and proven performance in downstream GPCR assays such as cAMP regulation and calcium flux assays (4,5).

This case study demonstrates the use of large-scale, bulk transfection as a means of over expressing the β2 adrenergic receptor. Two independent large-scale transfections of over one billion cells produced high quality, consistent results with cell viability of greater than 97%. Electroporation did not significantly affect cell viability or assay quality as the level of PI exclusion and assay S/B ratios were nearly identical to those of a reference stable cell line. Importantly, transiently transfected cells performed similarly to the reference cell line in a cAMP assay as demonstrated by comparable isoproternol EC_{50} values (Figure 4, table). These results highlight the capacity of the MaxCyte STX to produce functionally relevant cells at the multi-billion cell scale required for HTS.



Cells	Viability (%)	S/B	EC_{50} (nM)
1 x 10^9 Transfected	97.5	3.8	3.00
3 x 10^9 Transfected	97.2	5.0	2.83
CHO β2A Stable Cell Line	96.4	4.0	19.49

Figure 4: *CHO K1 cells were transiently transfected with a plasmid encoding the β2 adrenergic receptor:eGFP fusion protein. Transfected cells and CHO cells stably expressing the β2A receptor were stimulated with various concentrations of isoproternol and functional responses assessed using the DiscoveRx cAMP kit. Cell viability assessed via PI exclusion.*

Case Study 2: Ion Channel Screening Multi-subunit Protein Expression

Increased attention is being paid to voltage and ligand-gated ion channels as higher throughput, cell-based assays of channel activity have emerged. Current ion channel assays rely heavily on the use of stable cell lines, which are challenging to create due to the multi-subunit nature of many ion channel targets. The use of multiple selection agents during cell line creation can impair cell health and proliferation. Furthermore, ion channels can be toxic when expressed at high levels, creating the need for inducible promoters, which adds an additional layer of complexity.

Transient transfection offers an attractive alternative. Multiple plasmids can be co-transfected in defined stoichiometric ratios to create specific functional ion channel complexes using the MaxCyte STX (4,6).

Calcium Channels

Four cDNAs encoding the pore-forming alpha subunit of the voltage-gated calcium channel Cav2.2, the modulatory β subunit, the modulatory α2δ subunit and an inward rectifier potassium channel (Kir2.1) were co-transfected into HEK293 cells using the MaxCyte STX and assayed using the FLIPR® calcium influx assay (Figure 5). Strong calcium flux was evident in cells transfected with all four plasmids, but not in cells transfected without the inward rectifier plasmid or in untransfected control cells. Inclusion of the inward rectifier allowed modulation of resting membrane potential by external potassium and was essential for the FLIPR assay. The Cav2.2 channel was inhibited by omega-conotoxin, a known calcium channel specific antagonist, illustrating the functionality of the channel and the applicability of transiently transfected cells for compound screening of ion channels.

Potassium Channels

Potassium channels are generally considered the most complex class of voltage-gated ion channels both functionally and structurally. The Kv1.5 potassium channel is widely expressed in heart, colon, kidney, vascular smooth muscle and CNS, and is a therapeutic targets in many disease areas.

CHO K1 cells were transfected with a plasmid encoding the alpha subunit of the Kv1.5 voltage gated potassium channel and functionally characterized on the PatchXpress®, an automated electrophysiology assay platform, in both single hole and population patch clamp mode. Transfected cells showed strong, consistent current tracings in response to the application of standard voltage step protocols (4). To demonstrate the suitability of transiently transfected ion channels for drug screening applications, three independent channel inhibitors, capsaicin, nifedipine and bupivicaine, were tested using transfected cells as well as a Kv1.5 stable cell line (Table 2). The calculated IC_{50} were comparable between transiently transfected and stable cells and were in agreement with literature values. Thus, the MaxCyte STX provides a high fidelity alternative to creating stable cells lines which translates into shorter time to screen while using fewer valuable resources.

Compound	MaxCyte transfected cells End Step IC_{50}	Stable cell line End Step IC_{50}	Literature IC_{50}
Capsaicin	12 μM	48 μM	23 μM
Nifedipine	10 μM	16 μM	27 μM
Bupivicaine	49 μM	66 μM	13 μM

Table 2: *CHO K1 cells were transfected with a plasmid encoding the Kv1.5 potassium channel. IC_{50} values for three potassium channel inhibitors were calculated for transfected cells and a reference stable cell line using the PatchXpress platform.*

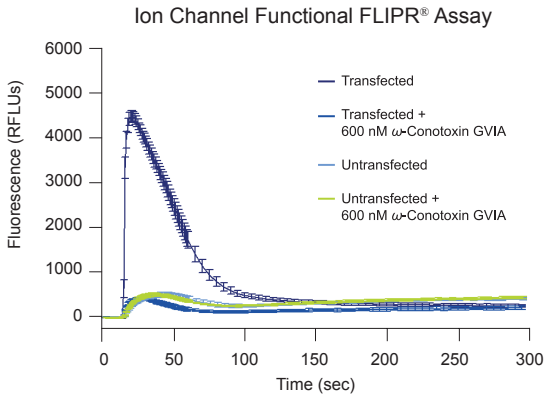


Figure 5: *HEK293 cells were transfected in bulk with multiple cDNA constructs encoding 3 calcium channel subunits ± a plasmid expressing the inward rectifier potassium channel, Kir2.1. Calcium influx FLIPR assays were conducted 24 hrs post transfection. Dye was added in a low potassium, low calcium solution and loaded for 30 mins. Vehicle control or antagonist (600 nM ω-Conotoxin GVIA) was added for an additional 30 mins. Cells were depolarized with high external K^+ (up to 135 mM).*

Conclusions

It is fast becoming accepted that electroporation is the most effective way to introduce DNA, RNA, siRNA or proteins into standard cell lines and difficult-to-transfect cells, such as primary and stem cells. The MaxCyte STX produces superior quality transfection using its proprietary flow electroporation and is uniquely positioned to fulfill the needs of researchers throughout early phase drug discovery and development. Transfect any molecule of interest, into any cell, at any scale in a timely, cost effective manner.

References

1. J. Fratantoni, *et al.* A Scalable Cell-Loading System for Non-Viral Gene Delivery and Other Applications. *Bioprocess J*, (2004) **3**, 49-53.
2. L. Bonetta. The Inside Scoop, Evaluating Gene Delivery Methods. *Nat. Methods*, (2005) **2**, 875-883.
3. L-H Li, *et al.* Highly Efficient, Large Volume Flow Electroporation. *Technology in Cancer Research & Treatment*, (2002) **1**, 341-349.
4. J. Brady, *et al.* Creating Cell-Based Assays for Screening GPCRs, Ion Channels and Other Targets in Cell Lines and Primary Cells Using the MaxCyte STX Scalable Transfection System. *Presented at 16th Annual SBS Conference* (2010).
5. MaxCyte. Developing Assays for Screening GPCRs with Transiently Transfected Cells Using the MaxCyte STX System. *App. Note*, **2**.
6. X. Jiang, *et al.* Improved Assay of Transiently Transfected Ion Channels on an Automated Electrophysiology System. *Presented at 54th Annual Biophysical Society Meeting, (2010).*

22 Firstfield Rd, Suite 110 • Gaithersburg, MD 20878
Email: info@maxcyte.com
Tel: (301) 944-1700

MaxCyte®
www.maxcyte.com