Rapid Automated Development of Cell Based Assays for Screening GPCRs, Ion Channels and Other Target Molecules Using the MaxCyte® STX™ Scalable Transient Transfection System

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Abstract

The MaxCyte® STX™ Scalable Transient Transfection system is an automated high throughput transient transfection technology, based on electroporation, that can transfect up to $1x10^{10}$ cells with DNA, mRNA, siRNA or proteins in less than thirty minutes. Levels of transfection efficiency and post EP viability exceed 90% with many cell types. The MaxCyte STX system was introduced to meet the needs of pharmaceutical and biotechnology companies, which are becoming increasingly reliant on cell based assays for target validation and drug screening. This increased application of cell based assays, along with a trend toward using physiological relevant primary cells and stem/progenitor cells, has led to an urgent need for new methods to generate large batches of transfected cells in quantities that are sufficient to screen entire compound libraries. To demonstrate utility of the MaxCyte STX technology for cell based assay development, we transfected HEK 293, CHO and U2OS cells with plasmid DNA encoding GPCRs, multi-subunit ion channels and fluorescent reporter molecules. By loading cells with increasing concentrations of plasmid DNA, we showed how assay optimization with the MaxCyte STX system is typically a straightforward process of identifying a DNA concentration that maximizes the dynamic range of the assay, while minimizing loss of viability that can result from overloading the cell with DNA. We tested transiently transfected cells in assay platforms that are commonly used for high throughput screening, including automated calcium flux, patch clamp and high content screening assays. Cells transfected using the MaxCyte STX performed comparably to stable cell lines in all of the above assay platforms. Finally, we demonstrated that STX-transfected cells could be cryopreserved without impacting transgene expression or assay performance. These data demonstrate how the MaxCyte STX system can overcome the cost, time and labor issues associated with developing stable cell lines. The MaxCyte STX system works with primary cells and stem cells, in addition to established cell lines, and it allows users to assay targets that cannot be screened in stable cells due to problems with cellular toxicity.

Results

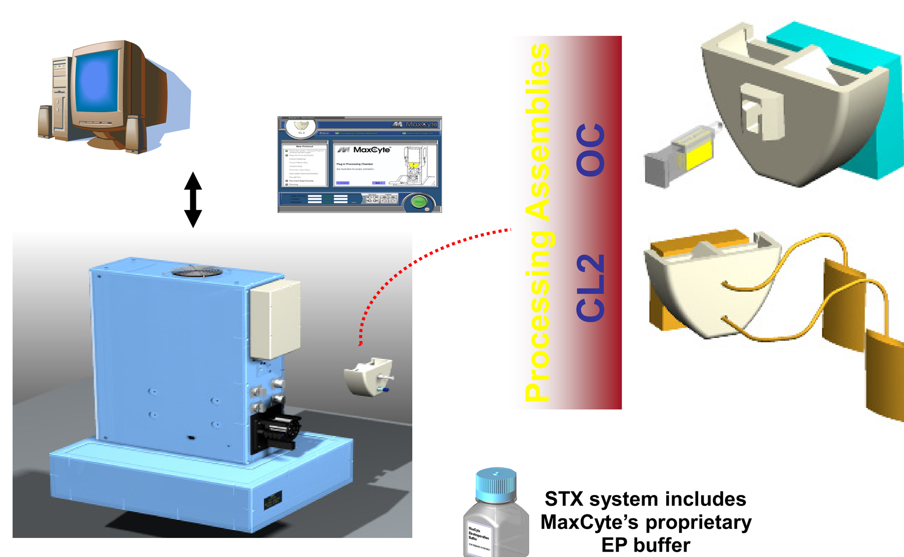


Figure 1. MaxCyte® STX™ system. The MaxCyte STX instrument uses a scalable electroporation (EP) technology to deliver DNA, mRNA, siRNA, protein or other molecules into primary cells and cell lines. Small scale transfections (up to $4x10^7$ cells) are performed by static EP in OC processing assemblies (PAs). Larger transfections ($1x10^8$ to $1x10^{10}$ cells) are performed by flow EP in CL2 PAs. The OC and CL2 PAs are sterile, closed, single use units, allowing the MaxCyte STX system to be installed anywhere and to be used by multiple users without worry of contamination. The EP process is controlled by software that includes pre-set protocols for transfecting a variety of cell types. No optimization of EP parameters is necessary.

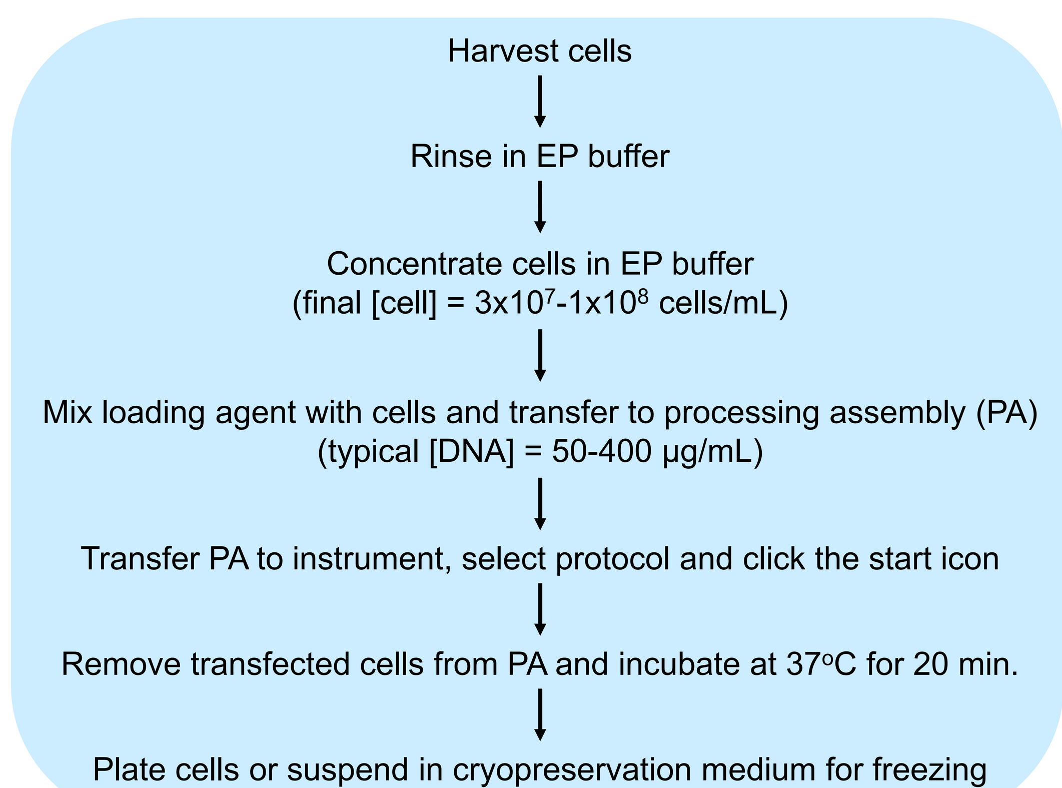


Figure 2. Protocol for transfecting cells using the MaxCyte STX system. During processing, cells are suspended in MaxCyte's EP buffer, a physiological salt solution that contains no proteins or biological agents. The same buffer is used for all cell types. Post transfection, cells can be diluted into culture medium without removing the EP buffer, or they can be pelleted and suspended in standard freezing medium for cryopreservation.

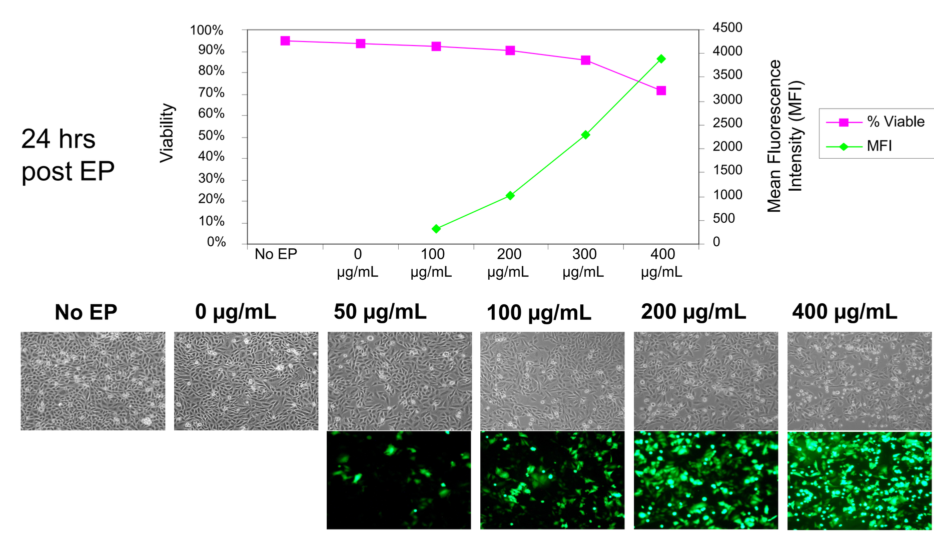
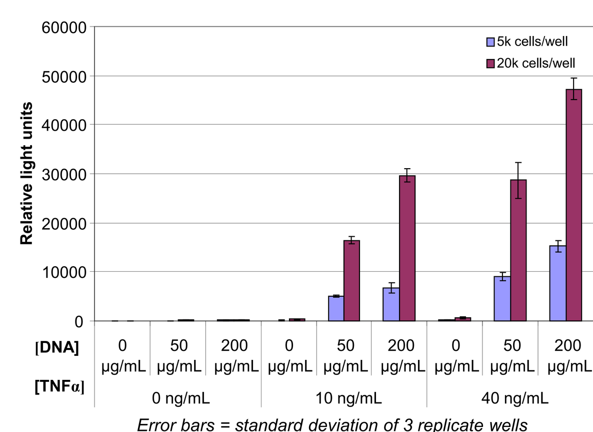


Figure 3. DNA titration with CHO K1 cells. CHO K1 cells were loaded with increasing concentrations of peGFP plasmid DNA in a series of small-scale, static EPs. Electroporation alone has negligible impact on viability, but viability is reduced by loading higher concentrations of DNA into the cell, a phenomenon known as DNA toxicity. The MaxCyte STX system comes preloaded with EP parameters that have been optimized for a variety of cell types. Therefore, when developing a cell based assay with the MaxCyte STX, the only optimization that is typically required involves identifying a DNA concentration that yields good assay performance while minimizing DNA toxicity.



Distinct ligand dose-response with both sets of transfected cells.

Assay sensitivity and dynamic range can be controlled via DNA concentration.

Figure 4. NFκB luciferase-based reporter assay with transiently transfected HEK 293 cells. HEK 293H cells were transfected with 2 different concentrations of a reporter plasmid containing tandem copies of the NFkB response element linked to a minimal promoter driving expression of firefly luciferase (SA Biosciences). Cells were plated at 2 densities in a 96 well dish immediately after EP and treated with 2 concentrations of TNFα 6 hrs later. Luciferase activity was measured the following day using a Dual-Glo® luciferase assay kit (Promega) on a FLUOstar OPTIMA plate reader (BMG Labtech).

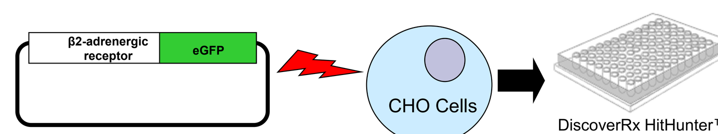


Figure 5. Developing a cell based assay for GPCR screening with the MaxCyte STX system. CHO K1 cells were transfected via static electroporation with plasmid DNA encoding a β2 adrenergic receptor:eGFP fusion protein. Response to the agonist isoproternol was determined by quantifying intracellular cAMP levels in a 96 well enzyme complementation assay.

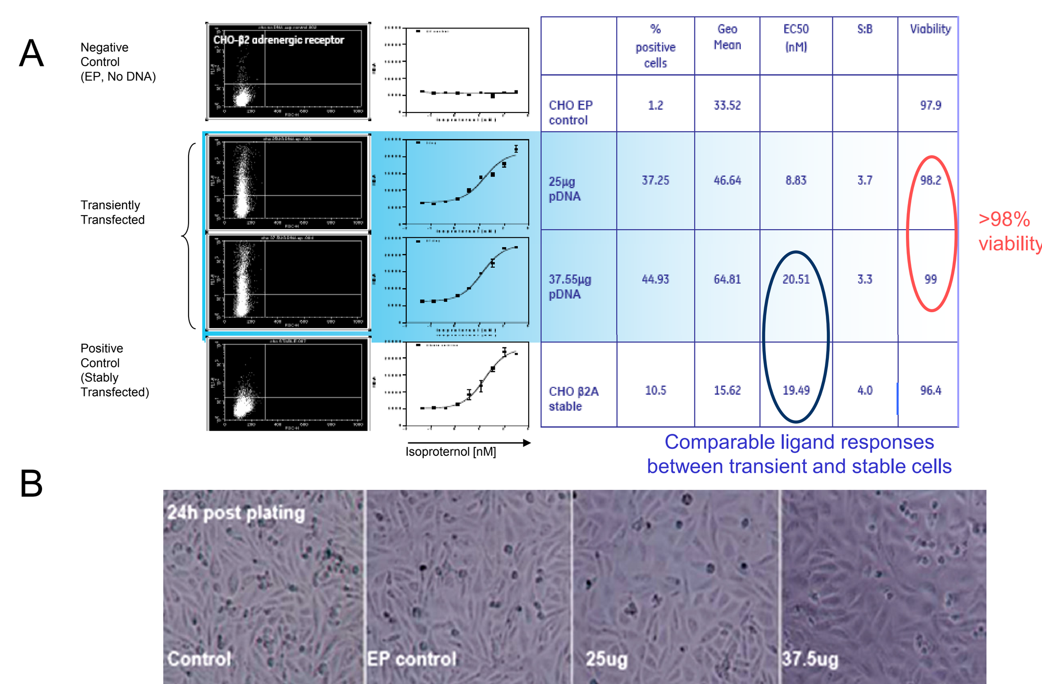


Figure 6. STX transfected cells perform comparably to stable cells in a GPCR assay. A. FACS analysis and enzyme complementation assay results for cells transiently transfected with 2 different concentrations of plasmid encoding a β2 adrenergic receptor:eGFP fusion protein. FACS data demonstrate that transgene expression correlates with DNA concentration. Cells transfected with the higher DNA concentration exhibit dose response curves, EC_{50} values and signal-to-background ratios comparable to those of stable cells. B. Transiently transfected cells exhibit normal morphology relative to non-transfected and stable cells.

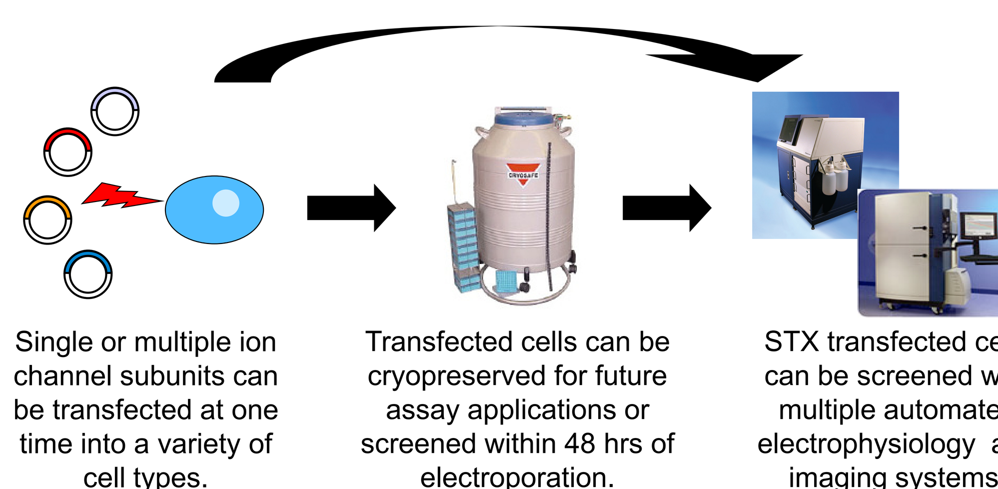


Figure 7. Enabling ion channel screening with the MaxCyte STX system.

Condition	% seals (>100 MΩ)		Seal resistance (mean±SD)		% expression (>0.5 nA)		Current amplitude (mean±SD)	
	24 hrs	48 hrs	24 hrs	48 hrs	24hrs	48 hrs	24 hrs	48hrs
Non-transfected	90%	97%	212±51 MΩ	322±136 MΩ	-	-	-	-
50 µg/ml cDNA	88%	87%	245±62 MΩ	295±101 MΩ	45%	54%	2.1±1.4 nA	1.8±1.5 nA
100 µg/ml cDNA	79%	84%	239±69 MΩ	278±83 MΩ	70%	89%	2.3±1.6 nA	2.9±1.8 nA
150 µg/ml cDNA	77%	96%	247±67 MΩ	246±78 MΩ	82%	95%	3.1±2.1 nA	3.2±2.1 nA

High seal efficiency & resistance

% Expression correlates with DNA concentration

Figure 8. Transiently transfected CHO K1 cells perform well in automated patch-clamp assays. CHO K1 cells transfected with DNA encoding the α-subunit of the $K_v1.5$ ion channel were analyzed on the IonWorks® Quattro™ recording in single hole mode at 24 & 48 hrs post EP.

Condition	PatchPlate	% seals (>100 MΩ)[1]	Seal resistance (mean±SD)	% expression (>0.5 nA)	Current amplitude (mean±SD)
Lipid-Mediated Transfection (20 ug DNA + 60 ul commercial lipid reagent)	Single Hole (SH)	77%	191±46 MΩ	4%	1.1±1.0 nA
150 µg/ml cDNA 48 hrs post-transfection	Single Hole (SH)	82%	248±87 MΩ	93%	2.8±1.4 nA
	Population Patch Clamp (PPC)	100%	72±31 MΩ	98%	1.3±0.3 nA

[1]PPC seals >20 MΩ

Figure 9. STX transfected cells perform better than lipid transfected cells for automated ion channel screening. CHO K1 cells were transfected with $K_v1.5$ α-subunit plasmid DNA using a commercial lipid-based transfection reagent or with the MaxCyte STX system. Cells were analyzed on the IonWorks® Quattro™ system.

PatchPlate	% seals (>100 MΩ)[1]	Seal resistance (mean±SD)	% expression (>0.5 nA)	Current amplitude (mean±SD)
SH	50%	202±77 MΩ	86%	2.7±1.3 nA
PPC	98%	38±11 MΩ	99%	1.5±0.3 nA

[1]PPC seals >20 MΩ.

Figure 10. Cells can be transfected in bulk with the MaxCyte STX system and cryopreserved for future use in automated electrophysiology assays. $8x10^8$ CHO K1 cells were transfected with 150 µg/mL of $K_v1.5$ α-subunit plasmid DNA via flow electroporation. Transfected cells were cultured @ 37°C for 24 hrs followed by an additional 24 hr incubation @ 28°C. After 48 hrs, cells were suspended in freezing medium @ $2x10^6$ cells/mL, aliquoted and cryopreserved in liquid nitrogen. Thawed cells were assayed in single hole (SH) and population patch clamp (PPC) mode on the IonWorks® Quattro™ system.

Summary

- ✓ The MaxCyte® STX™ system allows users to optimize cell based assays by varying the concentration of loading agent during electroporation.
- ✓ The MaxCyte STX transfection process is scalable, allowing users to optimize assay performance via small scale, static electroporation and achieve identical results at large scale via flow electroporation.
- ✓ Transfected cells can be used to screen a variety of target molecules, including GPCRs and ion channels, using multiple high throughput assay platforms.
- ✓ Transfected cells can be used immediately in cell based assays or cryopreserved for future applications.

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