Simple and Effective Generation of Cell-based Assays for Ion Channels, Transporters, and Kinase Screening in Biologically Relevant Cells Using Scalable Transient Transfection

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Abstract

Cell-based assays utilizing mammalian cells to express receptor targets are widely used to rapidly identify molecular mechanisms of human disease and to develop novel therapeutics. It is often challenging to engineer cells to co-express multiple transgenes and multiple protein complexes to generate these functional receptor assays. MaxCyte electroporation provides an extremely effective and flexible means of co-transfecting multiple plasmids to construct a variety of receptors and other assay targets, including GPCRs, ion channels, transporters, and kinases. The MaxCyte STX® Scalable Transfection System uses proprietary flow electroporation to transiently transfect from 5E5 cells in seconds up to 2E10 cells in less than 30 minutes, yielding high levels of transfection efficiency and cell viability. The technology is applicable to a wide range of cell types, including primary cells and stem cells. In this poster, we present MaxCyte transient-transfected assays including a CFTR transporter assay, a HCS casein kinase assay, and ion channel assays. The results demonstrated that MaxCyte transfection provides a rapid and robust means for conducting cell-based assay screening.

MaxCyte Transient Transfection Systems

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.

- High efficiency & high cell viability
- Broad cell compatibility including primary cells
- Streamlined scalability requiring no re-optimization
- Rapid assay development

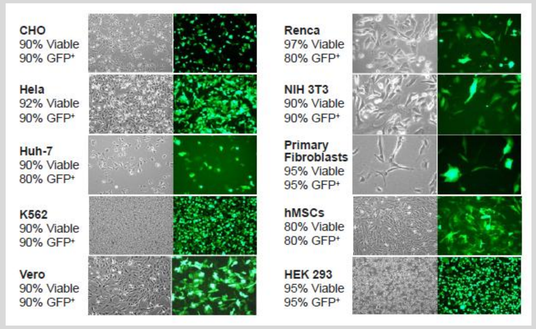

Figure 1. High Cell Viability and Transfection Efficiency Using MaxCyte Electroporation. Ten different cell types were transfected with 2 µg/1E6 cells of pGFP DNA using the appropriate MaxCyte STX protocol. 24 hours post transfection cells were examined for cell viability (% cells excluding propidium iodide, PI) and transfection efficiency (% cells GFP+) using FACS analysis.

Summary

- MaxCyte electroporation enables rapid transfection that yields high cell viabilities & transfection efficiencies with a variety of cell lines commonly using for cell-based screening, as well as primary cells.
- MaxCyte electroporation can (co)transfect multiple expression plasmids to generate functional ion channels and to enable transporter, and kinase assays with sensitive performance in compound screening.
- MaxCyte transient transfection provides a rapid and cost-effect alternative to stable cell line generation for assay development. MaxCyte transfected cells show comparable assay performance to stable cells in ion channel assays.
- MaxCyte offers seamless scalability and minimal impact on cell health/membrane integrity, two key advantages over lipid-based transfection.
- MaxCyte transfected cells can be used immediately in cell-based assays or cryopreserved for use in future downstream functional assays without a significant loss in expression or performance.
- Pre-optimized, cell-type specific electroporation protocols enable rapid and easy assay optimization via titration of DNA concentration.

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Ion Channel Screening

Robust Sodium Channel Screening

100% Expression in Planar Patch Clamp Assay Following MaxCyte Transfection

A. Nav1.5 Assay Development

[DNA]	Transfection efficiency	Average current level*	TTX block**
[µg/1E6 cells)]	[%]	[nA]	[%]
1.0	57	-6.3	65
1.5	100	-5.7	71
2.0	80	-3.6	82
2.5	75	-3.5	86

* Measured @ 0 mV in simple depolarizing step protocol
** Percentage block compared to saline period of a 20 uM TTX single addition

B. Seal and Transfection Efficiency Comparison with Lipid Transfection

	Transfection efficiency (%)	Average current level* (nA)	TTX block** (%)
Fresh Cells	93	-6.5	85
Frozen Cells	89	-5.4	81

* Measured @ 0 mV in simple depolarizing step protocol
** Percentage block compared to saline period of a 20 uM TTX single addition

C. $Na_V1.5$: Representative QPatch Data

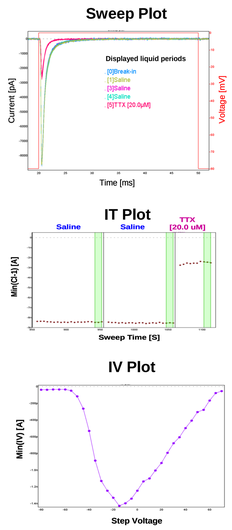
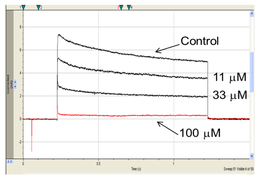


Figure 2. Nav1.5 Assay Development. A). HEK293 cells were transiently transfected with a Nav1.5 expression plasmid via small-scale electroporation (OC-100 processing assemblies). Transfected cells were cultured at 37°C for 24 hours, cultured at 28°C for an additional 24 hours, then assayed on the Sophion QPatch in single hole mode. Optimization of the DNA concentration yielded expression of channel activity in 100% of the assayed cells. B). HEK293 cells transfected with a Nav1.5 expression plasmid (1.5 µg/1E6 cells) were cultured for 48 hours, and then they were either assayed using the Sophion QPatch or cryopreserved. Cryopreserved cells were assayed immediately after thawing. Comparable assay results were achieved with freshly transfected and frozen cells. C). Representative plots for Sophion QPatch assays show expected current levels and response to TTX block.

Potassium Channel Pharmacology Testing

MaxCyte Transfected Cells Are Comparable to Stable Cell Lines and Superior to Lipid-Transfected Cells

A. Capsaicin Inhibition – Representative Sweep Plots



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

B. $K_V1.5$ Assay: Equivalent Results Pre & Post Cryopreservation

Condition	PatchPlate	% seals (>100 MΩ)[1]	Seal resistance (mean±SD)	% expression (>0.5 nA)	Current amplitude (mean±SD)
Lipid-Mediated Transfection (20 µg DNA + 60 ul commercial lipid reagent)	Single Hole (SH)	77%	191±46 MΩ	4%	1.1±1.0 nA
1.5 µg/1e6 cells 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

Figure 3. Pharmacology Assays with Transiently Transfected Cells. A). CHO K1 cells transiently transfected with a $K_V1.5$ plasmid were incubated with varying concentrations of three compounds and assayed on the PatchXpress. Representative sweep traces of $K_V1.5$ current in response to increasing concentrations of the capsaicin (left). IC_{50} values were consistent with data obtained using a stable cell line and reported values in the literature (right). B). MaxCyte-transfected cells showed higher seal resistance and percentage of expression compared to lipid-transfected cells. Data obtained from IonWorks Quattro. Data courtesy of BioFocus.

Calcium Channels FLIPR Assay

Co-transfection of 4 Plasmids for Modulator Studies with Ca_V Channels

- 4 different Ca_V pore-forming α subunits($Ca_V1.2$, $Ca_V2.2$, $Ca_V2.1$, and $Ca_V3.2$)
- Modulatory β subunit
- Modulatory α2δ subunit
- Inward rectifier potassium channel (Kir2.1) to allow modulation of resting membrane potential by external K^+

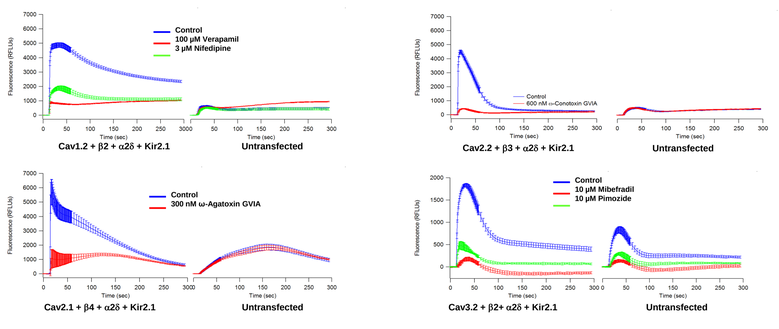
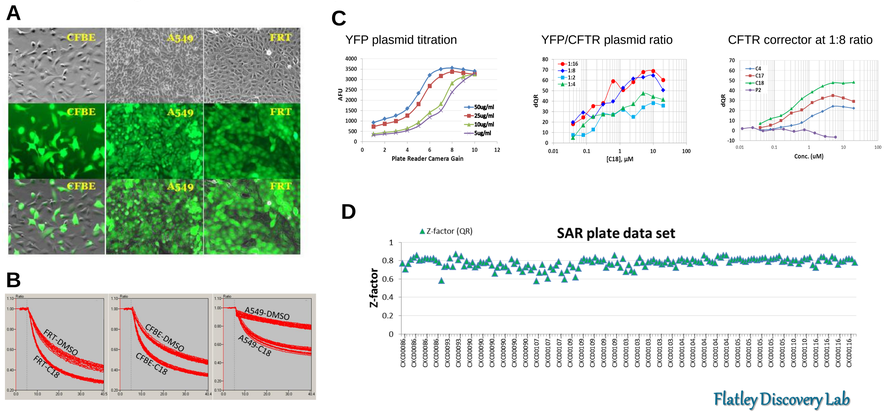


Figure 4. Co-transfection of 4 plasmids for Cav Channels Assay. HEK cells were transiently transfected with different Ca_V pore-forming α subunits ($Ca_V1.2$, $Ca_V2.2$, $Ca_V2.1$, and $Ca_V3.2$), a modulatory β subunit, a modulatory α2δ subunit and an inward rectifier potassium channel (Kir2.1). FLIPR assays performed using Calcium-4 No Wash Kit (Molecular Devices) showed robust calcium influx in all four sets of transfected cells in response to modulation of membrane potential. Response to inhibitor compounds was also seen in all four sets of transfected cells, indicating suitability of transfected cells for screening. *Data courtesy ChanTest Corporation, 14656 Neo Parkway, Cleveland, Ohio, 44128.*

Transporter Assay Screening

Sensitive Primary Screening Assay for CFTR Correctors & Modulators

Co-transfecting Human Bronchial Epithelial Cells with YFP & CFTR Plasmids



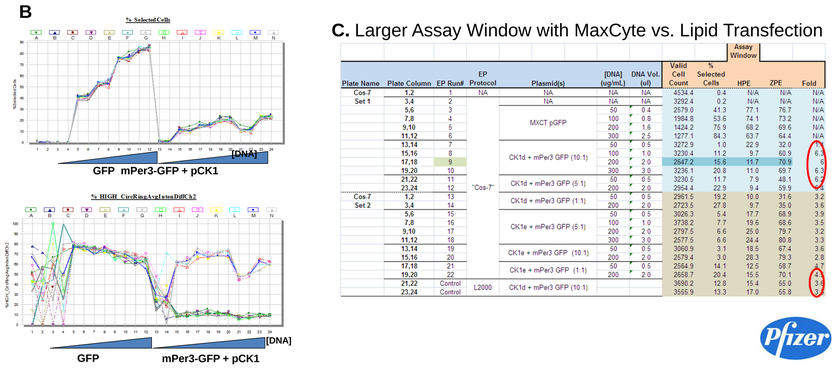
Flatley Discovery Lab

Figure 5. CFTR-YFP Iodide Flux Assay with Transiently Transfected Human and Rat Epithelial Cells A). Microscope images of transfected CFBE, A549, and FRT cells in phase-contrast view (*top panel*), fluorescence view (*middle panel*), and merged view (*lower panel*), showing high transfection efficiency in all three cell types. B). Iodide flux assay response to negative control (DMSO) and modulator. C).Transfection optimization performed via plasmid titration and sample dose-response plots with three positive compounds and one negative compound in CFBE cells. D). High Z' factors (0.4~0.8) over 50 plates demonstrate the robustness of this assay in transiently transfected CFBE cells. *Data courtesy of Flatley Discovery Lab., Suite 208, The Schrafft Center, 529 Main St., Charlestown, MA 02129.*

Casein Kinase (CK1) Screening

High Content Screening of CK1/mPER-Transfected COS-7 Cells

Robust HCS Kinase Assay Following Co-transfection with mPer3-GFP & CK1 Plasmids



Pfizer

Figure 6. High Content Screening of Casein Kinase Modulators using Transiently Transfected COS-7 cells. A). COS-7 cells were co-transfected with mPer3-GFP & CK1 expression plasmids then treated with CK1 modulator and control compounds. Imaging of treated cells using Cellomics ArrayScan VTi shows 100% inhibition of phosphorylation (GFP-negative nuclei) in response to the inhibitor compound (HPE) but no changes in nuclear GFP levels in response to control compound (ZPE). B) Assay sensitivity can be adjusted easily by increasing the DNA concentration in the electroporation reaction (left panel). Control cells transfected with GFP showed no changes in nuclear GFP levels, whereas cells co-transfected with mPER-GFP and CK1 exhibited loss of nuclear GFP in response to a CK1 modulator (*right panel*). C) Comparison of MaxCyte electroporation vs. lipid transfection shows a larger assay window for MaxCyte transfected cells. *Data courtesy of Pfizer, 235 East 42nd Street NY, NY 10017.*

Primary Cell Transfection

High-Performance Transfection of Primary Cells

High Primary Cell Viability and Transfection Efficiency Using MaxCyte Electroporation

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%

Figure 7. Highly Efficient Transfection of Primary Cells. Table: Results of transfecting primary cells with DNA plasmid encoding GFP. Efficiency expressed as % cells GFP^+ at 24 hours post electroporation; viability as % cells excluding propidium iodide. A). Human Skeletal Muscle Cells (hSkMCs) were isolated from adult biopsy samples and transfected with 2 µg/1E6 cells 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. B). E18 rat hippocampal, cortical and ventricular neurons were electroporated with either 0 or 2 µg/1E6 cells pGFP. 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.