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 proteins completely to mimic these functional receptor assays. MaxCyte electroporation provides an extremely effective and flexible means of co-transfecting multiple plasmids to construct a variety of receptor 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 S5S cells in seconds up to 2×10^6 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 transfectant-assayed including a CFTR transporter assay, a H2C2 canine 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®
S5S Cells in Seconds
Up to 1×10^6 Cells in 30 min.

MaxCyte VLX®
Up to 2×10^6 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

Summary
- MaxCyte electroporation enables rapid transfection that yields high cell viabilities & transfection efficiencies with a variety of cell lines commonly used 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 terms of low basal expression.
- MaxCyte transient transfection provides a rapid and cost-effective alternative to stable cell line generation for assay development.
- MaxCyte electroporation systems 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 transfectant 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.

Ion Channel Screening

Robust Sodium Channel Screening
100% Expression in Planar Patch Clamp Assay Following MaxCyte Transfection

A. Nav1.5 Assay Development

<table>
<thead>
<tr>
<th>[µg/10^6 cells]</th>
<th>Transfection efficiency (%)</th>
<th>Average current (nA)</th>
<th>TTX block (%)</th>
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<tbody>
<tr>
<td>1.0</td>
<td>57</td>
<td>-6.3</td>
<td>65</td>
</tr>
<tr>
<td>1.5</td>
<td>100</td>
<td>-5.7</td>
<td>71</td>
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<td>2.0</td>
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<tr>
<td>2.5</td>
<td>75</td>
<td>-3.5</td>
<td>86</td>
</tr>
</tbody>
</table>

* Measured with 0 mM TTX in single depolarizing step protocol.
** Percentage block compared to a stable 9.5 mM TTX single addition.

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 level of channel activity in 100% of the transfected cells. B) HEK293 cells transfected with a Nav1.5 expression plasmid (1.5 µg/10^6 cells) were cultured for 48 hours, and then they were either assayed using the QPatch QPatch or cryopreserved. Cryopreserved cells were assayed immediately after thawing. Comparable assay results achieved with freshly transfected and frozen cells. C) Representative plots for QPatch 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. Caspacin Inhibition – Representative Sweep Plots B. Kv1.5 Assay: Equivalent Results Pre & Post Cryopreservation

Figure 3. Pharmacology Assays with Transfected Transfected Cells. A) CHO K1 cells transiently transfected with a Kv1.5 plasmid were incubated with varying concentrations of three compounds and assayed on the PatchClamp. Representative sweep traces of Kv1.5 current in response to increasing concentrations of the caspacin (left). Kv1.5 values were consistent with data obtained using a stable cell line and reported values in the literature (right). B) MaxCyte-transfected cells showed higher signal 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 Ca2+ Channels
- 4 different Ca2+ pore-forming α subunits (Ca2+1, Ca2+2, Ca2+1.1, and Ca2+3.2)
- Modulatory β subunit
- Modulatory δ2 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 Cav2.1 pore-forming α subunits (Ca2+1.2, Ca2+2, Ca2+1.1, and Ca2+3.2), a modulatory β subunit, a modulatory δ2 subunit and an inward rectifier potassium channel (Kir2.1). FLIPR assays performed using Calcium-4 Na-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 of ChanTest Corporation, 14865 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
A

Figure 5. CFTR-YFP Iodide Flux Assay with Transfected Human and Rat Epithelial Cells A). Microscopic images of transfected CFBE, AS49, 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 rapid 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 PabodyDiscovery Lab., Suite 208, The Schratt Center, 529 Main St., Charleston, WV 25312.

Casein Kinase (CK1) Screening
High Content Screening of CK1/mPER-Transfected COS-7 Cells
Robust HCS Kinase Assay Following Co-transfection with mPER-3-GFP & CK1 Plasmids
A

Figure 6. High Content Screening of Casein Kinase Modulators using Transfected Transfected COS-7 cells. A) COS-7 cells were co-transfected with mPER-3-GFP & CK1 expression plasmids then treated with CK1 modulator and control compounds. Imaging of treated cells using Celomics AmiScan V11 shows 100% inhibition of phosphorylation (GFP-negative nuclei) in response to the inhibitor compound (HPE) but no changes in GFP level in response to control compounds (B). B) Assy 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 nGFP level 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 10117.

Primary Cell Transfection
High-Performance Transfection of Primary Cells
High Primary Cell Viability and Transfection Efficiency Using MaxCyte Electroporation

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 Myocytes (n=35) were isolated from adult human samples and transfected with 2µg/10^6 cells of pGFP. Cells were either examined 1 day post EP (fresh) or cryopreserved 1 day following cell thawing (frozen). GFP expression was assessed via microscopy and FACs analysis. B) 18% rat hippocampal cortical and ventricular neurons were electroporated with either 0 or 2µg/10^6 cells pGFP. 5 days post EP cells were assessed for cell viability and GFP expression. Cell viability was greater than 85% with approximately 50% of cells positive for GFP expression.