

Creating Cell-Based Assays for Screening GPCRs, Ion Channels and Other Targets in Cell Lines and Primary Cells Using the MaxCyte® STX™ Scalable Transient Transfection System

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Abstract

The MaxCyte® STX™ Scalable Transient Transfection system uses proprietary flow electroporation technology that enables transfection of up to 1x10¹⁰ cells with DNA, mRNA, siRNA or protein in less than thirty minutes. Levels of transfection efficiency and viability exceed 90% with many cell types. Here we present a series of case studies illustrating how the MaxCyte STX system enables rapid development of assays for screening single and multi-subunit ion channels, GPCRs and other molecules in HEK, CHO and other cell types using automated electrophysiology and other high throughput screening platforms. The data illustrate typical examples of assay development based on transfecting small numbers of cells with increasing amounts of plasmid DNA using static electroporation to identify an optimal DNA concentration that yields good viability and sensitive assay performance. Loading conditions optimized at small scale can then be used to scale up the transfection reaction via flow electroporation without altering transfection efficiency, viability or assay performance. We also show that transfected cells can be used immediately or aliquoted and cryopreserved for future assay applications. Through the application of flow electroporation, MaxCyte STX users can eliminate the costly, time consuming and labor intensive process of stable cell line development by transiently transfecting target and/or reporter molecules into cell lines and physiologically relevant primary cells or stem cells.

Results

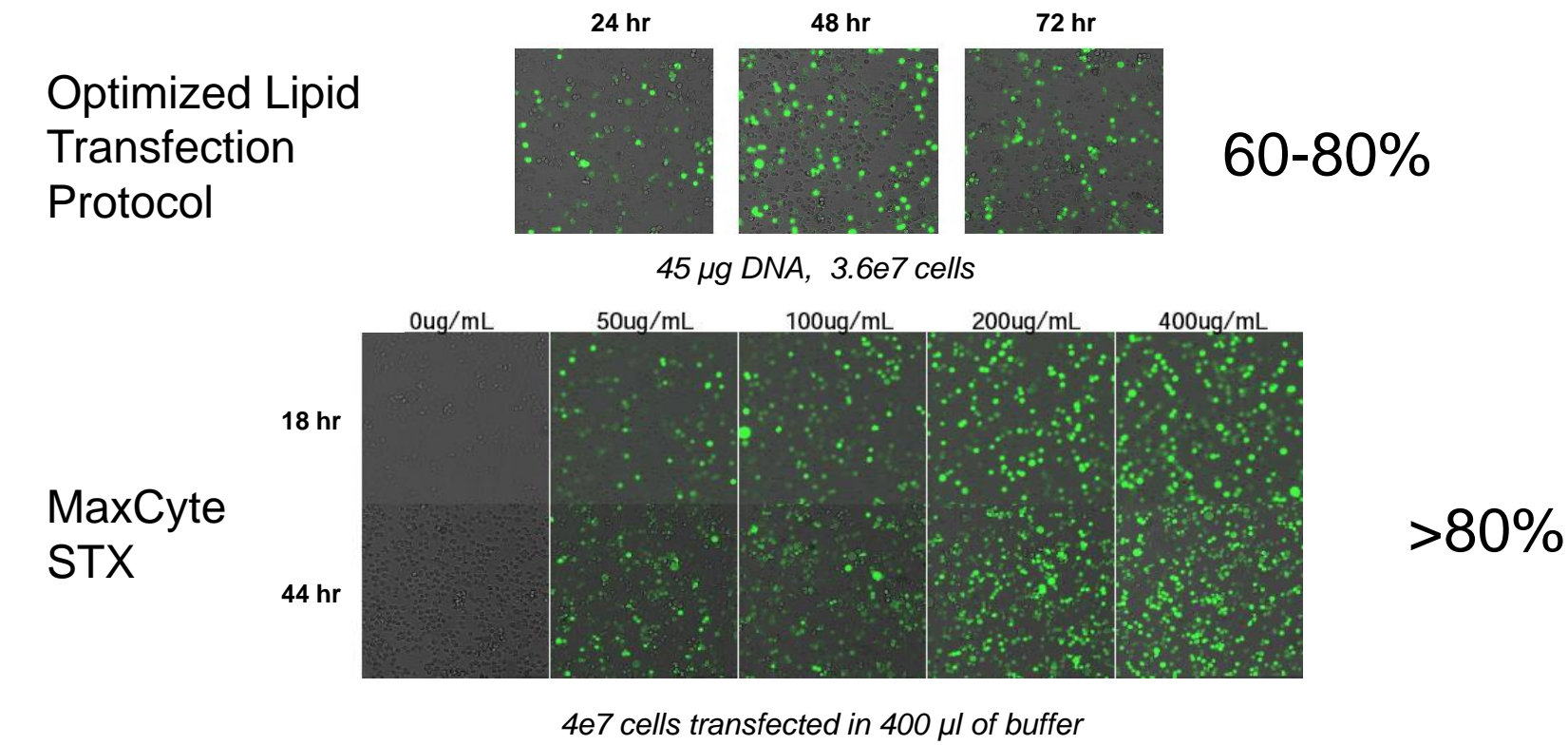
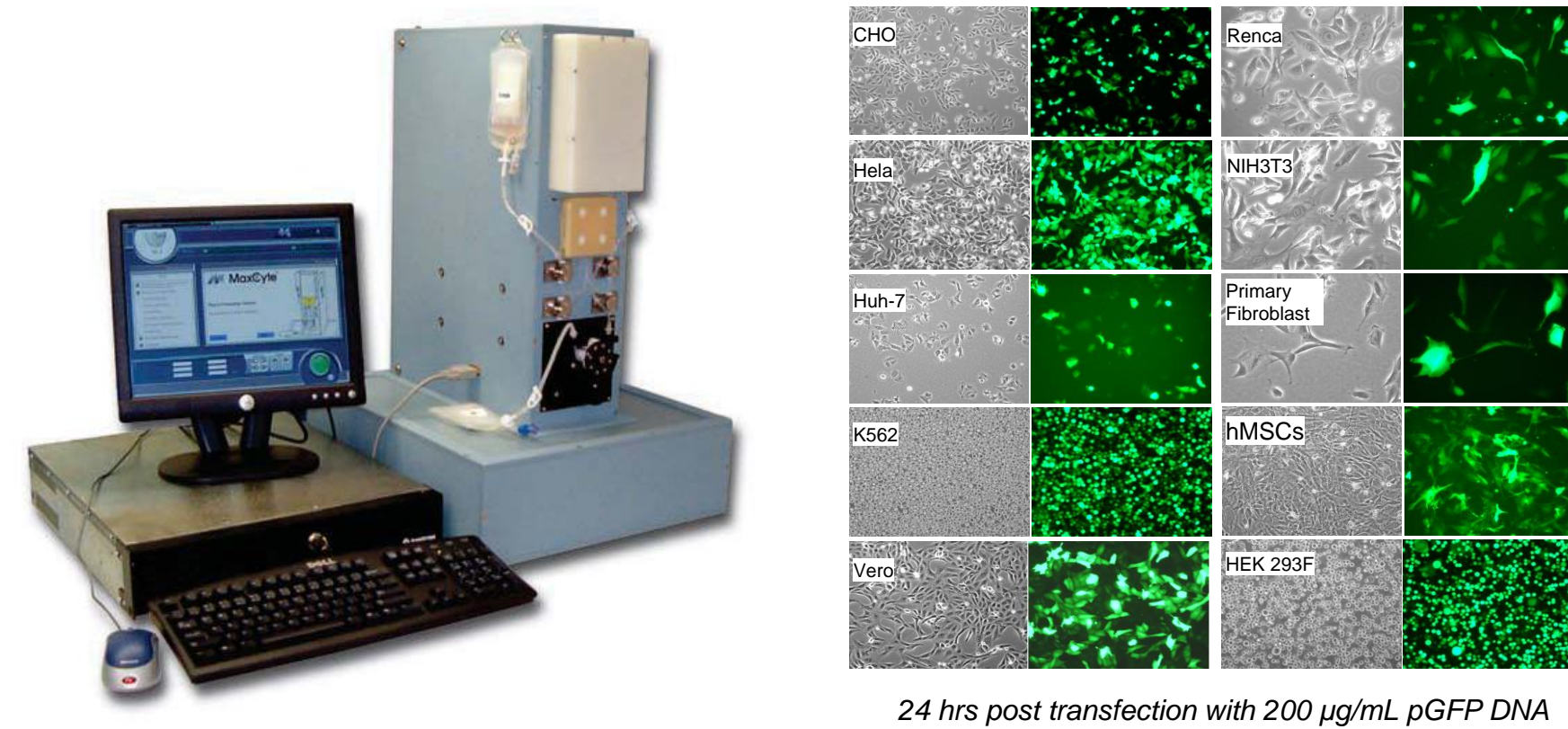


Figure 2. Transfecting HEK 293F suspension cells: MaxCyte STX vs. lipids. The MaxCyte STX system provides an efficient and reproducible method for loading molecules into both adherent and suspension adapted cells. In this experiment, conducted during an independent evaluation of the MaxCyte STX system, HEK 293F cells cultured in FreeStyle™ medium (Invitrogen) were transfected with a plasmid encoding the fluorescent protein Azami Green using a commercial lipid reagent or by static EP with the MaxCyte STX. Note that fluorescent protein expression directly correlates with plasmid DNA concentration in EP'd cells, illustrating how MaxCyte STX users can tightly control expression levels and assay performance by varying loading agent concentrations.

MaxCyte STX Instrument

Representative Results



Features & Attributes

Simple:	Preoptimized protocols for many cell types
High Yield:	>90% viability & recovery
High Efficiency:	>90% cell loading & transfection efficiency
Safe:	Chemically defined buffer; sterile, closed processing assemblies
Scalable:	5 x 10 ⁵ - 4x10 ⁷ (Static EP); up to 1x10 ¹⁰ cells (Flow EP)
Rapid:	1x10 ¹⁰ cells processed in <30 min.
Rugged:	Reproducible processing
Quality:	cGMP compliant, ISO 9001 certification, CE marking

Figure 1. Introduction to the MaxCyte® STX™ scalable transfection system. The MaxCyte STX system uses a clinically validated, scalable electroporation (EP) technology to load DNA, mRNA, siRNA, protein or other molecules into primary cells and cell lines with high levels of efficiency and with minimal impact on cell viability. The instrument is computer controlled and comes preloaded with optimized transfection protocols for a variety of cell types. The same protocols are used for every type of molecule, allowing cotransfection of multiple loading agents and enabling applications such as assay development with multi-subunit drug targets and testing siRNAs. Transfection takes place in sterile, single use processing assemblies. Small scale transfections (up to 4x10⁷ cells) are performed by static EP; larger transfections (1x10⁸ to 1x10¹⁰ cells) are performed by flow EP. Static and flow EPs yield identical levels of loading efficiency and cell viability.

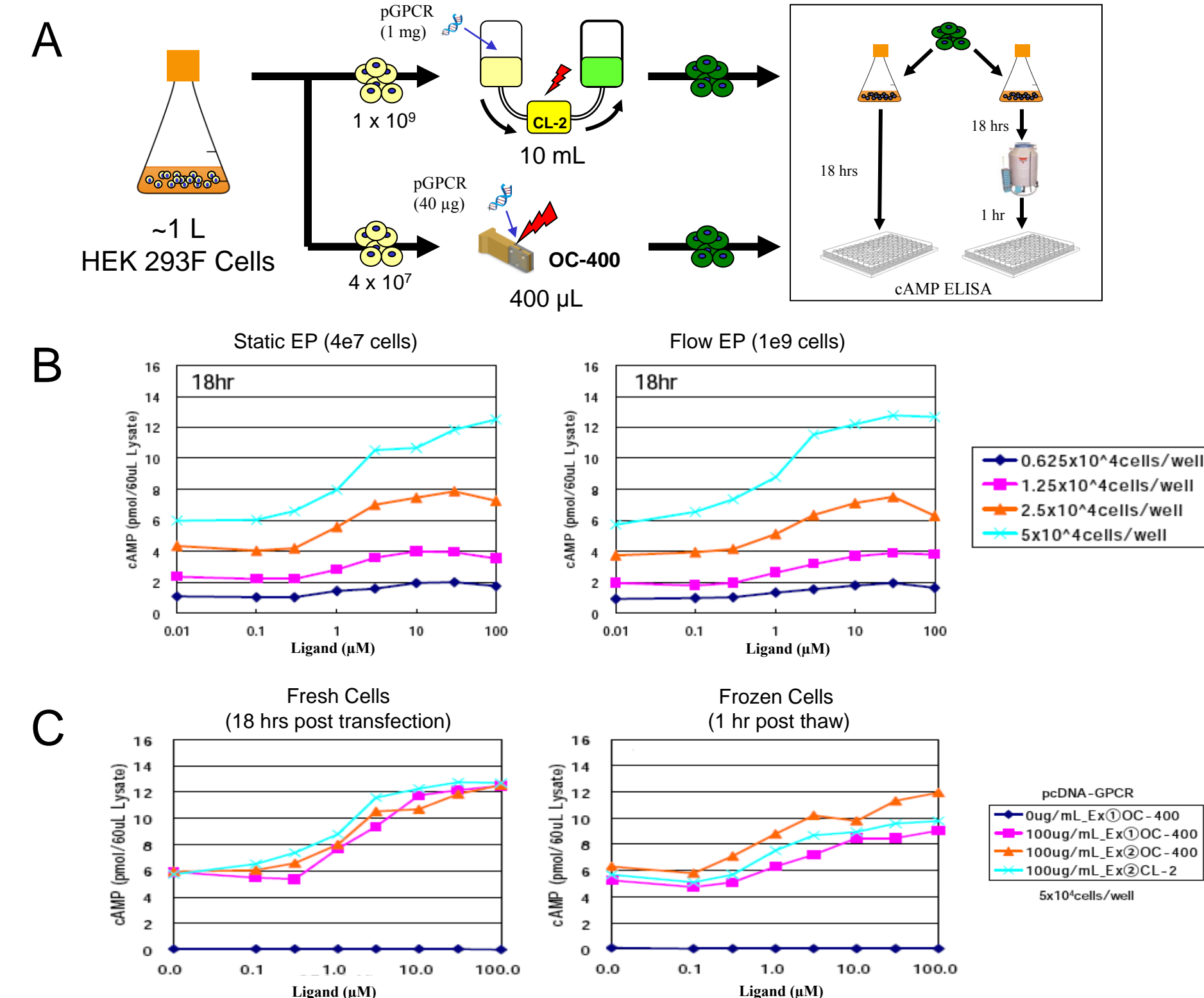


Figure 3. GPCR Assay in HEK 293F suspension cells. A. One billion HEK 293F cells suspended in MaxCyte EP buffer (1x10⁸ cells/mL) were mixed with a GPCR expression plasmid (100 µg/mL) and transfected at both large and small scales using flow and static EP, respectively. Some of the cells were cryopreserved 18 hrs post transfection. GPCR activity was assayed by cAMP ELISA at 18 hrs post EP (fresh cells) or at 1 hr post thawing (frozen cells). B. Cells transfected at small and large scales showed comparable concentration-dependent responses to a natural GPCR ligand. C. Cells transfected in two separate small scale experiments and in one large scale experiment exhibited reproducible ligand responses before and after cryopreservation.

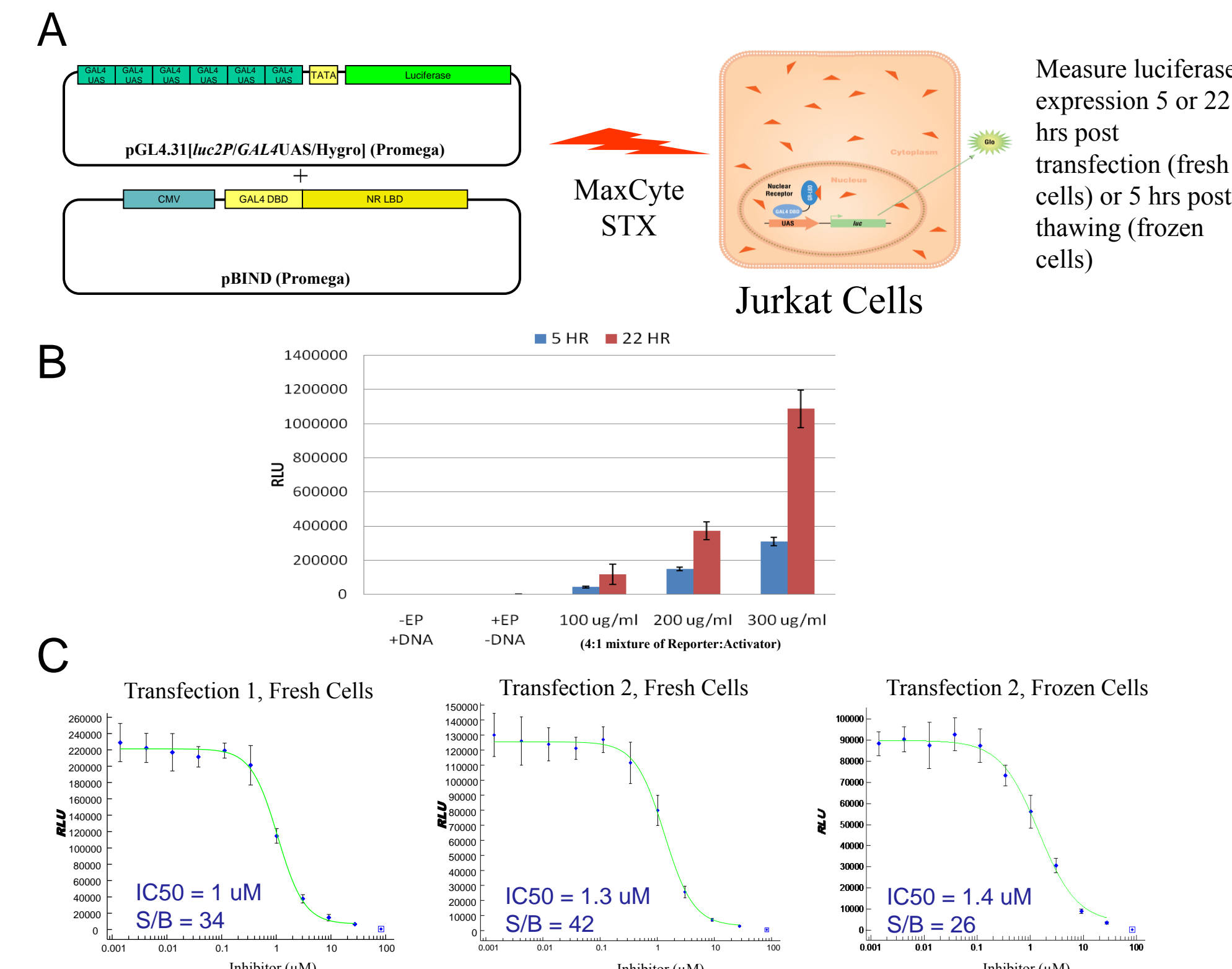


Figure 4. Nuclear receptor assay in Jurkat cells. A. Jurkat cells were cotransfected via static EP with a reporter plasmid in which luciferase was expressed from a minimal promoter containing multiple GAL4 UAS sequences and with an activator plasmid encoding a constitutively expressed fusion protein in which a GAL4 DNA binding domain was linked to a nuclear receptor ligand binding domain. B. Cells transfected with three concentrations of the plasmid mixture were plated in 384 well plates (~15k cells/well) and assayed at 5 & 22 hrs post EP. Assay sensitivity directly correlated with DNA concentration. C. Cells transfected with 200 µg/mL of the plasmid mixture were plated in 384 well plates immediately after EP (fresh cells) or cryopreserved immediately after EP and plated immediately after thawing (frozen cells). Cells were treated with varying concentrations of inhibitor immediately after plating, and luciferase activity was measured 5 hrs later. Fresh cells from two separate EPs and frozen cells showed reproducible inhibition profiles. These data illustrate the consistency of MaxCyte's transfection process and demonstrate that STX transfected cells can be cryopreserved without impacting assay performance.

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
Mock-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
150 µg/ml cDNA	77%	96%	247±67 MΩ	246±78 MΩ	82%	95%	3.1±2.1 nA	3.2±2.1 nA

Figure 5. Analysis of K_v1.5 ion channel activity in transiently transfected CHO cells. CHO K1 cells were transfected via static EP with two different concentrations of a K_v1.5 α-subunit expression plasmid and assayed on the IonWorks® Quattro™ (IWQ) recording in single hole mode. 24 hr evaluation: cells were cultured at 37°C for 6 hrs followed by 28°C for 18 hrs; 48 hr evaluation: culture at 37°C for 24 hrs followed by 28°C for 24 hrs. High seal resistances and strong current amplitudes in control and DNA transfected cells demonstrate that the EP process has no discernible impact on membrane integrity. The percentage of cells expressing currents >0.5 nA correlates with the amount of DNA used in the EP reaction.

Time post transfection	PatchPlate	% seals (>100 MΩ) ¹	Seal resistance (mean±SD)	% expression (>0.5 nA)	Current amplitude (mean±SD)
<1 month	SH	50%	202±77 MΩ	86%	2.7±1.3 nA
	PPC	98%	38±11 MΩ	99%	1.5±0.3 nA
5 months	SH	53%	197±80 MΩ	81%	2.4±1.2 nA
	PPC	93%	30±8 MΩ	97%	1.4±0.3 nA
	PPC	90%	32±8 MΩ	100%	1.9±0.2 nA
	PPC	99%	34±8 MΩ	100%	1.4±0.2 nA

¹ PPC seals >20MΩ
 • Consistent current amplitude
 • Defrost conditions to be optimised

Figure 6. Large scale transfection and cryopreservation of CHO cells for ion channel assays. 8x10⁸ CHO K1 cells were transfected with 150 µg/mL of K_v1.5 α-subunit plasmid DNA via flow EP. Transfected cells were cultured @ 37°C for 24 hrs, transferred to a 28°C incubator for an additional 24 hrs, then suspended in freezing medium and cryopreserved in liquid nitrogen using standard methodology. Thawed cells were assayed in single hole (SH) and population patch clamp (PPC) modes on the IWQ. Following cryopreservation, cells exhibited seal resistances, expression levels and current amplitudes comparable to those of freshly transfected cells.

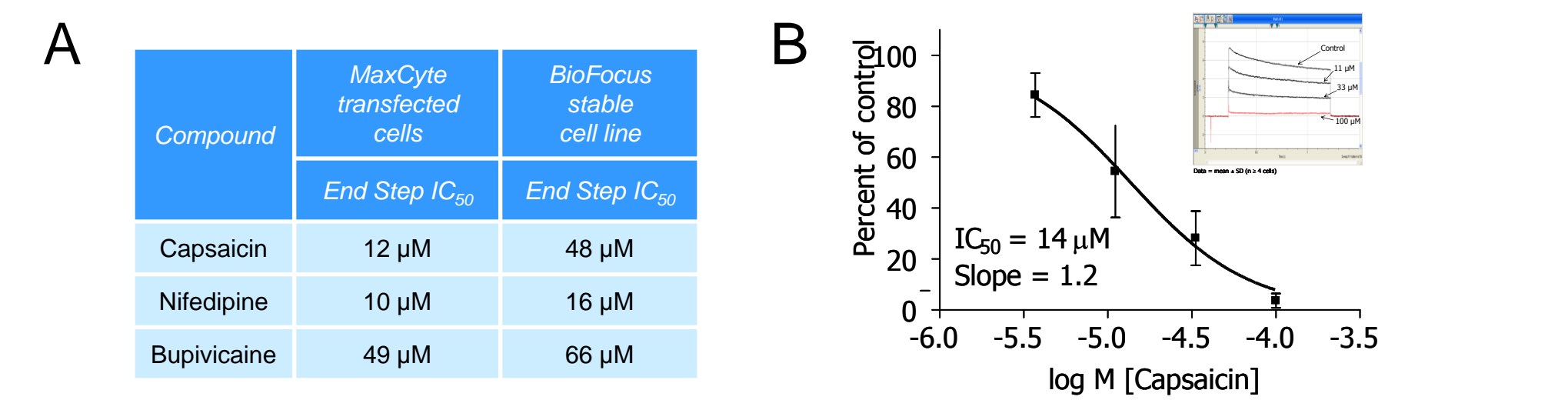


Figure 9. Pharmacological assays with transiently transfected cells. A. CHO K1 cells transiently transfected with a K_v1.5 expression plasmid were exposed to varying concentrations of three ion channel inhibitors and assayed on the IWQ in PPC mode. IC₅₀ values for each compound compared favorably to PPC mode data obtained using the stable cell line. B. Pharmacological analysis of transiently transfected cells using the PatchXpress® instrument. Inset shows representative current tracings that depict a step-wise loss of K_v1.5 activity in response to increasing concentrations of the inhibitor capsaicin.

Summary

- ✓ The MaxCyte STX system enables rapid development of cell based assays with standard cell lines, such as HEK and CHO, and with physiologically relevant cells, such as Jurkats.
- ✓ The MaxCyte STX transfection process is scalable and consistent. Reproducible assay results are seen with multiple batches of transfected cells, regardless of the number of cells transfected.
- ✓ Transfected cells can be cryopreserved without impacting assay performance.
- ✓ Cells transfected with ion channel plasmids exhibit high levels of ion channel expression, strong seal resistances and robust current tracings on multiple automated electrophysiology platforms. Transiently transfected cells perform comparably to stable cells in pharmacological assays.