

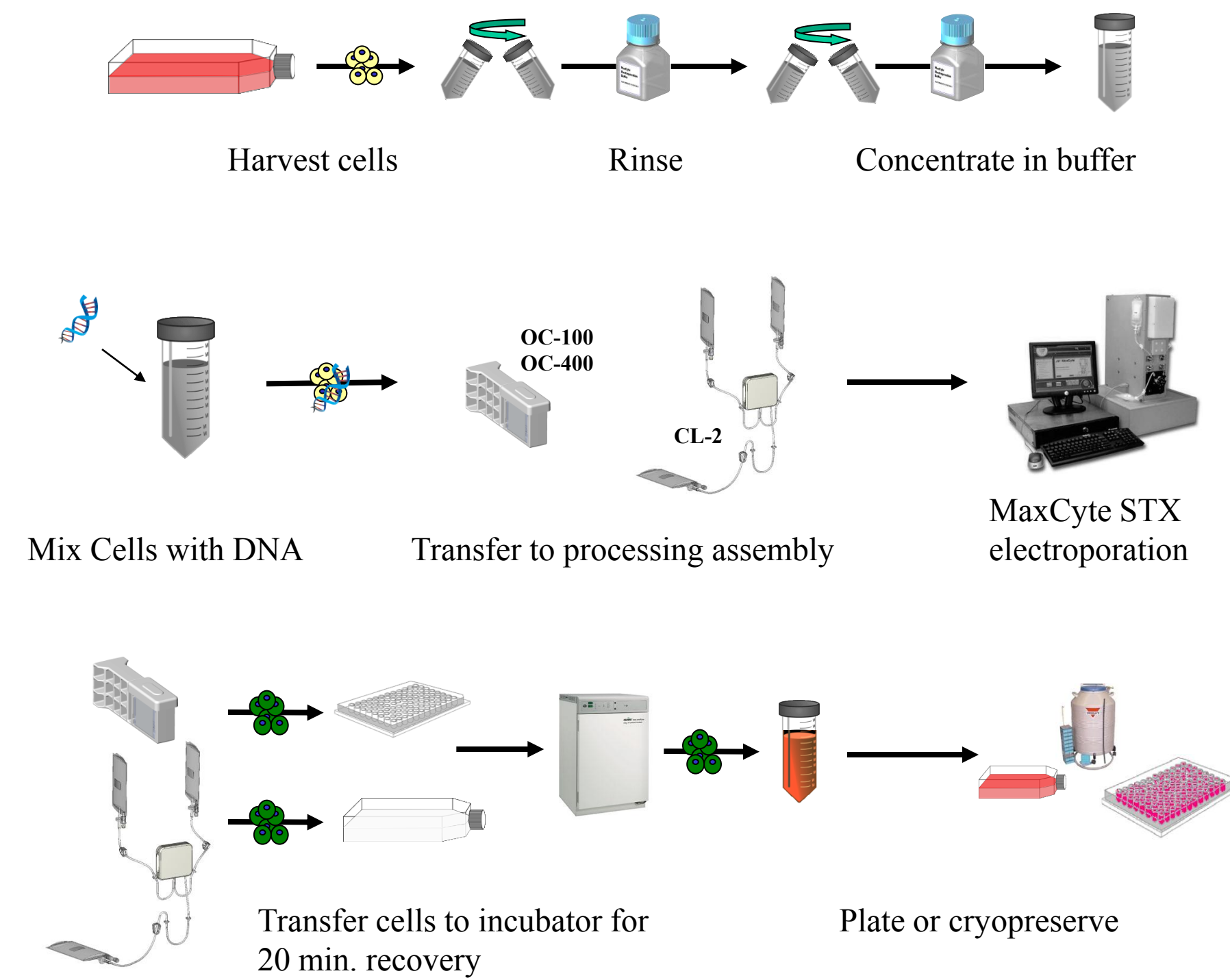
# Advancing Drug Discovery with the MaxCyte® STX™ Scalable Transient Transfection System: Expression of Intracellular, Membrane-Bound and Secreted Proteins in Physiologically Relevant Cell Lines, Primary Cells and Stem Cells

James Brady, Linhong Li, Cornell Allen, Rama Shivakumar, Angelia Viley and Madhusudan Peshwa  
MaxCyte Inc, 22 Firstfield Rd, Suite 110, Gaithersburg, Maryland, USA



## Abstract

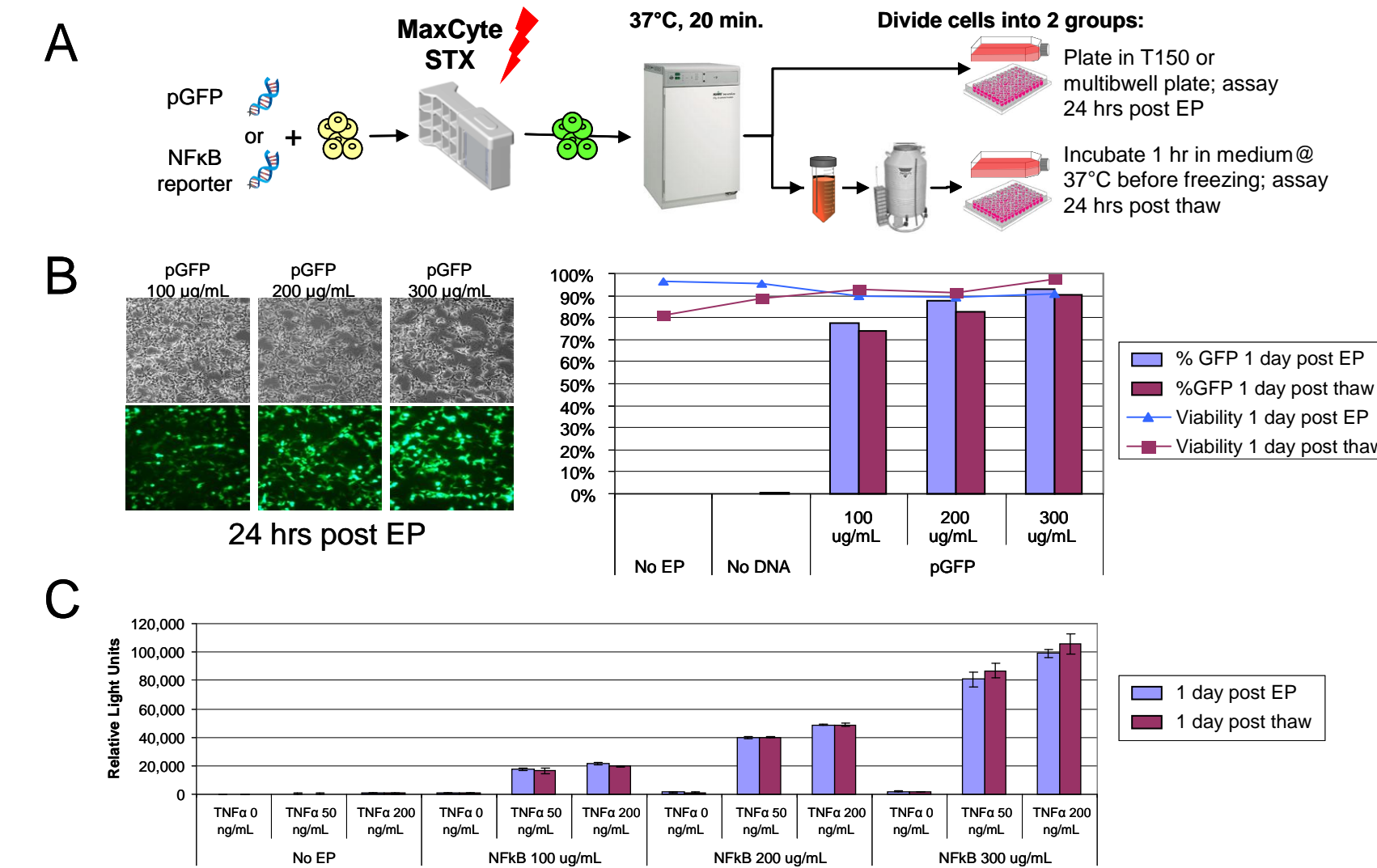
The MaxCyte® STX™ Scalable Transfection System is a bench-top instrument that can transfect small scale (5x10<sup>5</sup> cells) to HTS scale (1x10<sup>6</sup> cells) using its proprietary flow electroporation technology with DNA, mRNA, siRNA or protein in less than thirty minutes. The cell type-specific electroporation protocols allow users to consistently transfect cells on large and small scale with high viability, transfection efficiency, and cell membrane integrity for high quality results in downstream HTS/HCS functional assays for ion channels, GPCRs, and other targets of interest. The technology works very well with commonly used cell lines, such as HEK 293 and CHO, but one of its primary advantages is that it allows efficient transfection of challenging cell lines and primary cells that are not normally amenable to transient transfection. Here we present data on how to use the MaxCyte STX system to transfect various cell types at small and large scale for rapid development of cell based assays, and we illustrate the capabilities of the MaxCyte STX system for protein production at both small and large scale.



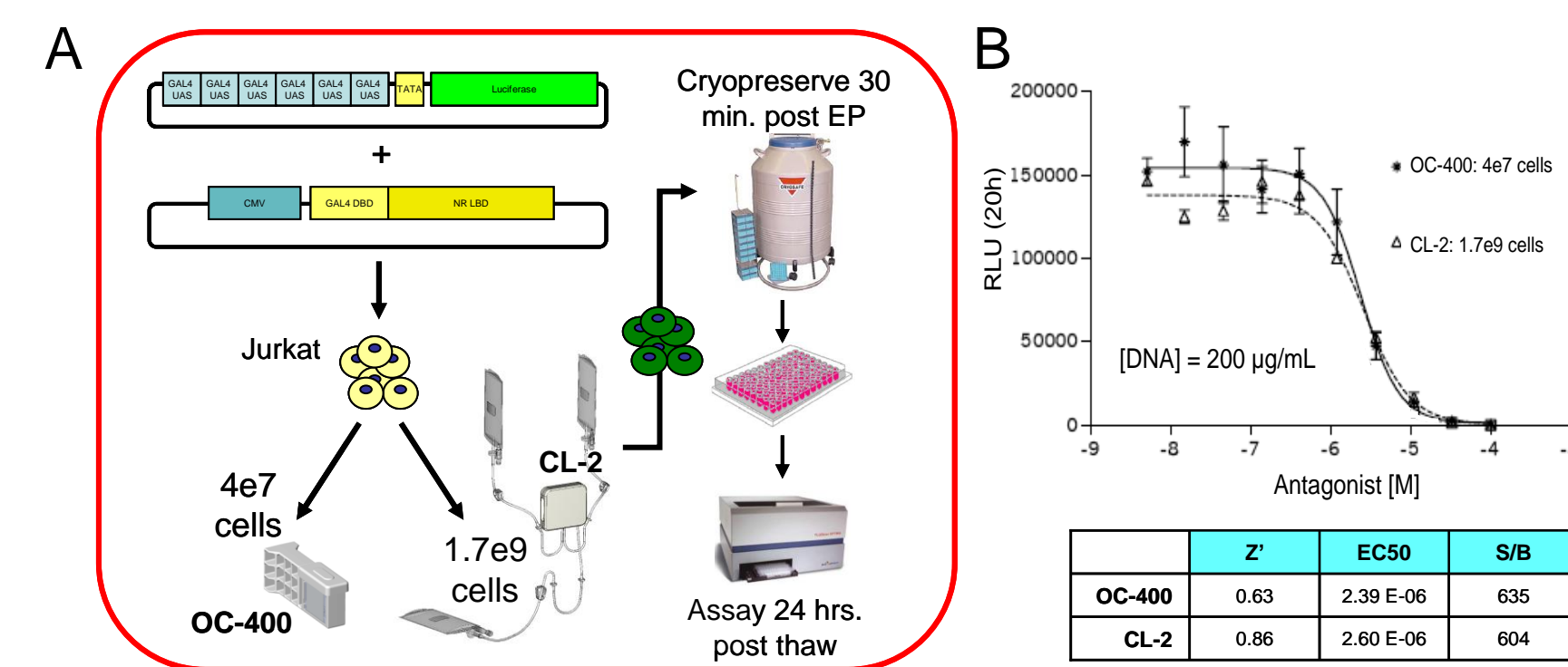
**Figure 1. Transfecting cells with the MaxCyte® STX™ scalable transfection system.** Cells are harvested from culture using standard methods and reagents and suspended at high density (typically 5e7-1e8 cells/mL) in MaxCyte's electroporation (EP) buffer, a physiologically balanced salt solution that contains no biological agents. The same buffer formulation is used for every cell type. Next, cells are mixed with loading agents and transferred to sterile, single use processing assemblies (PAs). Cells can be transfected with multiple types and combinations of loading agents, including DNA, mRNA, siRNA, proteins and small molecules. Small scale transfections (5e5-4e7 cells) are performed by static EP (completed in seconds) in OC-100 and OC-400 PAs; large scale transfections (up to 1e10 cells) are performed by flow EP (less than 30 minutes) in CL-2 PAs. The STX instrument comes loaded with a variety of EP protocols that are optimized for individual cell types; the user does not need to make any adjustments to the electrical parameters. After EP, cells are transferred from the PA to a sterile, multiwell dish (for OC-100/400s) or T-flask (for CL-2 EPs) and allowed to recover for 20 minutes at 37°C. Finally, the cells are suspended in standard tissue culture medium and either plated for assays or cryopreserved for future use.

© 2011 MaxCyte, Inc. All Rights Reserved.  
Presented at LabAutomation2011  
Palm Springs, CA January 30-February 2, 2011

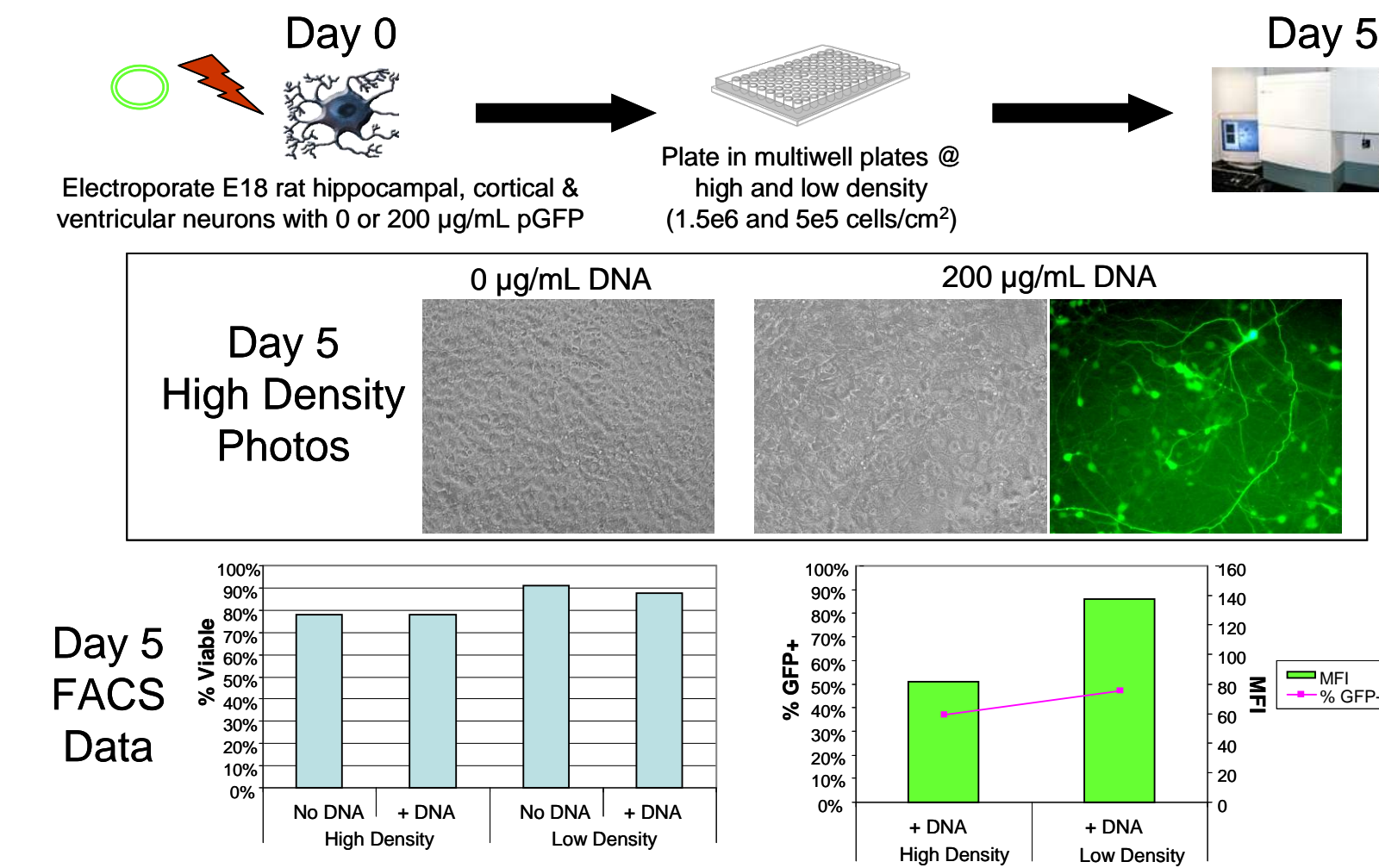
## Results



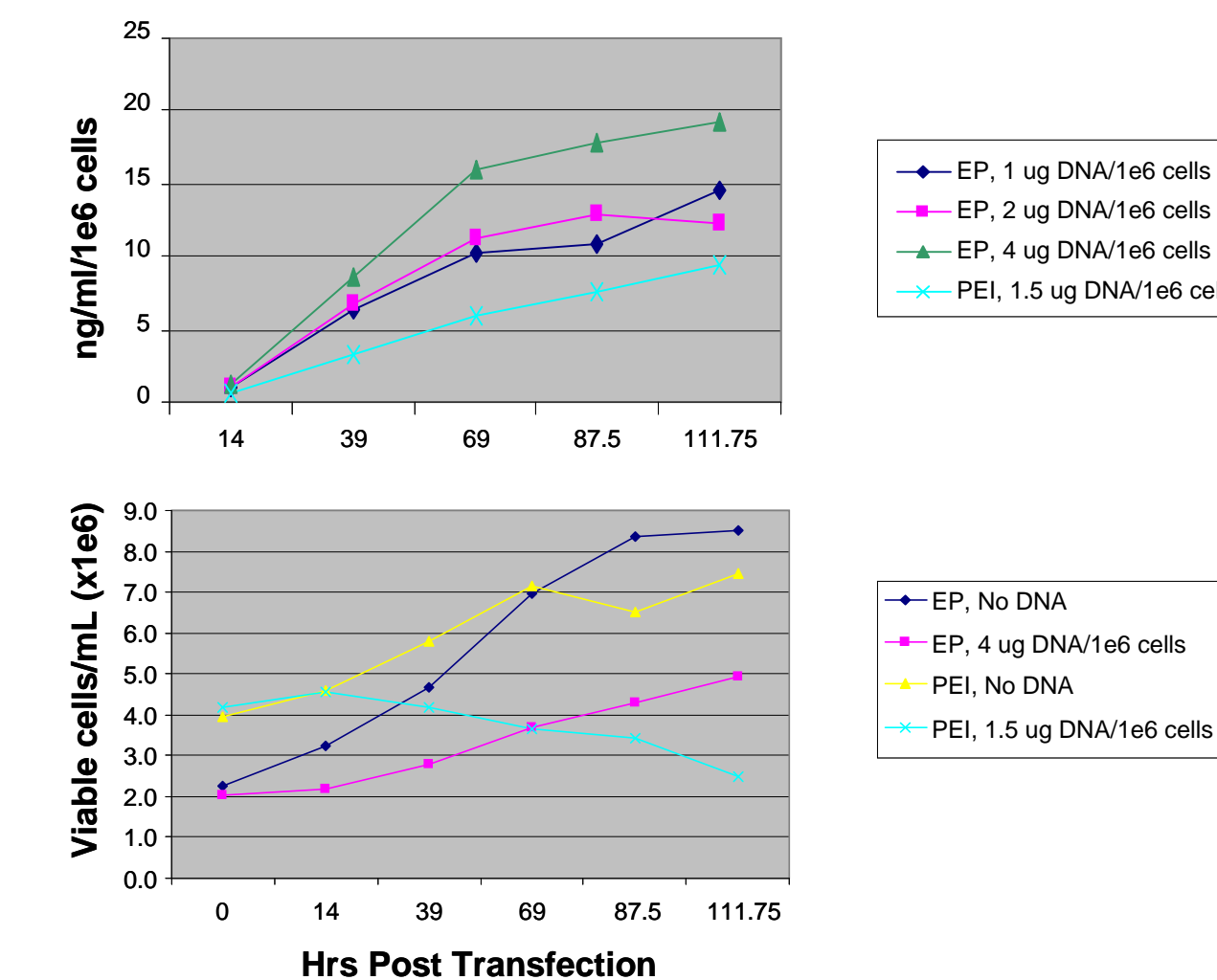
**Figure 2. Cryopreservation of transiently transfected HEK cells does not impact gene expression or viability.** A. HEK 293T cells were transfected in OC-400 PAs with either pGFP or a NFκB reporter plasmid and divided into two groups: 1) plate immediately for assays, or 2) cryopreserve and assay post thaw. For the NFκB assays, cells were seeded in 96 well plates (50k cells/well) and treated with TNFα 4 hrs post plating; luciferase activity was measured the following morning. GFP expression was analyzed by FACS. B. GFP expression correlated with plasmid DNA concentration before and after freezing. Good viability was obtained with all conditions. C. Assay sensitivity correlated with DNA concentration. Frozen and non-frozen cells showed comparable concentration-dependent responses to TNFα.



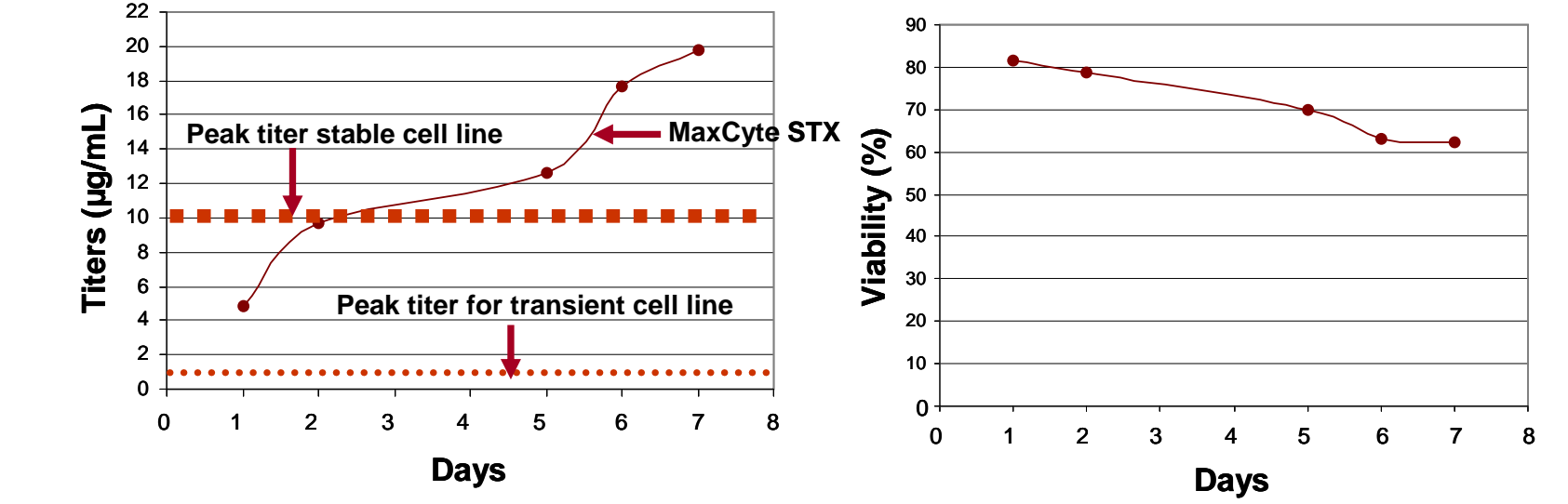
**Figure 3. Dual plasmid nuclear receptor assay in transiently transfected Jurkat cells.** A. Jurkat cells were suspended in MaxCyte EP buffer with 200 µg/mL of a dual plasmid mixture containing a luciferase reporter plasmid with GAL4 binding sites and an activator plasmid encoding a GAL4 DNA binding domain-nuclear receptor ligand binding domain fusion protein. 4e7 cells were transfected by static EP in an OC-400 PA; 1.7e9 cells were transfected by flow EP in a CL-2 PA. Cells were cryopreserved 30 minutes post EP. Cells were plated with compound immediately after thawing and assayed on the following day. B. Both sets of transfected cells showed comparable responses to compound, illustrating the scalability and consistency of MaxCyte's transfection process.



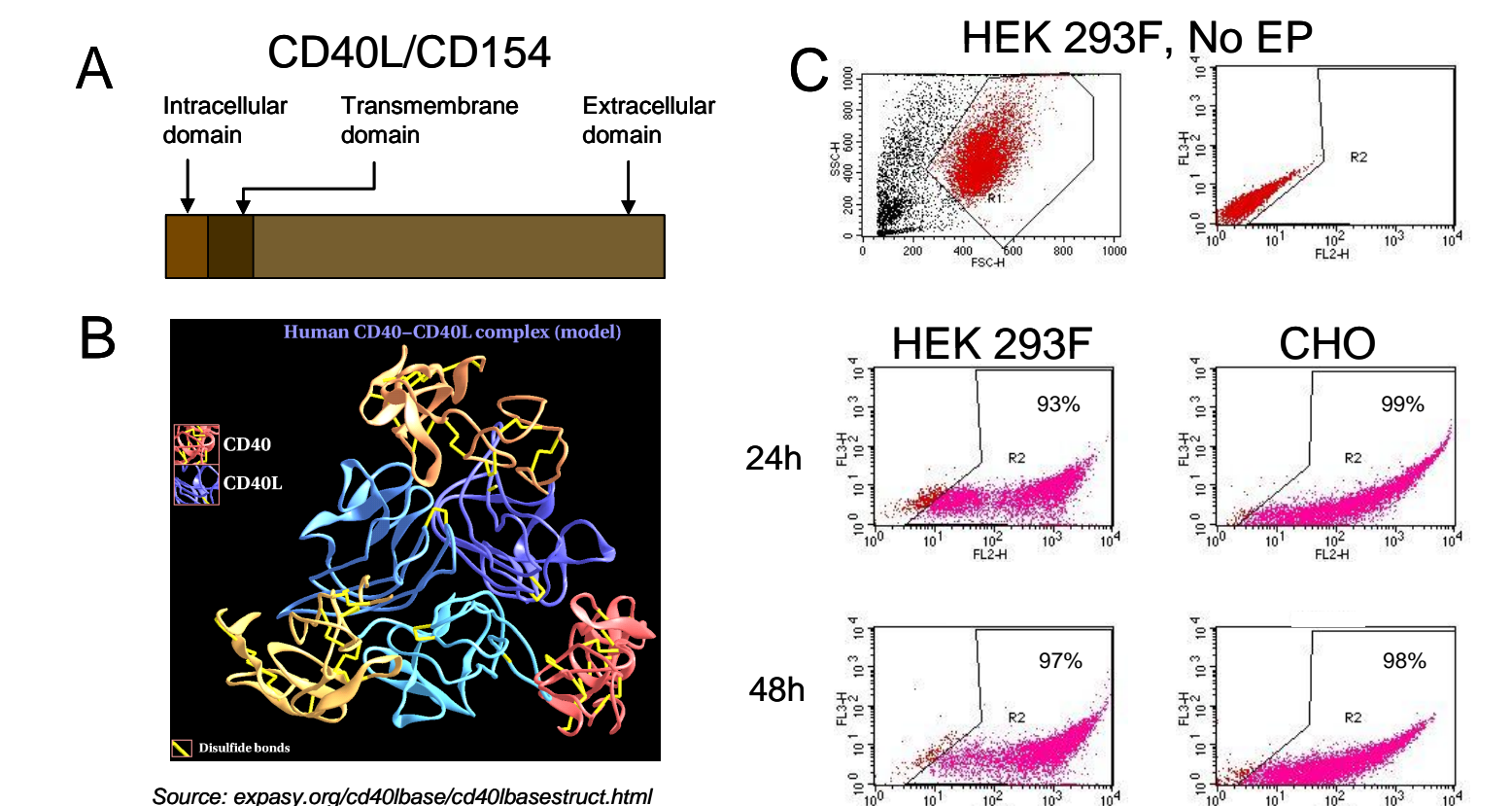
**Figure 4. Efficient transfection of primary neurons with the MaxCyte STX technology.** Primary neurons were isolated from the ventricular, cortical and hippocampal regions of day 18 embryonic rat brains by dissociation with trypsin and suspended in electroporation buffer at a density of 3e7 cells/mL. The neurons were electroporated in an OC-100 PA with 200 µg/mL of a GFP expression plasmid and plated at two different densities in a poly-D lysine coated 96 well plate. Five days after electroporation, cells were photographed, harvested with trypsin and assayed by FACS to quantify GFP expression and viability. After 5 days in culture, the cells still exhibited ~50% GFP expression, and they showed good viability with normal neuronal morphology.



**Figure 5. Protein production with transiently transfected HEK cells.** Suspension adapted HEK 293F cells were suspended in electroporation buffer at a density of 1e8 cells/mL and transfected with two concentrations of an expression plasmid encoding a secreted viral coat protein. A parallel transfection was performed using PEI. Electroporated cells were seeded in shake flasks with serum free media at a density of 2e6 cells/mL; PEI transfections were performed with cells that were seeded at a starting density of 4e6 cells/mL. Media samples were collected without replacement at the indicated time points, and protein expression was quantified by ELISA. The top graph shows that protein expression levels correlated with DNA concentration in the electroporated cells, and they exceeded protein titers obtained with an optimized PEI transfection protocol. The bottom graph demonstrates that electroporation has less impact on growth kinetics relative to PEI transfection.



**Figure 6. Antibody production in transiently transfected CHO cells.** Forty million suspension adapted CHO cells were transfected in an OC-400 PA with a monoclonal antibody expression plasmid and seeded in a shake flask with serum free medium at a density of 4e6 cells/mL. The graph on the left shows that antibody titers, quantified by immunoassay, increased steadily over a seven day period and exceeded the peak titer previously determined for a cell line that was stably transfected with the same plasmid. The dotted line at the bottom of the graph shows the peak titer that was previously achieved with this antibody using a reagent-based transfection method.



**Figure 7. Expression of CD40 ligand on the surface of transiently transfected HEK 293F and CHO K1 cells.** Suspension-adapted HEK 293F cells and adherent CHO K1 cells were transfected with 200 µg/mL of a plasmid encoding CD40 ligand (CD40L/CD154) via static electroporation in OC-100 PAs. Cells were analyzed by FACS using a fluorescently labeled anti-CD40L antibody at 24 & 48 hrs post electroporation. A. Structure of CD40L protein. B. Model of homotrimeric CD40L protein interacting with CD40. C. FACS analyses of transfected 293F and CHO K1 cells. Almost all transfected cells exhibited CD40L on their surfaces, demonstrating utility of the MaxCyte STX system for efficient expression of cell surface proteins.

## Summary

- ✓ The MaxCyte® STX™ system provides an efficient and reproducible method to transfect suspension adapted and adherent cell lines and primary cells with DNA and other molecules.
- ✓ The STX transfection process produces consistent results at small and large scale, even with multi-plasmid assays.
- ✓ Transfected cells can be plated for immediate use in cell based assays or cryopreserved for future applications.
- ✓ The MaxCyte scalable transfection process enables high titer, large scale recombinant protein production in HEK, CHO and other cell types.

Correspondence: Jim Brady at (301) 944-1639  
Jamesb@maxcyte.com