Development of Fully Scalable Reporter Gene Assays for Studying Transcriptional Regulation & Receptor Activation Using Flow Electroporation.

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Abstract

Reporter gene assays are commonly used to assess transcriptional regulation. For use in high throughput screening campaigns these assays need to be scalable in nature requiring either a large number of transiently transfected cells or creation of stable cell lines. There are major drawbacks to working with stably transfected cells, including extensive time and expenses associated with cell line generation, limited applicability with physiologically relevant cells and challenges related to working with multi-subunit and/or toxic proteins. The MaxCyte STX system uses a novel flow electroporation-based technology to transiently (co)transfect from 5E5 in seconds to 1E10 cells in less than 30 minutes with drug targets or reporter molecules, yielding levels of transfection efficiency, cell viability and assay sensitivity that match or exceed those of most stable cell lines. The technology works with a wide range of cell types, including primary cells and stem cells expressing a variety of druggable targets including GPCRs, ion channels, cytokine receptors, kinases and nuclear receptors. In this poster we highlight the use of MaxCyte electroporation to develop and scale up a variety of reporter gene assays including expression of TNFα inducible NF-kB reporters & NFAT reporter for examining receptor transcriptional activation. In addition we demonstrate that MaxCyte transiently transfected cells can be cryopreserved without impacting assay performance enabling large scale, bulk transfections for future screening.

MaxCyte Fully Scalable Flow Electroporation

High Performance: >95% Viability & Transfection Efficiency

MaxCyte STX®

5E5 Cells in Seconds
Up to 1E10 Cells in <30 Min.

- Rapid & simple to use
- High efficiency & high viability
- Broad cell compatibility including cell lines, primary cells & other difficult-to-transfect cells
- Streamlined scalability

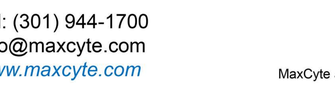
Figure 1. The MaxCyte® STX™ uses scalable electroporation technology to (co)transfect a variety of cell types, including primary cells and other difficult-to-transfect cells, with DNA, RNA, siRNA, proteins or other biomolecules of interest. Transfection efficiencies and cell viability are routinely greater than 95%. Transfected cells can be used immediately following electroporation or cryopreserved for future use. The MaxCyte STX can perform small-scale transfections for basic research and assay development or perform large scale, bulk transfections for use in full-scale, screening and profiling.

Transfection Methods & Constructs

Rapid, Streamlined Transient Transfection Processing

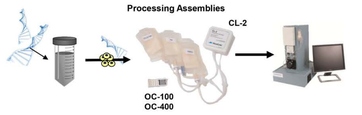
>95% Viability & Transfection Efficiency

Cell Harvesting



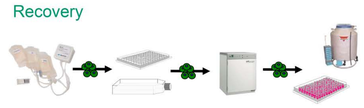
- Collect cells
- Rinse
- Concentrate in EP buffer

Electroporation



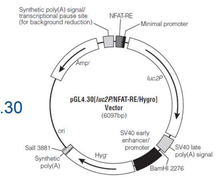
- Mix Cells with DNA
- Transfer to PAs
- STX electroporation

Recovery



- Transfect cells to flask or plate
- Incubate 20 min @ 37°C
- Resuspend cells
- Run reporter gene assays or cryopreserve

pGL4.30



pGL4.32

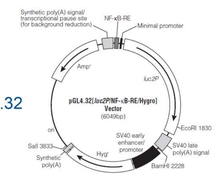


Figure 2. Constructs used are Promega designed vectors that report the activity of a variety of pathways using the optimized luc2 firefly luciferase gene in the pGL4 backbone. Luc2P is a synthetically-derived luciferase sequence with humanized codon optimization that is designed for high expression and reduced anomalous transcription.

pGL4.32[luc2P/NF-κB-RE/Hygro] contains five copies of an NF-κB response element (NF-κB-RE) that drives transcription of luciferase reporter gene luc2P.

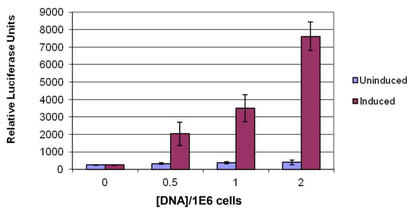
pGL4.30[luc2P/NFAT-RE/Hygro] contains an NFAT response element (NFAT-RE) that drives transcription of the luciferase reporter gene luc2P.

NFAT Reporter Assays

NFAT Reporter Measures Cellular Stimulation

Assay Performance Comparable Pre and Post Cell Cryopreservation

A. Fresh Cells



B. Cryopreserved Cells

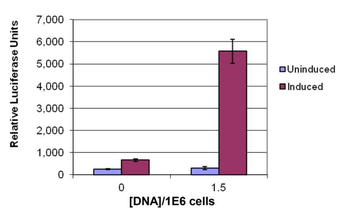


Figure 3. Excellent NFAT Assay Performance Following Transfected Cell Cryopreservation. **A.** HEK 293T cells were transfected using the MaxCyte STX with 3 concentrations of the pGL4.30 (NFAT reporter) plasmid and plated in a 96 well plate (50K cells/well). 24 hrs after plating, cells were treated with Induction Solution (10 ng/mL PMA, 1 uM iononmycin) and incubated overnight. Luciferase activity was measured using Promega Bright-Glo™ reagent following the standard protocol. **B.** HEK cells were transfected with 1.5 μg/1E6 cells pGL4.30, and the cells cryopreserved in 90% FBS/10% DMS0 following a 20 minute post electroporation (EP) recovery period. Transfected cells were thawed, plated in a 96 well plate (50K cells/well), and treated with Induction Solution 4 hours after plating. Luciferase activity was measured following an overnight incubation. Error bars denote standard deviation from 3 replicate wells.

Electroporated cells perform well in an NFAT reporter gene assay. Data showed that transgene expression and assay sensitivity can be controlled by varying DNA concentration used for transfection. Transfected cells can be cryopreserved without affecting cell performance in future assays.

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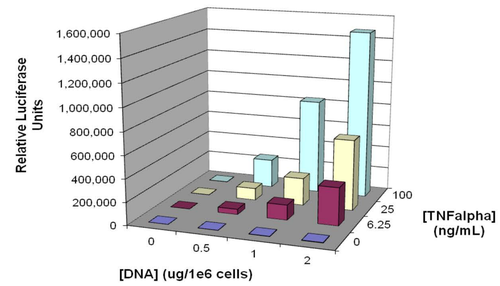
Nf-kB Reporter Gene Assays

Straightforward Assay Development & Scale-up

NF-kB Cellular Assay Results Equivalent Upon Transfection Scale-up

A. DNA & TNFα Dose-dependent Responses



B. Seamless Scalability

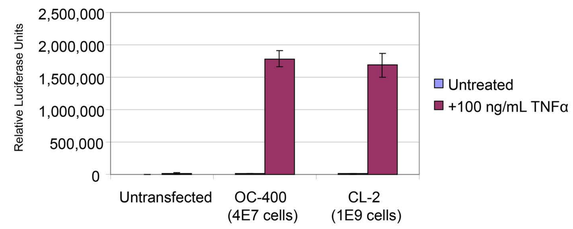
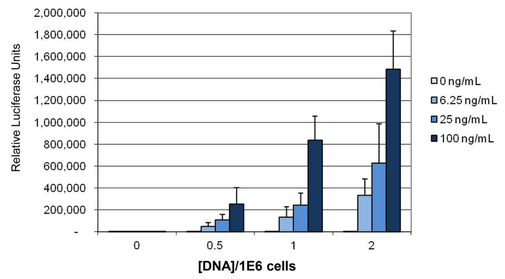


Figure 4. Cytokine Receptor Stimulation Elicits Dose-dependent Luciferase Expression. A. HEK 293T cells were transfected via small scale EP with 3 concentrations of the pGL4.32 (NF-kB) plasmid and plated in a 96 well plate (50K cells/well). 4 hrs after plating, cells were treated with TNFα and incubated overnight. Luciferase activity was measured using Promega Bright-Glo® reagent following the standard protocol. **B.** 1E9 cells were suspended in MaxCyte EP buffer at a density of 1E8 cells/mL, and pGL4.32 plasmid (1 μg/1e6 cells) added. 400 ul of the cell/plasmid mixture was transfected by static EP using an OC-400 processing assembly (PA); the remaining cells were transfected by flow EP in a CL-2 PA. Transfected cells were cryopreserved in 90% FBS/10% DMS0 following a 20 minute post EP recovery period. Cells were thawed, plated in a 96 well plate (50K cells/well), and treated with TNFα 4 hours after plating. Luciferase activity was measured following an overnight incubation. Error bars denote standard deviations from 6 replicate wells. These data show DNA dose-dependent and TNFα-dependent NFkB reporter gene assay results. Additionally, these data clearly demonstrate the ability to scale up cellular assays without affecting downstream assay performance.

Cell Cryopreservation Enables Bulk Transfection

NF-kB Cellular Assay Results Equivalent Upon Cell Cryopreservation

Fresh Cells



Cryopreserved Cells

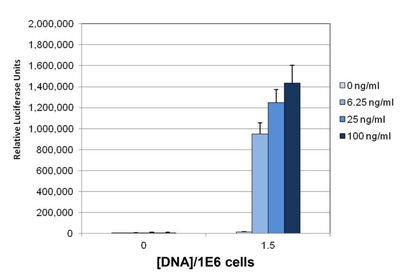


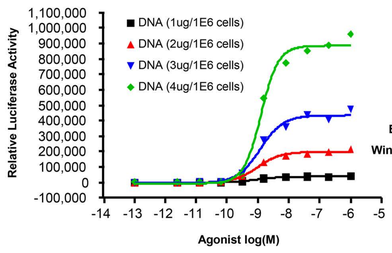
Figure 5 Transfected Cells Can be Cryopreserved Without Affecting NFkB Reporter Gene Assay Result. HEK 293T cells were transfected with the pGL4.32 (NF-κB) plasmid (1.5 μg/1E6 cells), and cryopreserved in 90% FBS/10% DMS0 following a standard 20 min. post EP recovery. Transfected cells were thawed, plated in a 96 well plate (50K cells/well), and treated with TNFα 4 hours after plating. Luciferase activity was measured following an overnight incubation. Error bars denote standard deviations from 3 replicate wells. These data indicate that cell cryopreservation following electroporation does not affect their performance in future cellular assays.

Dual Plasmid Luciferase Assay

Seamless Scale-up of Nuclear Receptor Reporter Gene Assay

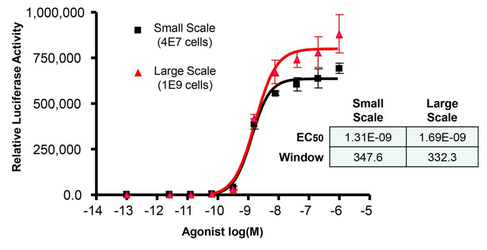
Agonist-induced Responses & Assay Window Equivalent Upon Transfection Scale-up

A. Assay Window Correlates with DNA Concentration



	1μg/1E6 cells	2μg/1E6 cells	3μg/1E6 cells	4μg/1E6 cells
EC50	9.33E-10	9.78E-10	1.20E-10	1.27E-10
Window	66.67	204.61	310.14	378.38

B. Agonist Responses



	Small Scale	Large Scale
EC50	1.31E-09	1.69E-09
Window	347.6	332.3

Figure 6 Dual Plasmid Nuclear Receptor Assay Development and Scale-up. **A.** U2OS cells were co-transfected in OC-400 PAs (4E7 cells/condition) with four concentrations of a DNA solution containing a 1:1 mixture of a nuclear receptor protein expression plasmid and a luciferase reporter plasmid. Following a standard 20 minute incubation at 37°C, the transfected cells were plated in 96 well plates and treated with varying concentrations of agonist compound. Measurements of luciferase activity on the following day revealed that cells transfected with all four concentrations of the plasmid mixture showed concentration-dependent ligand responses with consistent EC50 values. Assay sensitivity correlated directly with [DNA]. **B.** Large scale transfection was performed using flow electroporation in a CL-2 PA with 1E9 cells and 3μg/1E6 cells of the plasmid mixture; an additional small scale was performed. The two sets of transfected cells produced overlapping concentration response curves with comparable EC50 values and assay windows, demonstrating scalability and consistency of MaxCyte transfection.

Summary

- The MaxCyte STX system provides a rapid and cost effective alternative to stable cell lines for reporter assays. High levels of transfection efficiency and cell viability result in assay responses that are equivalent to or greater than those of stably transfected cells.
- STX users have tight control over assay sensitivity simply by varying the DNA concentration.
- Multiple plasmids or other loading agents can be co-transfected to develop complex assays.
- Rapid transgene expression in STX transfected cells provides assay data within one day of transfection.
- The STX provides seamless scalability for assay production. Assay results with 5E6-4E7 cells transfected at small scale via static electroporation are faithfully reproduced with 5E8-1E10 cells transfected at large scale via flow electroporation.
- Cells transfected with the STX can be cryopreserved shortly after electroporation and thawed for assays to be conducted at future time points. Cells from large scale transfections can be aliquoted during cryopreservation, allowing users to run many assays with the same batch of transfected cells, greatly reducing run-to-run variability that is inherent with other transient transfection methods.