

MaxCyte Electroporation Enables the Production of Assay Ready Cells Suitable for Ion Channel Studies

Victoria McClelland, Debra Barberini, Marianna Romito and James Brady, MaxCyte, Inc., Rockville, MD, USA



Abstract

Advances in technology for automated electrophysiology have simplified the implementation of high throughput screening for ion channel research and drug discovery. However, a continued, widespread reliance on stable cell lines remains a bottleneck to progress.

Stable cell line development is a lengthy, laborious process, and creating stable cell lines for ion channel expression can be particularly challenging. Ion channels are complex, multi-subunit proteins and cell line development may require inducible promoters or multiple rounds of selection with harsh selection agents, potentially altering cell health and growth characteristics.

The MaxCyte® electroporation platform enables the efficient transfection of virtually any cell type for the rapid, scalable

transient expression of multi-subunit protein complexes, including toxic or intractable ion channels. Unlike other electroporation methods or chemical or lipid reagents, MaxCyte electroporation delivers high transfection efficiency while maintaining high cell viability and membrane integrity.

MaxCyte transfected cells are suitable for immediate use in cell-based assays, or they can be aliquoted and cryopreserved as stocks of Assay Ready Cells for later use. MaxCyte's Flow Electroporation® technology can transfect up to 2x10¹⁰ cells in under 30 minutes to produce Assay Ready Cells in the most appropriate cell type. Here we show scalable, highly efficient transfection in a variety of cell lines and stem cells and present case studies demonstrating the suitability of Assay Ready Cells as an alternative to stable cell lines for ion channel research, assay development and drug discovery.

1 MaxCyte Electroporation Enables the Use of the Most Appropriate Cell Context

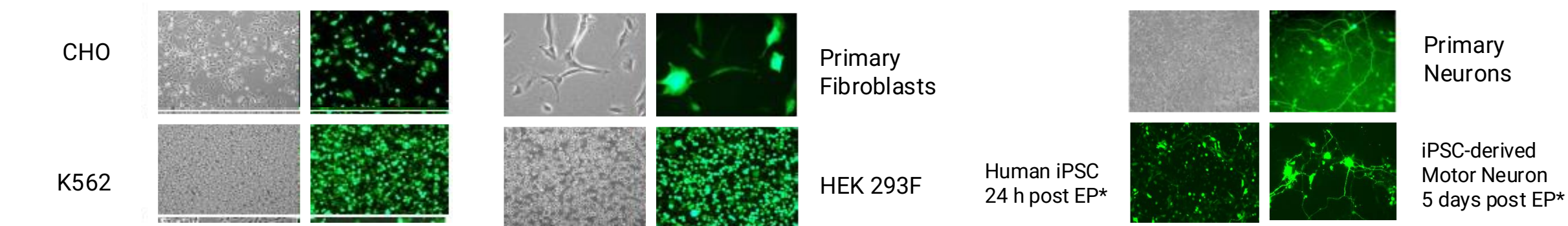


Figure 1: High Transfection Efficiency in Virtually Any Cell Type with MaxCyte Electroporation. Cells were transfected with 2 µg of pGFP DNA per 1x10⁶ cells using the appropriate pre-loaded MaxCyte electroporation protocol. 24 hours post transfection cells were examined for cell viability and transfection efficiency. *Data courtesy of iXCells

2 MaxCyte Electroporation is Scalable

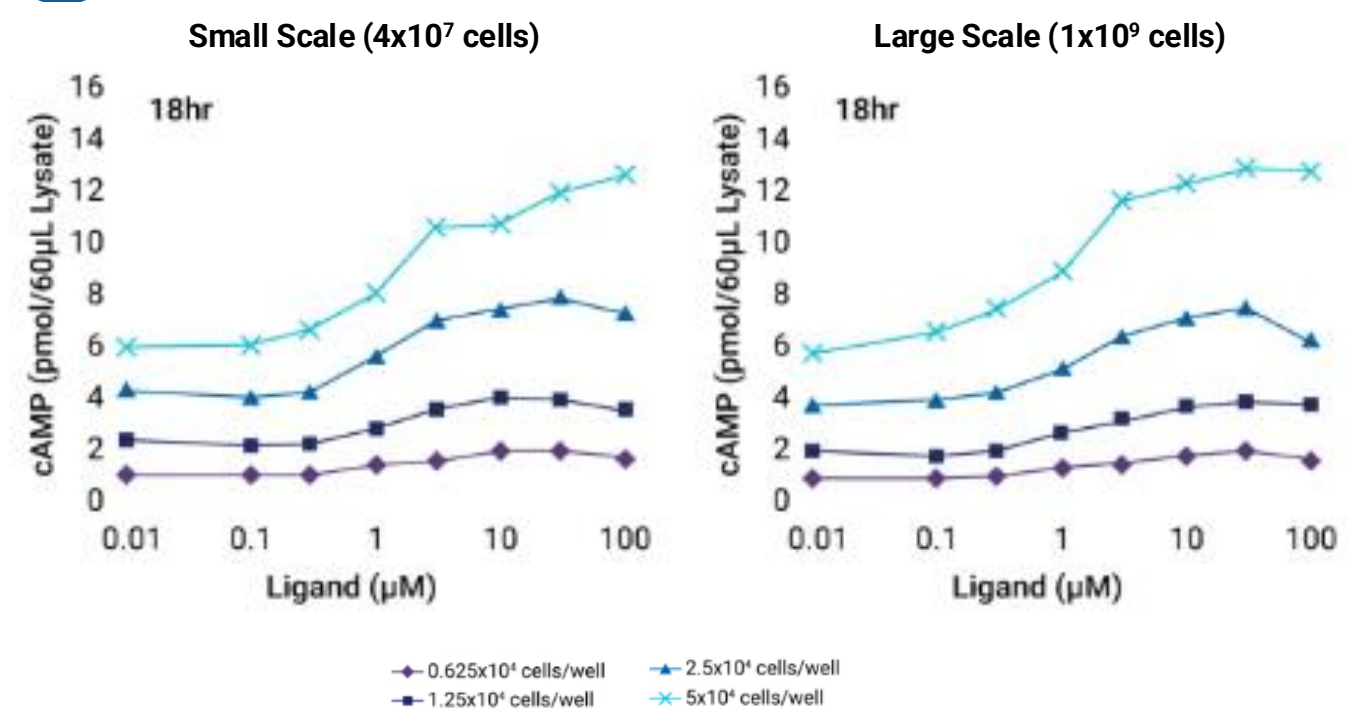


Figure 2: Consistent assay performance between cells prepared by small or large-scale electroporation. HEK 293F suspension cells were transfected with a GPCR expression plasmid using either electroporation at either small-scale (4x10⁷ cells) or large-scale (1x10⁹ cells). Post electroporation, cells were seeded in 96-well plates at the indicated cell density. GPCR activity was assayed using a cAMP ELISA at 18 hours post transfection. The data demonstrates consistent performance in cells transfected at both small and large scale by MaxCyte electroporation.

3 MaxCyte Transiently Transfected Cells are Suitable for Calcium Channel Studies

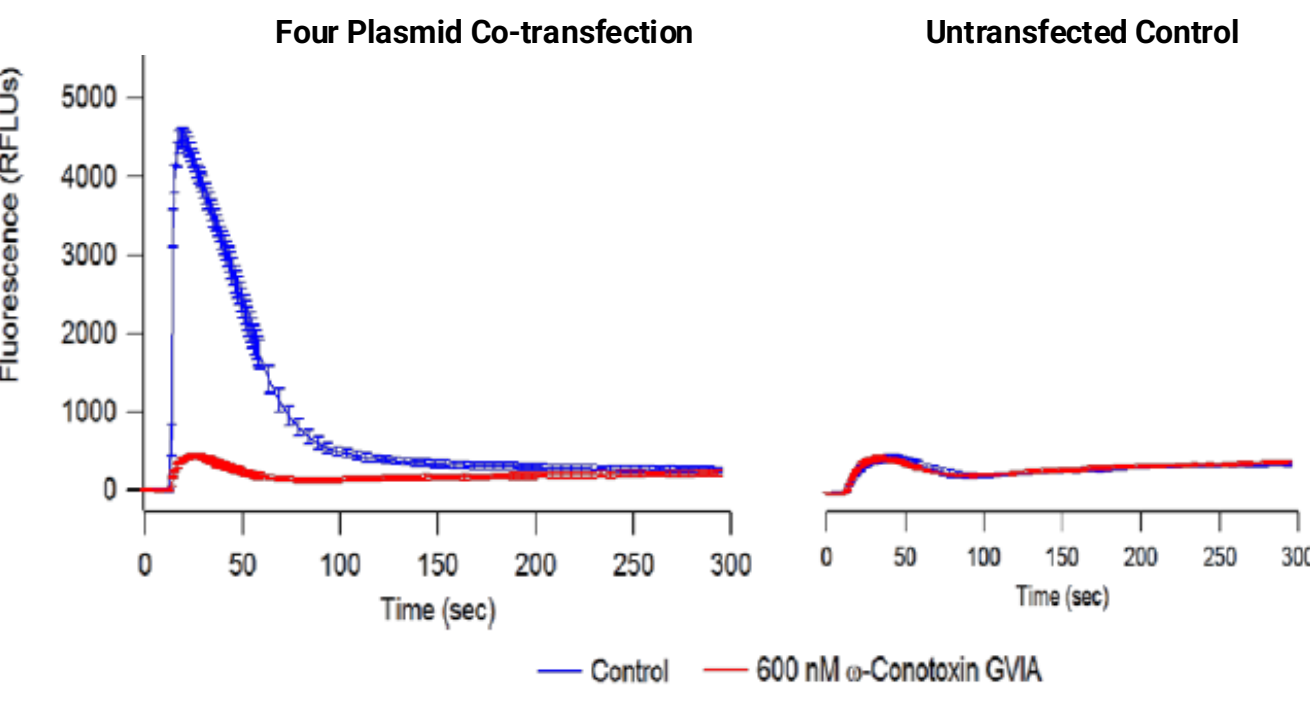


Figure 3: Mean fluorescent signal in K⁺ stimulated cells treated with a calcium channel antagonist. HEK 293 cells were either transfected (A) with the Cav2.2 pore-forming alpha subunit (5-6kb cDNA) and two modulatory subunits, (the modulatory β3 subunit, the modulatory α2δ subunit) and an inward rectifier potassium channel, Kir2.1, or untransfected (B). At 24 hours post electroporation, cells were depolarized with high external potassium, then treated, or untreated, with the calcium channel antagonist, conotoxin and FLIPR® assays were carried out. Together, the results confirm that the signal seen in panel A is due to calcium channel activity, confirming that MaxCyte transiently transfected cells are suitable for calcium channel studies.

Data courtesy of ChanTest, now part of Charles River Laboratories.

4 High Throughput Screening for Compounds that Restore Functional CFTR Expression

Mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR) may disrupt the function of an ion channel involved in hydration maintenance, leading to cystic fibrosis. A stop mutation (G542X) in the CFTR gene is present in around 5% of cystic fibrosis patients. Aminoglycosides, such as G418, promote translational readthrough of non-sense mutations, including the G542X mutation. A high-throughput screening method was developed to identify compounds that promote readthrough of the G542X stop mutation, potentially restoring functional CFTR expression

A) Preparation of Assay Ready Cells for Rapid Assay Development

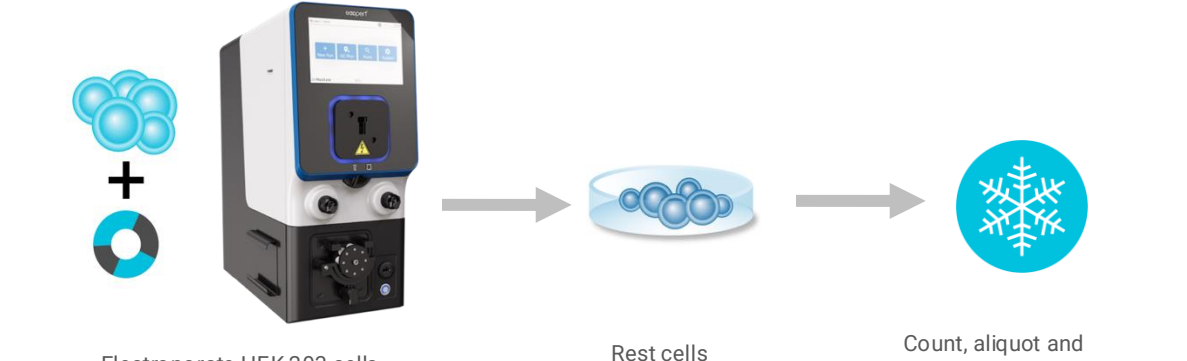
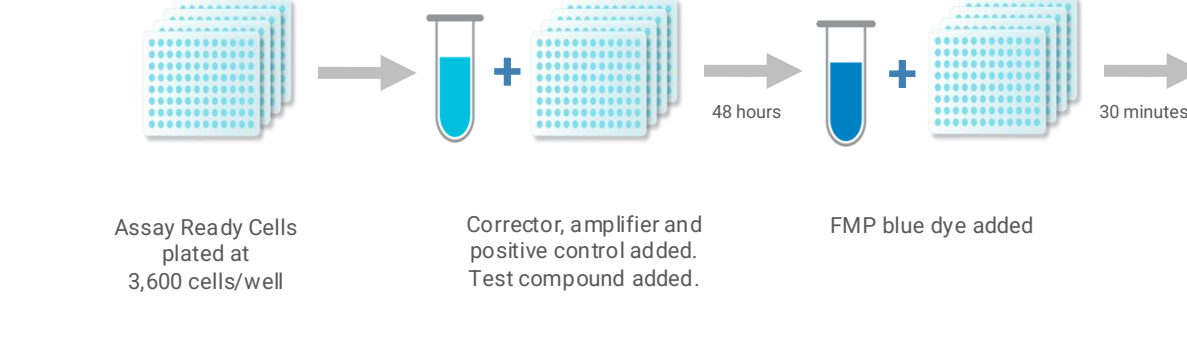


Figure 4: HEK 293 cells were transfected with a plasmid encoding a CFTR minigene including the G542X stop mutation. A large stock of matched, validated Assay Ready Cells was prepared and cryopreserved (Figure 4A) to enable rapid assay development, optimization, initial validation and miniaturization. Assay Ready Cells were used to develop the High-Throughput Screening (HTS) assay in 1536-well format (Figure 4B) while a stable cell line was developed.

B) Optimized High-Throughput Screening Assay Workflow



C) MaxCyte Assay Ready Cells Outperform Lipid Transfection

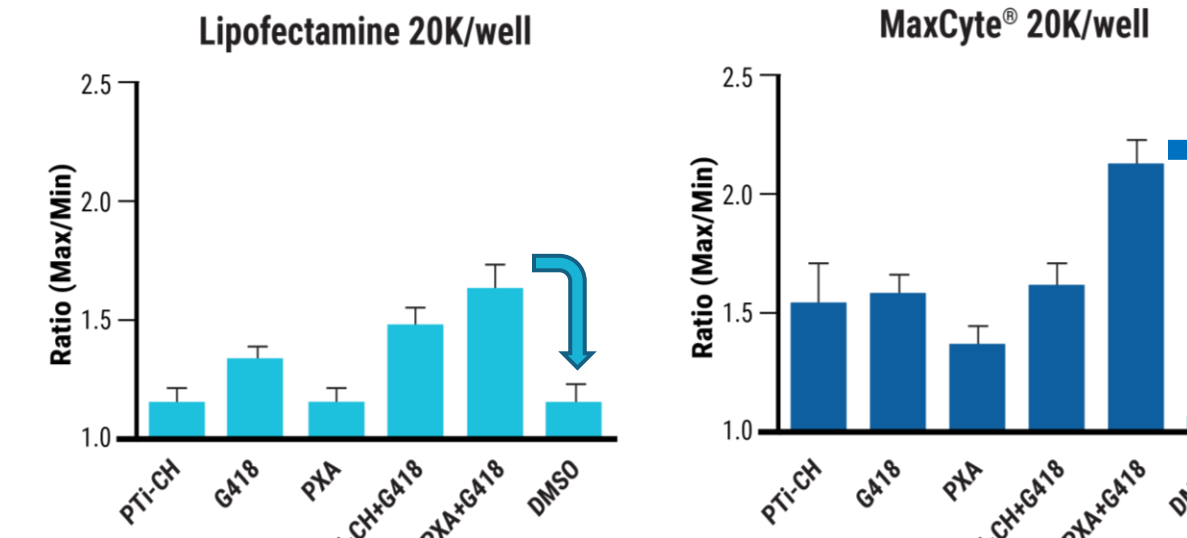
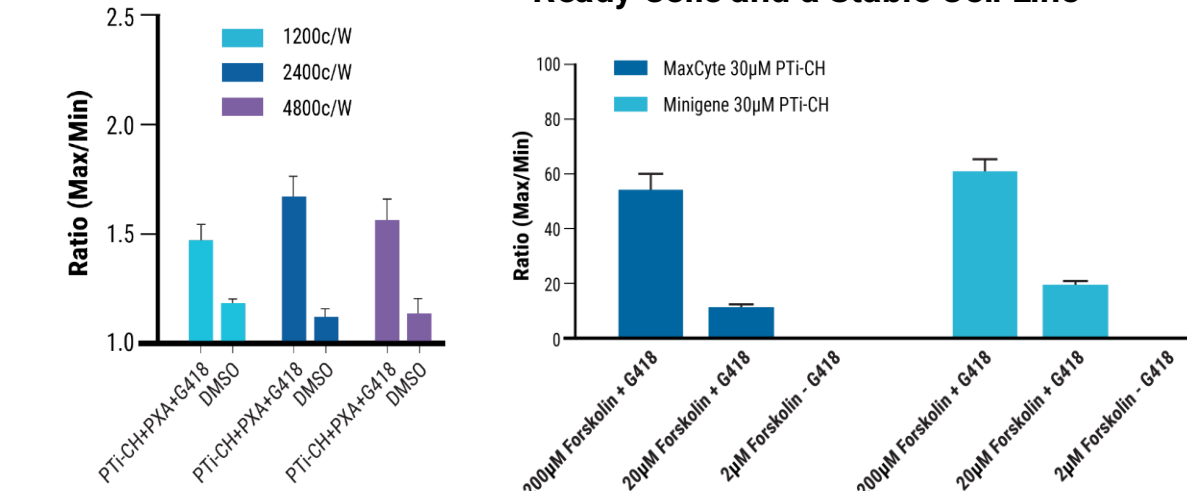


Figure 4 (continued): HEK cells transfected with the CFTR G542X minigene using either lipid or MaxCyte electroporation were compared in the initial 384-well assay format. Transfected cells were treated with combinations of PT-CH (amplifier), VX-809 (corrector) and G418 (positive control) or DMSO (negative control). MaxCyte electroporated cells had a larger assay window than lipid transfected cells (Figure 4C). MaxCyte electroporation was thus used to prepare Assay Ready Cells for cost-effective assay development, miniaturization and optimization.

D) Assay Miniaturization



E) Comparable Performance of Assay Ready Cells and a Stable Cell Line

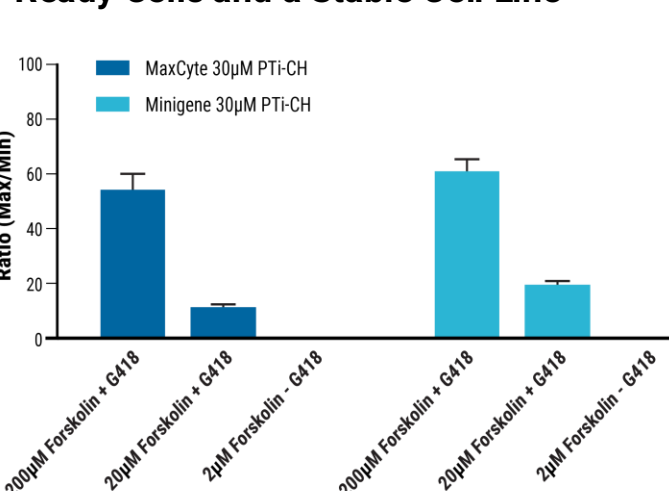


Table 1: MaxCyte Flow Electroporation Could Enable HTS of the Scripps SDDL

Screening Round	Cells/Well	Sample #	Doses	Replicates	Total Cell #
Pilot	3,600	1,280	1	3	1.38x10 ⁷
Primary	3,600	666,120	1	1	2.40x10 ⁹
Confirmation	3,600	6,395	1	3	6.91x10 ⁷
Counter	3,600	6,395	1	3	6.91x10 ⁷
Titration 1	3,600	652	10	3	7.04x10 ⁷
Titration 2	3,600	652	10	3	7.04x10 ⁷
Titration 3	3,600	652	10	3	7.04x10 ⁷
Total Cell # Needed for HTS Campaign					2.76x10 ⁹

References

- Smith E, Dukovski D, Shumate J, Scampavia L, Miller JP, Spicer TP. Identification of Compounds That Promote Readthrough of Premature Termination Codons in the CFTR. *SLAS Discov*. 2021 Feb;26(2):205-215. doi: 10.1177/2472555220962001. Epub 2020 Oct 5. PMID: 33016182; PMCID: PMC7838340.
- Vanoye CG, Desai RR, Ji Z, Adusumilli S, Jairom N, Ghabra N, Joshi N, Fitch E, Helbig KL, McKnight D, Lindy AS, Zou F, Helbig T, Cooper EC, George AL Jr. High-throughput evaluation of epilepsy-associated KCNQ2 variants reveals functional and pharmacological heterogeneity. *JCI Insight*. 2022 Mar 8;7(5):e156314. doi: 10.1172/jci.insight.156314.

Corresponding Author: James Brady, jamesb@maxcyte.com, MaxCyte, Inc., Tel: (301) 944-1700 info@maxcyte.com • www.maxcyte.com
©2024 MaxCyte, Inc. All Rights Reserved. MaxCyte®, EXPERT ATx®, EXPERT GTx®, EXPERT STx®, and Flow Electroporation® are trademarks of MaxCyte, Inc. registered in the U.S. Patent and Trademark Office. EXPERT™, GTx™, ATx™, and STx™ are trademarks of MaxCyte, Inc. FLIPR® is a registered trademark of Molecular Devices LLC, Sunnyvale, CA, US (a subsidiary of Danaher Corp.)

5 MaxCyte Electroporation Enables High-Throughput Evaluation of Ion Channel Variants

K_v7 is a family of tetrameric voltage-gated potassium channels encoded by five *KCNQ* genes expressing KCNQ1-5. The KCNQ2 subunit (Q2) can co-assemble with KCNQ3 (Q3) to form heterotetramers widely expressed in the nervous system. Hundreds of Q2 genetic variants are associated with epilepsy and developmental challenges, a fraction of these have been studied. Researchers from the George Lab (Northwestern University) developed and validated a method for the high-throughput functional evaluation of ion channel variants. Here we share their evaluation of 39 previously unstudied epilepsy-associated Q2 variants and their responses to a candidate therapeutic. MaxCyte electroporation was used to produce Assay Ready Cell banks suitable for automated patch clamping, enabling ion channel variant characterization on an unprecedented scale.

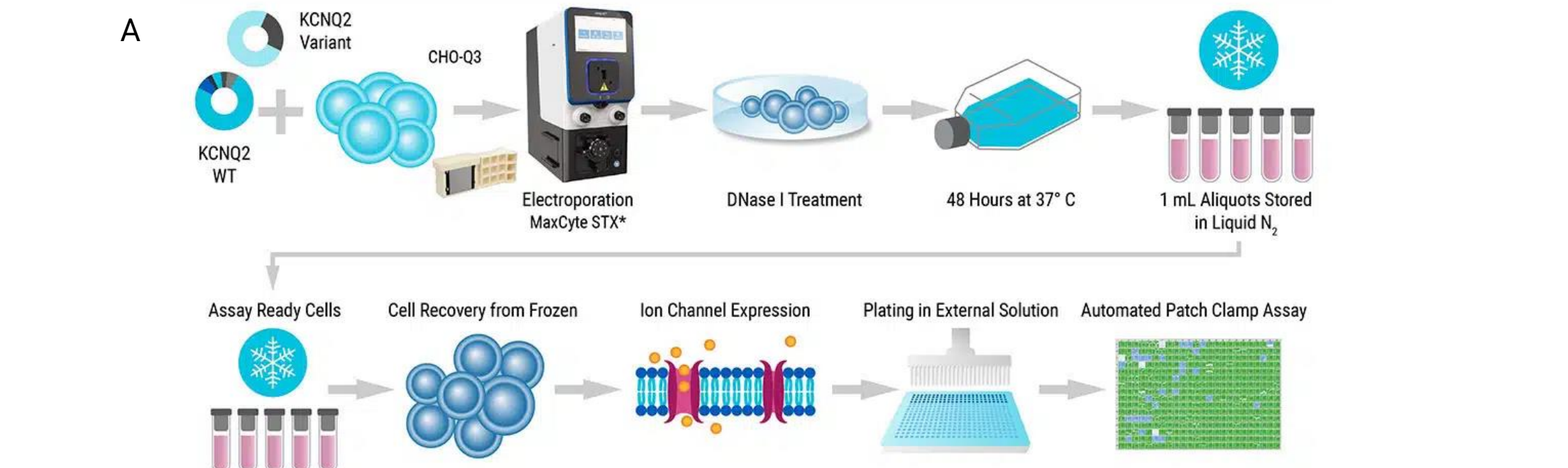
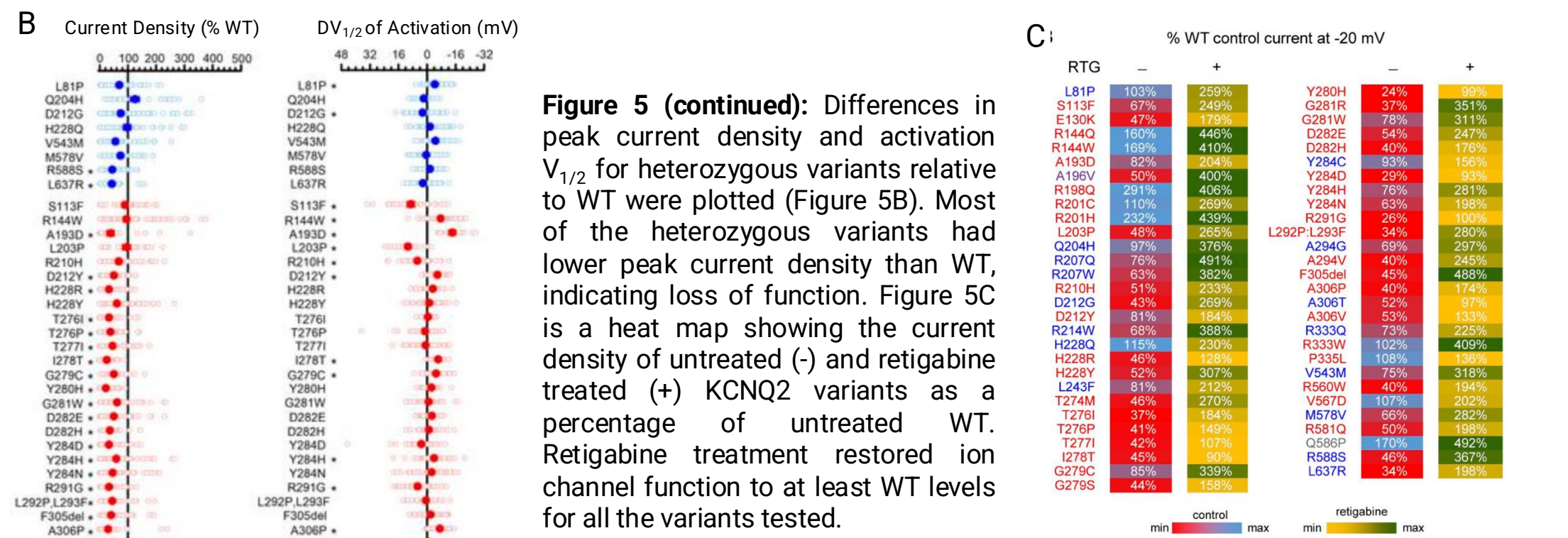


Figure 5: Workflow for Preparation of Assay Ready Cells and Evaluation of KCNQ2 Variants (Figure 5A). CHO-Q3 cells (stably express Q3 subunit) were electroporated with plasmids expressing Q2 variants to generate homozygous (variant Q2 alone) or heterozygous (variant Q2 + WT Q2; 1:1 ratio) Assay Ready Cell banks which were cryopreserved until needed. Assay Ready Cells were thawed and incubated for 10 hours at 37°C with 5% CO₂, then grown overnight at 28°C to increase membrane ion channel expression. Cells were passaged with 5% trypsin then diluted to 200,000 cells/mL in external solution and incubated for 60 minutes at 15°C with shaking. Automated patch clamp recordings were performed.



²Content adapted and reproduced from Vanoye et al JCI Insight. 2022 under Creative Commons License Attribution 4.0 International (CC BY 4.0)

Channel	Cell Type	Efficiency	EP Protocol	# Constructs	Notes
KCNQ1 (K _v 7.1)	CHO (stable KCNE1)	>90%	CHO-PE	291	Includes WT co-expression
KCNQ2 (K _v 7.2)	CHO (stable K _v 7.3)	>90%	CHO-PE	250	Includes WT co-expression
KCNH1 (K _v 10.1, eag1)	HEK293T	>90%	6	64	Includes WT co-expression
SCN1A (Nav1.1)	HEK293T	80-90%	6	61	Includes 1 splice isoform
SCN2A (Nav1.2)	HEK293T	~70%	4	225	Includes 2 splice isoforms
SCN4A (Nav1.4)	ND7/23-LoNav	80-95%	ND7		
SCN5A (Nav1.5)	HEK293T	85-95%	HEK		
SCN8A (Nav1.6)	ND7/23-LoNav	75-85%	4 or 6	35	Includes 2 splice isoforms
KCNE1				125	Includes WT co-expression
KCNB1 (K _v 2.1)				52	Includes WT co-expression

Table 2: Summary of ion channels variants (>1,100 constructs) studied by the George Lab (Northwestern University) using MaxCyte electroporated, transiently transfected Assay Ready Cells. Data reproduced courtesy of Professor Alfred George, Jr., Department of Pharmacology, Northwestern University Feinberg School of Medicine.

6 Rapid Development of Assay Ready Cells for hNav1.5 Studies

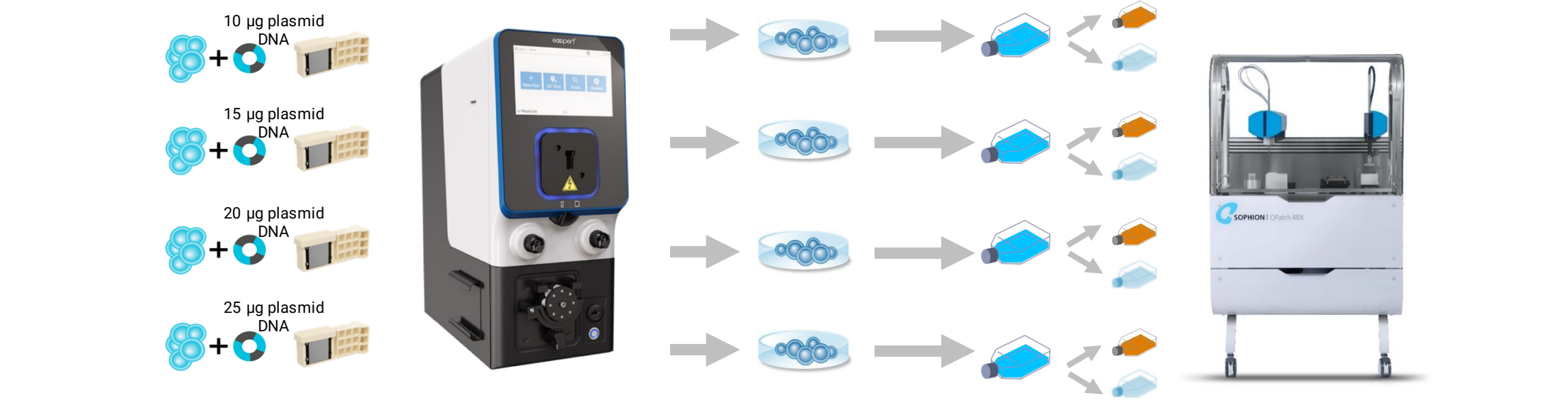


Figure 6: Workflow for Optimizing DNA Concentration and Cell Culture Conditions. HEK 293 cells were transfected with four different concentrations of plasmid encoding the human Nav1.5 ion channel (courtesy of Thomas Jespersen at Copenhagen University) using the MaxCyte scalable electroporation system. After transfection, cells were rested for 20-30 minutes then cultured at 37°C for 24 hours. Half of the cells were then transferred to 28°C for while the other half remained at 37°C until harvest. Cells were screened on the Sophion QPatch II in single hole mode.

DNA concentration (µg/mL)	Temperature (°C)	Transfection efficiency (%)	Average current level (nA)*	TTX block (%)**
100	28	57	-6.3	65
150	28	100	-5.7	71
200	28	80	-3.6	82
250	28	75	-3.5	86
100	37	82	-2.5	77
150	37	89	-2.0	79
200	37	83	-3.8	79
250	37	42	-3.0	82

*measured @ 0 mV in simple depolarizing step protocol **percentage block compared to saline period of a 20 µM TTX single addition

Table 3: Optimized DNA Concentration and Culture Temperature. A DNA concentration of 150 µg/mL was chosen to give optimal results. MaxCyte electroporation efficiently delivered the relatively large Nav1.5 gene to cells which were then suitable for use in automated electrophysiology studies. The current amplitude from cells incubated at 37°C is large enough to enable future studies to be carried out at a physiological temperature, the lower temperature of 28°C consistently produced larger peaks, which might be a useful attribute when studying low expressing channels.

Freshly Electroporated Cells			Cryopreserved Cells	
Temperature (°C)	Average current level (nA)*	TTX block (%)**	Average current level (nA)*	TTX block (%)**
28	-6.5	85	-5.4	81
37	-5.2	85	-4.8	79

*measured @ 0 mV in simple depolarizing step protocol **percentage block compared to saline period of a 20 µM TTX single addition

Table 4: Comparison of Fresh and Cryopreserved Assay Ready Cells for Automated Patch Clamp Screening HEK293 cells (four samples of 4x10⁷ cells) were electroporated with 150 µg/ml of the Nav1.5 expression construct using the MaxCyte scalable electroporation system. After resting, transfected cells were cultured in four T175 flasks at 37°C for 24 hours. Two flasks were then transferred to 28°C and two flasks were maintaining at 37°C. After 48 hours in final culture, the cells were tested on the QPatch II or aliquoted and frozen down according to Sophion's recommended cryopreservation strategy (QGUIDE 16153). The frozen cells were subsequently revived and tested on the QPatch II. The overall success rate on the QPatch II was 61% for the "fresh" cells and 60% for the "frozen" cells effectively showing no significant difference.

Conclusion: This study demonstrates that MaxCyte electroporation produces efficiently transfected cells compatible with QPatch II technology. MaxCyte electroporated Assay Ready Cells can be generated within 3 days post transfection. Cryopreserved Assay Ready Cells can be assayed on the QPatch II immediately after thawing with no significant decrease in performance. The compatibility of MaxCyte electroporated Assay Ready Cells with the QPatch II system enables researchers to efficiently generate and screen ion channel expressing cells without stable cell line generation.

Data reproduced courtesy of Sophion with thanks to Daniel Sauter.

Summary

- MaxCyte electroporation efficiently transfects cell lines, primary human cells and stem cells
- Transient transfection with MaxCyte electroporation enables simplified expression of complex, multi-subunit or toxic proteins
- MaxCyte electroporation enables scalability from assay development to compound library screening without re-optimization
- Transiently transfected, Assay Ready Cells are a suitable alternative to stable cell lines for ion channel research, assay development and drug discovery, decreasing reliance on costly stable cell lines