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Advancing High Throughput Screening of CFTR Modulators with Human Epithelial Cell Lines

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ABSTRACT

Human epithelial cell lines were adapted to the iodide flux HTS assay: A549 cells stably transfected with YFP and CFTR-F508del (Pedemonte et al., 2010, AJP 298), and CFBE41o- transiently transfected with YFP and CFTR-F508del. For CFBE41o- cells, a suitable stably transfected cell line has not been available. We introduced a high volume transient transfection method (STX by MaxCyte) for the CFBE41o- cells to express YFP and CFTR-F508del for the HTS. This method allows plasmid titration in order to achieve desirable expression levels and expression ratios of YFP and CFTR-F508del for best assay signals, with good transfection efficiency and cell viability. This method also demonstrated consistency across experiments and for various freezing durations. The high transfection volume and the consistent transfection quality make this method a suitable approach for the HTS. Data generated from FRT, A549, and from CFBE cells against a set of selected compounds are compared, showing the benefits of using human epithelial cells in the HTS assay. Advancing the iodide flux assay on the HTS platform from Fisher Rat Thyroid cells to human epithelial cells is a significant step towards the ideal CF cellular model. The human cell-based iodide flux assays are currently the primary HTS methods in the CFTR modulator screening campaign at Flatley Discovery Lab.

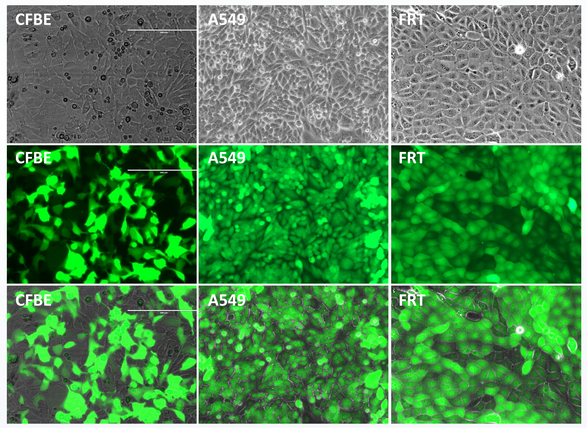


Figure 1. Sample microscope images of transfected CFBE, A549, and FRT cells in phase-contrast view (top panel), GFP view (middle panel), and merged view (lower panel), showing the expression of YFP in the cells under the conditions where they are ready for the experiments

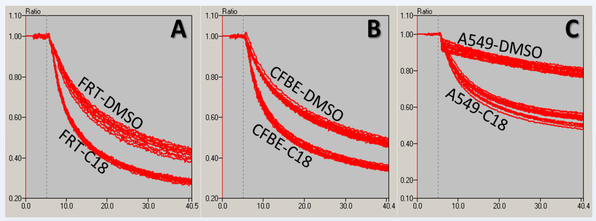


Figure 2. Sample records of YFP signals from FRT cells (A), CFBE cells (B), and A549 (C) cells, showing traces from negative control wells (cells were treated with DMSO only, DMSO) and from positive control wells (cells were treated with C18 for 24-hours, C18) respectively.

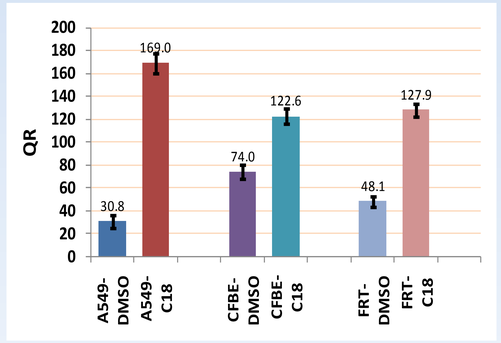


Figure 3. Comparison of YFP quenching rate (QR) signal windows between negative control (DMSO) and positive control (C18, 10 um) among the three tested cell lines (FRT, A549, and CFBE) in the YFP assay

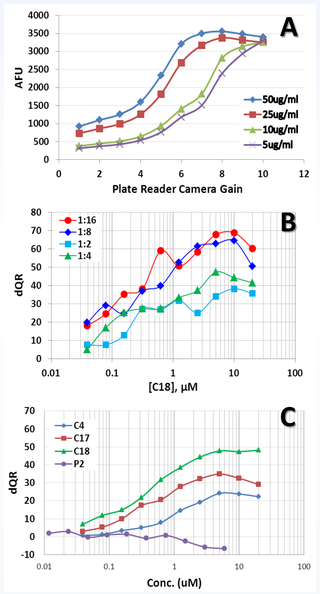
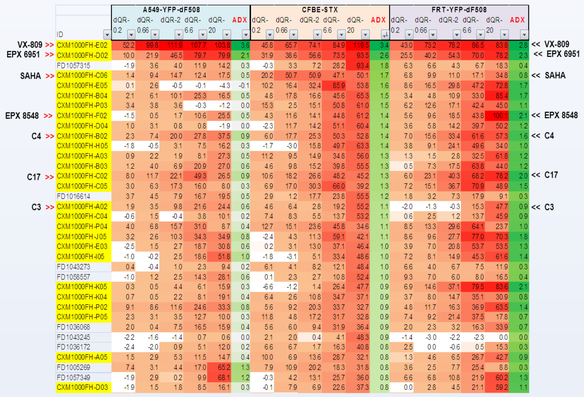


Figure 4. Plasmid titration for YFP and YFP:dF508 ratio in CFBE cell transfection

A. YFP plasmid titration experiment showing YFP signal varied with plasmid concentration used in MaxCyte STX transfection. 5μg/ml was selected in our following experiments. B. YFP:dF508 plasmid titration results, showing that increasing dF508 plasmid from 1:2 to 1:8 enhanced the assay signal; further increase of dF508 plasmid brought no further signal enhancement. C. Sample dose-response plots of control compounds (C4, C17, C18 and P2) tested with cells transfected with 5μg/ml YFP plasmid and 40μg/ml dF508 plasmid.



	ID	A549-YFP-dF508 dQR 0.2	dQR 0.66	dQR-2	dQR 6.6	dQR 20	ADX	CFBE-STX dQR 0.2	dQR 0.66	dQR-2	dQR 6.6	dQR 20	ADX	FRT-YFP-dF508 dQR 0.2	dQR 0.66	dQR-2	dQR 6.6	dQR 20	ADX	
VX-809 >>	CXM1000FH-E02	52.2	99.0	111.9	107.7	103.8	3.8	45.6	65.7	74.1	84.9	116.5	3.4	43.0	75.2	78.2	86.5	83.8	2	<< VX-809
EPX 6951 >>	CXM1000FH-D02	10.0	21.9	46.5	79.7	79.9	2.1	31.9	38.6	56.6	73.5	93.5	2.6	25.5	40.2	54.3	70.0	78.2	2	<< EPX 6951
	FD1007315	-1.9	3.6	9.8	11.9	14.2	0.3	-0.3	3.3	7.2	28.2	93.4	1.8	6.3	6.6	4.3	6.7	18.3	0.4	
SAHA >>	CXM1000FH-C06	1.4	9.4	14.7	12.4	17.5	0.5	20.2	50.7	50.9	47.1	50.1	1.7	6.8	9.9	11.0	17.1	34.8	0.9	<< SAHA
	CXM1000FH-E05	0.1	2.6	-0.5	-0.1	-4.3	-0.1	10.2	16.4	32.4	65.9	53.8	1.6	8.6	16.5	29.8	47.2	72.8	1.7	
	CXM1000FH-B04	2.7	6.1	10.1	25.3	16.8	0.5	4.8	17.8	16.6	45.6	65.3	1.5	3.4	4.8	10.9	33.0	85.4	1.7	
	CXM1000FH-P03	3.4	3.8	3.6	-0.3	-1.2	0.0	15.3	2.5	15.1	50.8	61.0	1.5	6.2	12.6	17.1	42.4	45.0	1.1	
EPX 8548 >>	CXM1000FH-F02	-1.5	0.5	1.7	10.6	25.6	0.5	4.3	11.6	14.1	44.6	61.2	1.4	5.6	9.6	16.5	43.6	100.1	2	<< EPX 8548
	CXM1000FH-D04	1.0	3.1	0.6	0.6	-1.9	0.0	-2.3	11.7	14.2	51.1	60.4	1.4	3.6	5.8	14.2	39.7	50.2	1.3	
C4 >>	CXM1000FH-B02	2.3	7.4	20.0	27.8	37.5	0.9	6.0	17.7	25.3	50.3	52.8	1.4	7.0	15.6	33.4	61.6	57.3	1.5	<< C4
	CXM1000FH-H05	-1.8	-0.5	3.1	7.5	16.2	0.3	-1.7	-3.0	15.8	49.7	63.3	1.4	3.8	9.1	24.1	49.6	34.0	1.0	
	CXM1000FH-A03	0.9	2.2	1.9	6.1	27.3	0.5	11.2	9.5	14.9	34.8	56.0	1.3	1.3	1.5	2.8	32.5	61.8	1.2	
	CXM1000FH-B03	1.2	4.0	6.9	20.9	27.0	0.6	4.6	9.8	15.2	39.8	55.5	1.3	0.5	7.3	17.5	63.8	44.0	1.2	
C17 >>	CXM1000FH-C02	8.0	11.7	22.1	49.3	26.5	0.9	10.6	18.2	26.6	48.2	45.2	1.3	6.0	23.1	40.3	68.2	78.2	2.0	<< C17
	CXM1000FH-C05	3.0	6.3	17.3	16.0	8.0	0.3	6.9	17.0	30.3	66.0	39.2	1.3	7.2	15.1	36.7	70.9	48.9	1.5	
	FD1018614	3.7	4.5	7.9	16.7	19.5	0.5	2.9	1.2	12.7	23.6	55.5	1.2	1.8	3.2	7.3	17.9	9.1	0.3	
C3 >>	CXM1000FH-A02	1.9	3.5	9.8	21.6	24.4	0.6	4.6	6.4	2.8	19.2	55.2	1.1	-2.0	-1.3	-0.3	15.3	47.7	0.9	<< C3
	CXM1000FH-C04	-0.6	1.5	-0.4	3.8	10.1	0.2	7.4	8.3	5.5	13.7	53.2	1.1	0.6	2.5	1.2	13.7	45.9	0.9	
	CXM1000FH-P04	4.0	6.8	15.7	31.0	8.7	0.4	12.7	15.1	23.6	45.8	34.6	1.1	8.5	13.3	29.6	64.1	23.7	1.0	
	CXM1000FH-H06	3.2	2.6	10.3	34.3	34.9	0.8	2.4	4.3	11.3	59.5	64.1	1.1	8.6	9.6	27.7	77.0	70.3	1.8	
	CXM1000FH-E03	-2.5	1.5	2.7	18.7	30.8	0.6	0.2	3.1	13.0	37.1	46.4	1.0	3.9	7.0	20.8	53.7	53.5	1.3	
	CXM1000FH-H05	-1.0	-0.2	2.5	18.6	51.8	1.0	-1.8	-3.1	5.1	33.4	49.6	1.0	7.2	8.1	14.9	45.3	61.6	1.4	
	FD1043273	0.4	-0.4	1.0	2.3	9.4	0.2	6.1	4.1	8.2	12.1	48.4	1.0	6.6	4.0	6.7	7.5	11.9	0.3	
	FD1036557	-1.0	1.2	2.5	14.3	28.1	0.6	0.1	2.3	2.7	10.8	42.4	1.0	9.3	7.0	6.0	8.0	16.5	0.4	
	CXM1000FH-A05	0.3	0.5	4.4	6.1	15.9	0.3	-6.6	-1.2	1.4	26.4	47.7	0.9	6.9	14.6	37.1	79.5	83.8	2.1	
	CXM1000FH-K04	0.7	0.5	2.2	8.1	19.1	0.4	6.4	2.6	10.8	34.7	37.1	0.9	3.7	8.0	14.7	35.1	30.9	0.8	
	CXM1000FH-P02	9.1	8.6	11.6	24.6	33.3	0.8	5.6	9.2	20.3	33.7	32.7	0.9	4.8	11.7	16.3	36.9	63.5	1.4	
	CXM1000FH-P06	2.3	3.1	3.5	12.7	10.0	0.3	11.8	4.8	17.2	31.7	32.8	0.9	7.4	9.2	21.4	37.5	17.8	0.7	
	FD1036068	2.0	0.4	7.5	16.5	15.9	0.4	5.6	6.0	9.4	31.9	36.4	0.9	2.0	2.3	3.2	16.3	33.9	0.7	
	FD1043245	-2.2	-1.6	-1.4	0.7	0.6	0.0	2.1	2.0	0.4	4.1	45.3	0.9	-1.4	-3.0	-2.2	-2.3	0.0	0.0	
	FD1036172	-2.4	-2.0	0.9	5.1	12.0	0.2	6.6	6.7	1.7	16.3	40.8	0.9	2.6	0.0	-0.6	0.5	15.3	0.3	
	CXM1000FH-A05	1.5	2.9	5.3	11.5	14.7	0.4	10.0	6.9	13.6	28.7	32.1	0.8	1.3	4.6	6.5	26.7	42.7	0.9	
	FD1006269	7.4	3.1	4.4	17.0	65.2	1.3	7.9	10.9	20.2	18.3	31.8	0.8	3.6	2.5	7.7	25.4	8.8	0.3	
	FD1037340	-1.9	2.9	0.2	9.9	68.1	0.8	-0.3	4.2	13.1	25.7	36.0	0.8	6.6	6.8	10.8	21.9	60.2	1.3	
	CXM1000FH-D03	-1.9	1.5	1.8	8.5	16.1	0.3	-0.1	7.9	6.9	22.6	37.9	0.8	0.0	2.8	4.5	21.1	59.2	1.1	

Figure 6. CFTR Corrector validation hits

Table data is sorted by ADX of CFBE cell data, from a sample data set from the iodide flux HTS assay with three different cell lines (FRT, A459, and CFBE41o-), showing the top performers in the assay including VX-809, C18, C17, SAHA, C4.

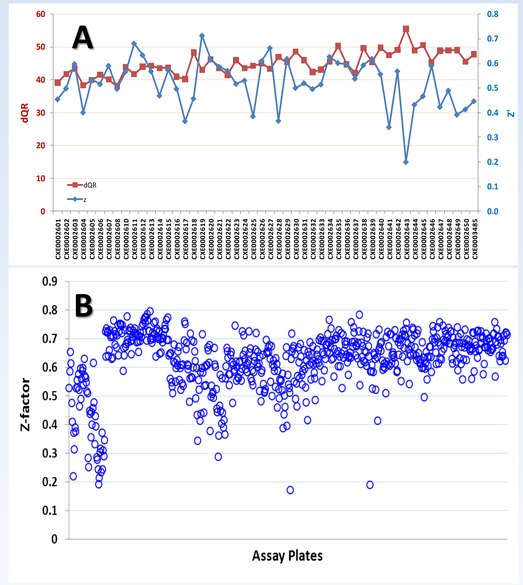


Figure 5. CFBE41o- iodide flux assay performance in one experiment and across multiple experiments.

A. plot of differential quenching rate (dQR) and z-factors from one experiment set of assay plates with CFBE41o- cells expressing YFP & CFTR-F508del transiently transfected with MaxCyte STX; B. z-factor plot of 672 assay plates across multiple experiments.

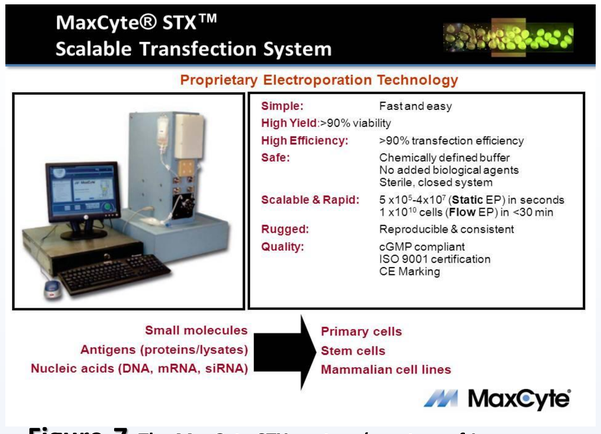


Figure 7. The MaxCyte STX system (courtesy of James Brady from MaxCyte Inc.)

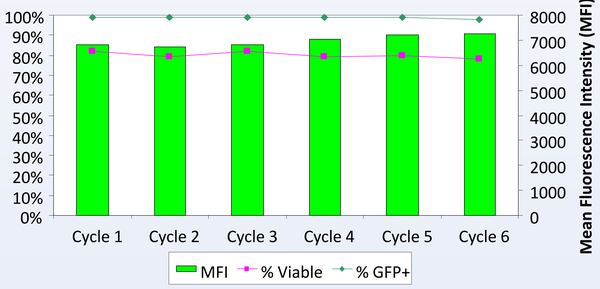


Figure 8. Large Scale transfection of GFP with CFBE41o- cells

EP protocol = "A549". [DNA] = 100 ug/mL, [cell] = 3e7/mL. FACS Data 24 hrs post electroporation (courtesy of James Brady from MaxCyte Inc.).

INTRODUCTION

The YFP-based iodide influx assay has been widely deployed in high-throughput screening (HTS) for small molecule CFTR modulators. It's a robust cell-based assay, developed with Fisher Rat Thyroid (FRT) cells expressing YFP and CFTR-F508del (Pedemonte et al., 2005, JCI 115; Sui et al., 2010, ADDT 8).

It has been highly desirable to use human epithelial cells replacing FRT cells in this assay to eliminate concerns over the species gap between rat and human. It may significantly boost our chance of finding clinically relevant HTS hits in our efforts seeking small molecule CFTR modulators. This work is to report our work along this line using A549 and CFBE cells, both of which are human bronchial cell lines, to replace FRT cells.

MATERIALS & METHODS

FRT cells and A549 cells stably expressing YFP and CFTR-F508del were kindly provided by Dr. Galietta. High volume transient transfection for HTS was developed with MaxCyte STX (see pictures 7 & 8). CFBE41o- cells were transfected and frozen immediately. Freshly thawed cells were seeded in assay plates and treated with testing compounds following the CFTR corrector protocol for the iodide influx assay (Sui et al, ADDT 2010).

YFP data analysis, HTS data quality control, and the HTS and chemical library data management software system were developed in-house. YFP signal quenching rate (QR) was derived by a non-linear curve fitting to the equation

$$f(t) = (A_0 - A_1)e^{-tQR} + A_1$$

Differential quenching rate (dQR) is given by dQR=QR-QR_{NC}, where QR_{NC} is the mean QR of the DMSO control wells in the assay plate. For each compound with the results of all three (0.2μM, 2μM, and 20μM) or five (0.2μM, 0.66μM, 2μM, 6.6μM, and 20μM) testing doses, the activity index (ADX) is calculated with the formula $ADX = \sum_{k=1}^{n}(A_k - A_{k-1})[-log(C_k)]$, where A_k is the activity measurement, C_k is the testing concentration.

CONCLUSION

- At Flatley Discovery Lab (FDL), we have adopted several human epithelial cell lines for the iodide flux HTS assays.
- A high volume transient transfection method (STX by MaxCyte) has been established to express YFP and CFTR-F508del in the human bronchial cells for the HTS assays. This method allows plasmid titration in order to achieve desirable expression levels, with consistently high transfection efficiency and cell viability.
- In the CFTR modulator screening campaign at FDL, we are using the human bronchial cells in the iodide flux HTS primary assays, and we have screened several hundreds of thousands of library compounds. This effort has yielded several interesting hits.

Acknowledgements

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References

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Seeking A Cure for Cystic Fibrosis