Identification of Compounds that Promote Readthrough of Premature Termination Codons in the CFTR

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Supplement Table 1: CFTR Readthrough Initial 1536 Issues to Address

Step	Issue for 1536 well implementation
1	*Transient transfected cells need to be made, since no stable cell line is available*
2	*Cell number needs optimized for 1536 well format*
3	*Aspiration is slow and variable in 1536 well format (not addressed, just eliminated)*
4	Must use either FLIPR pintool or 1536 well pipettor. Addition concentrations will need to be optimized. (Pintool did not work – data not shown, proceeded to pipettor)
5	Amplifier and stimulation concentration optimization
6	Incubation time needs to be optimized
7	Read times will be optimized after seeing kinetic traces of the responses

Supplement Table 2: CFTR Readthrough 1536 well Protocol

Step	Parameter	Value	Description
1	*Cell Plating*	3µl/well	3,600c/w, spin
2	Dispense PTI-CH, VX-809 and G418	1µl/well	Corrector, amplifier and positive control
3	Compound addition	30nl/well	Pintool addition
4	Compound incubation	48 hours	*37°C, 99% R.H., 5% CO2*
5	FMP Blue dye addition	3µl/well	Dispenser addition in assay buffer
6	Dye incubation	30 minutes	*37°C, 99% R.H., 5% CO2*
7	FLIPR Pre-Pin with basal read 1	5 seconds	Average baseline read on FLIPR
8	Forskolin and Genistein addition	2µl/well	1536 FLIPR Pipettor dispense
9	Forskolin incubation	30 minutes	RT in the dark
10	FLIPR Read 2	5 seconds	Average 30 minute read on FLIPR
11	Data analysis	510/545nm	Read 2/read 1