

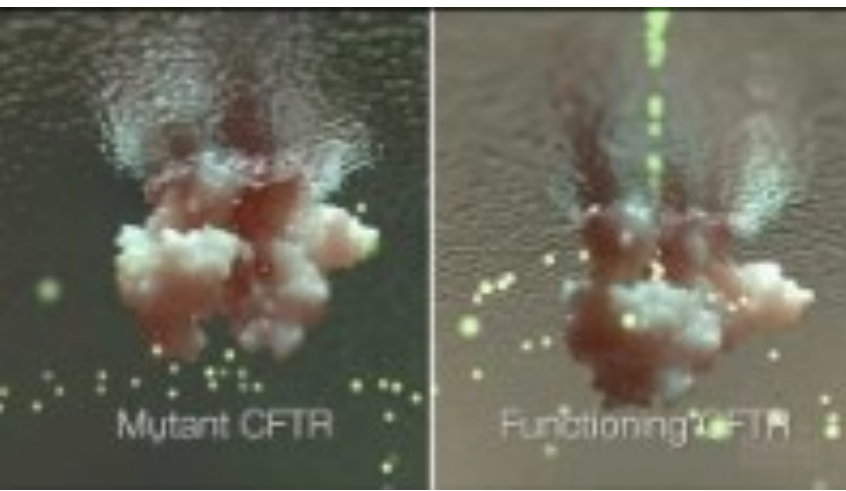
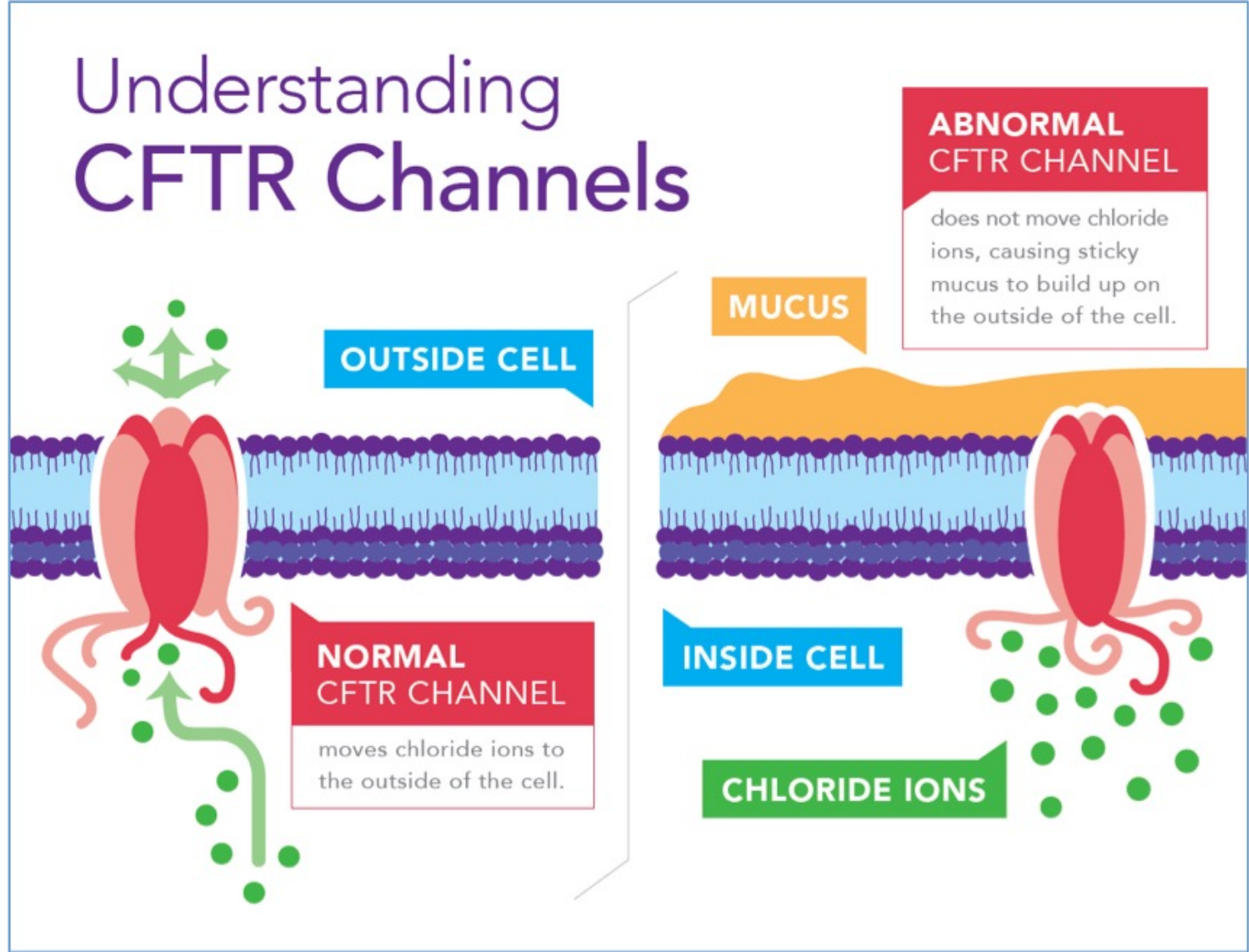
Università di Torino
Molecular Biotechnology Center

Il saggio della YFP per testare la funzione del CFTR e la risposta ai modulatori

Angela Della Sala, Ph.D.

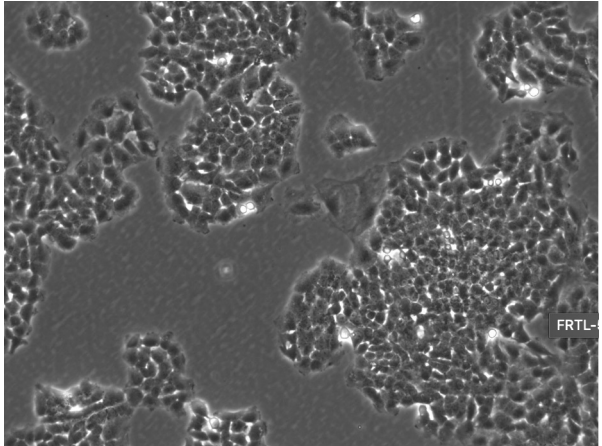
University of Torino
Molecular Biotechnology Center
Dept. of Molecular Biotechnology and Health Sciences
angela.dellasala@unito.it

Understanding CFTR Channels



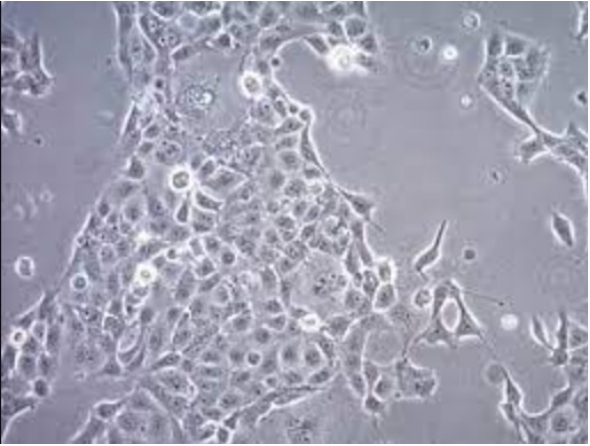
I Modelli *in-vitro*

- **Stable Cell Lines**
Fischer Rat Thyroid Cells



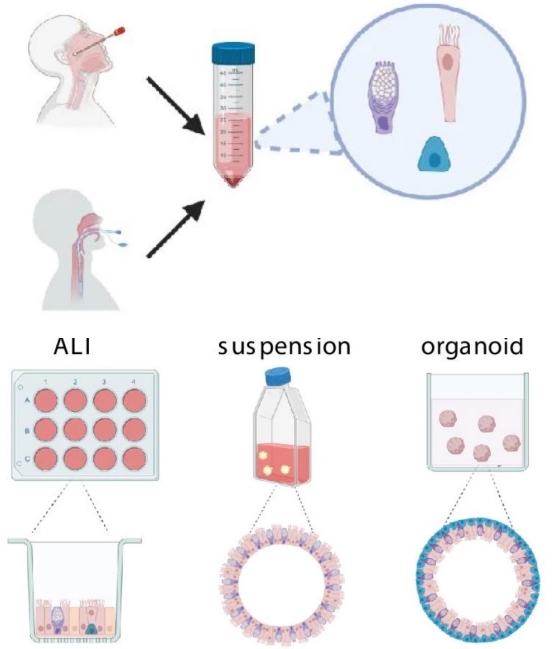
- FRTL cells are commonly used in:
- Electrophysiological studies:
 - Ussing chamber analysis
 - Patch clamp/single channel measurements
 - Medium-throughput conductance measurement
 - Western blot
 - Compound library screening in a high-throughput manner

- **Immortalized Human Respiratory Cell Lines**
CF and Non-CF Cells



- 16HBE14o-/ CFBE41o- cells are commonly used in:
- Electrophysiological studies:
 - Ussing chamber analysis
 - Patch clamp/single channel measurements
 - Western blot
 - Compound library screening in a high-throughput manner

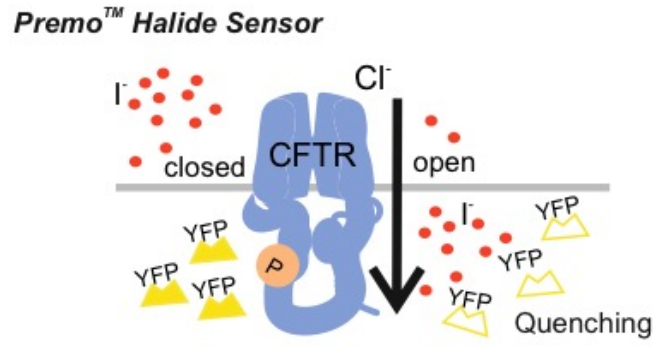
- **Primary Respiratory Cell Lines**
Bronchial and Nasal Cells



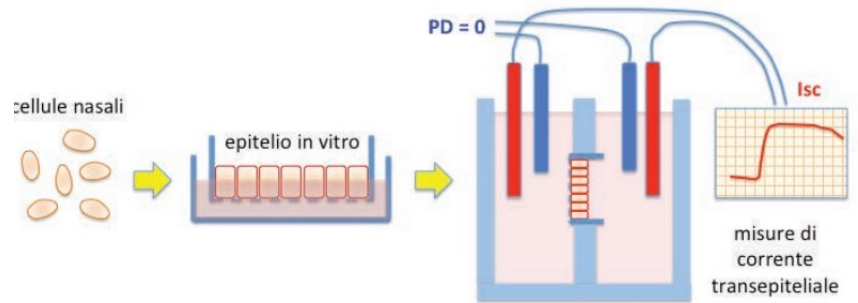
- Primary Respiratory cells are commonly used in:
- Short-circuit current (Isc)
 - Studying CFTR mutations in the native gene context
 - Western blot
 - Analysis of mRNA abundance

Test Cellulari per Valutare la Funzione del CFTR

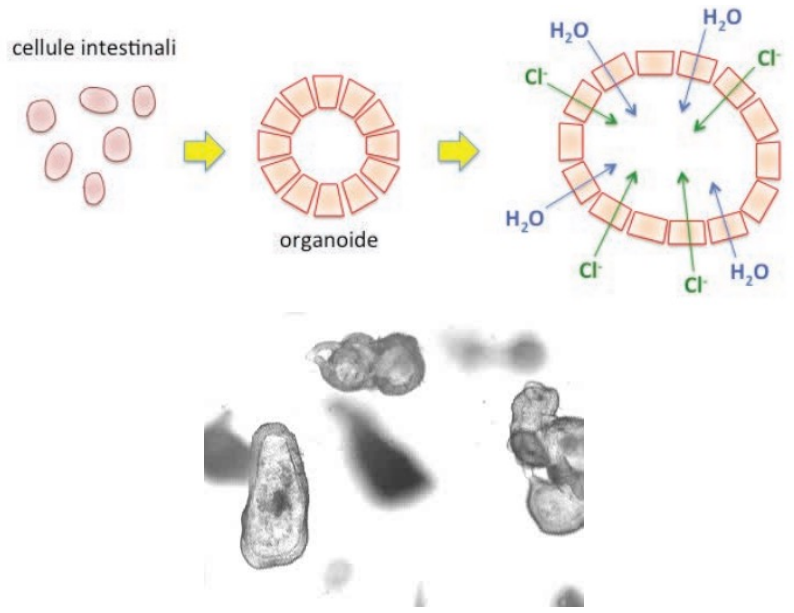
- YFP test in FRT cells by PremoHalide sensor



- Cl⁻ conductance in primary cells by ISC measurements

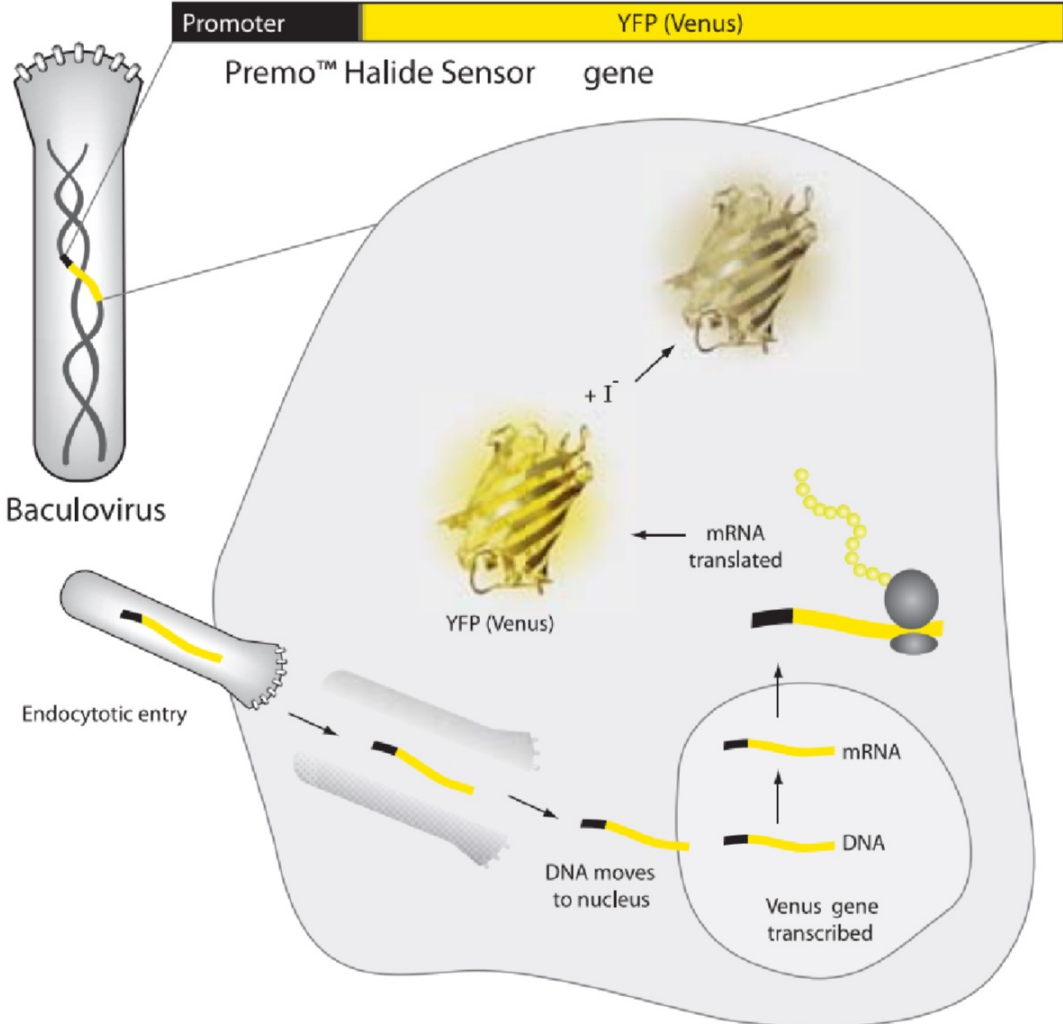


- Forskolin-induced swelling Assay in Patients derived Organoids



Saggio funzionale per CFTR basato sulla “proteina fluorescente gialla”

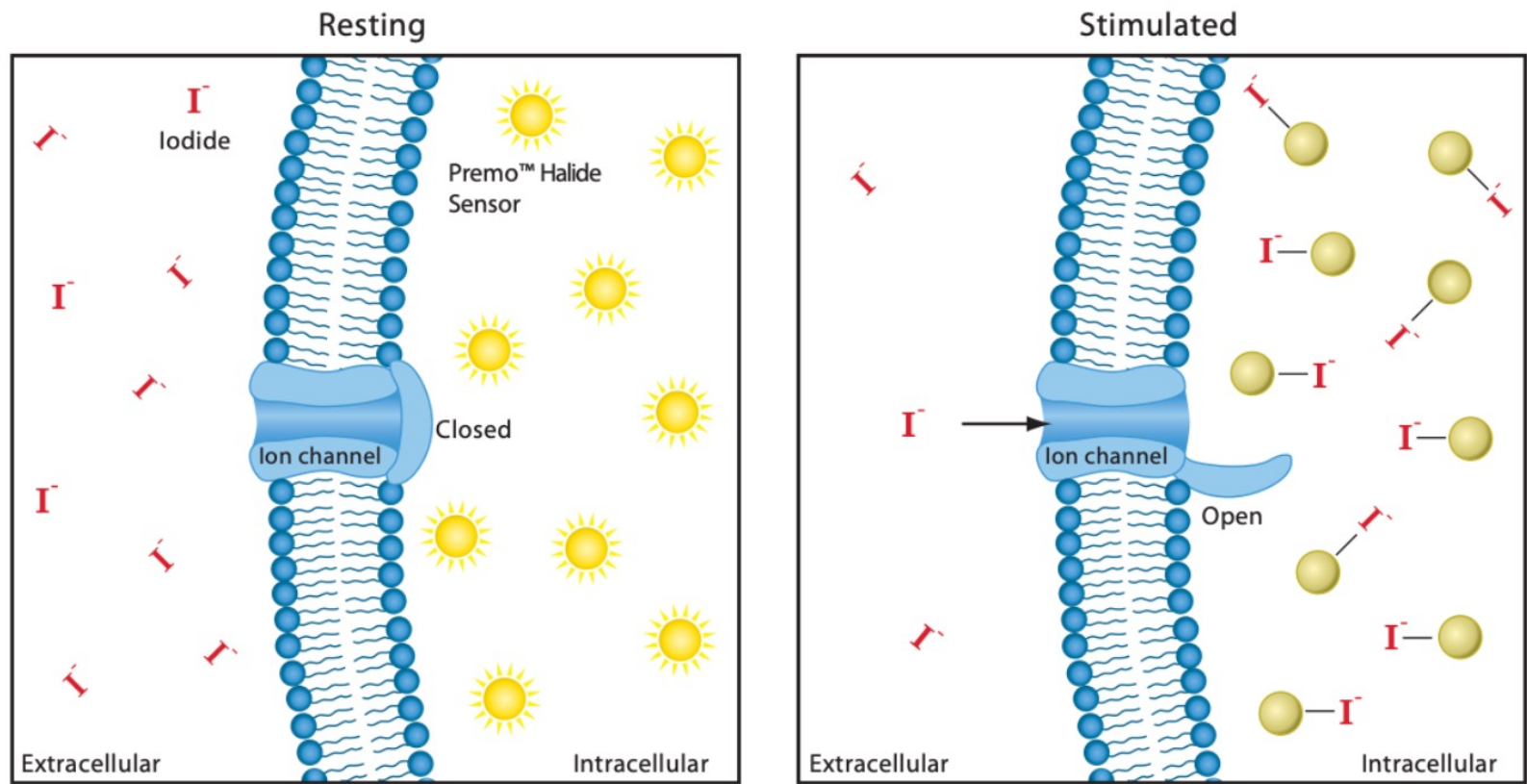
- PremoHalide sensor expression



Baculoviral particles encoding Premo™ Halide sensor enter the cells via an endocytotic pathway. Following cellular entry, the baculovirus moves to the nucleus where the Premo™ Halide sensor gene is expressed. The Premo™ Halide sensor protein is localized throughout the cytoplasm and is free to react with iodide ions upon chloride channel activation.

Saggio funzionale per CFTR basato sulla “proteina fluorescente gialla”

- Principle of PremoHalide sensor

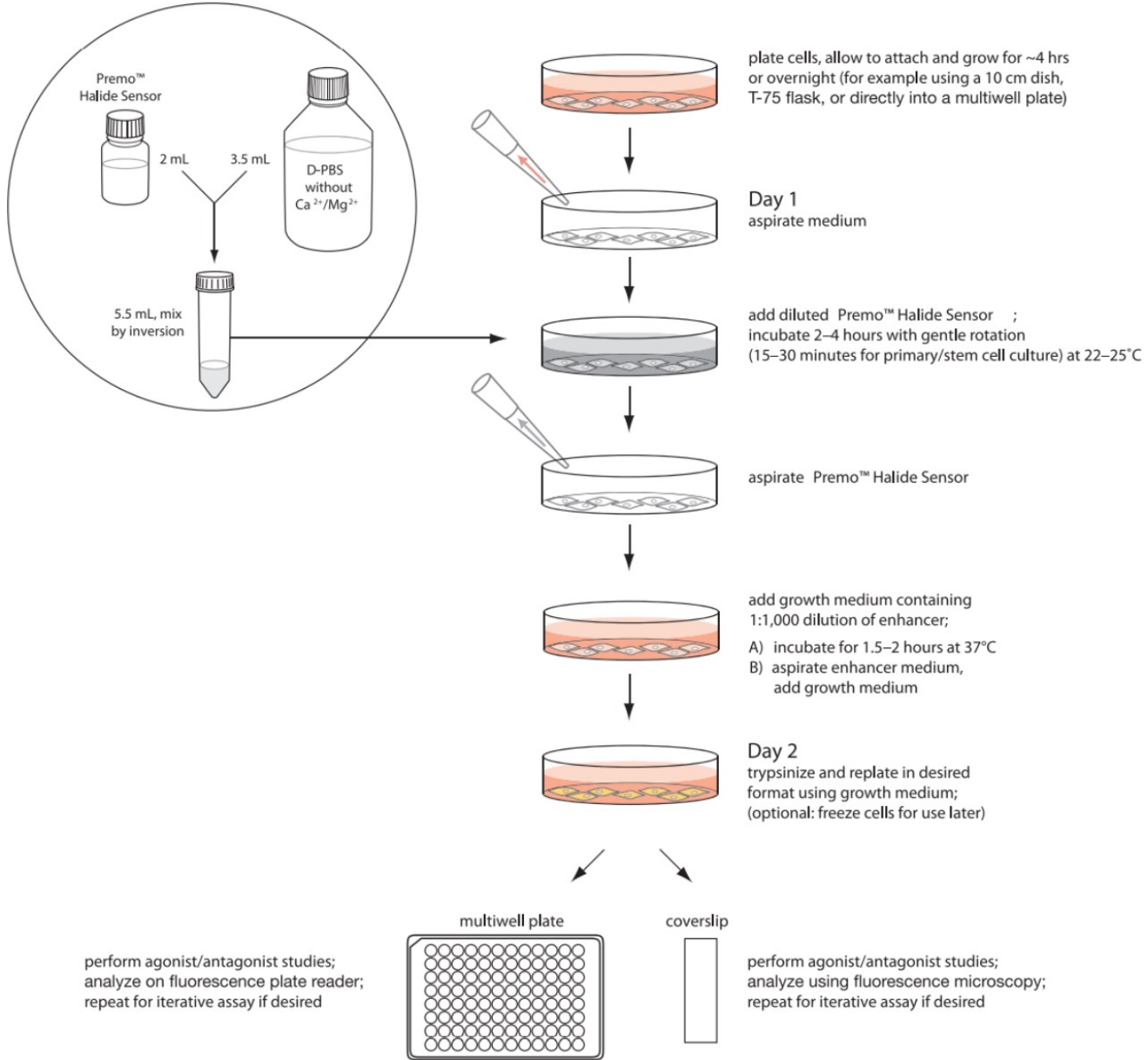


Upon activation (opening) of chloride channels, the iodide ions enter the cell, down its concentration gradient, and quench the fluorescence from the Premo™ Halide sensor.

Saggio funzionale per CFTR basato sulla “proteina fluorescente gialla”

- PremoHalide sensor**

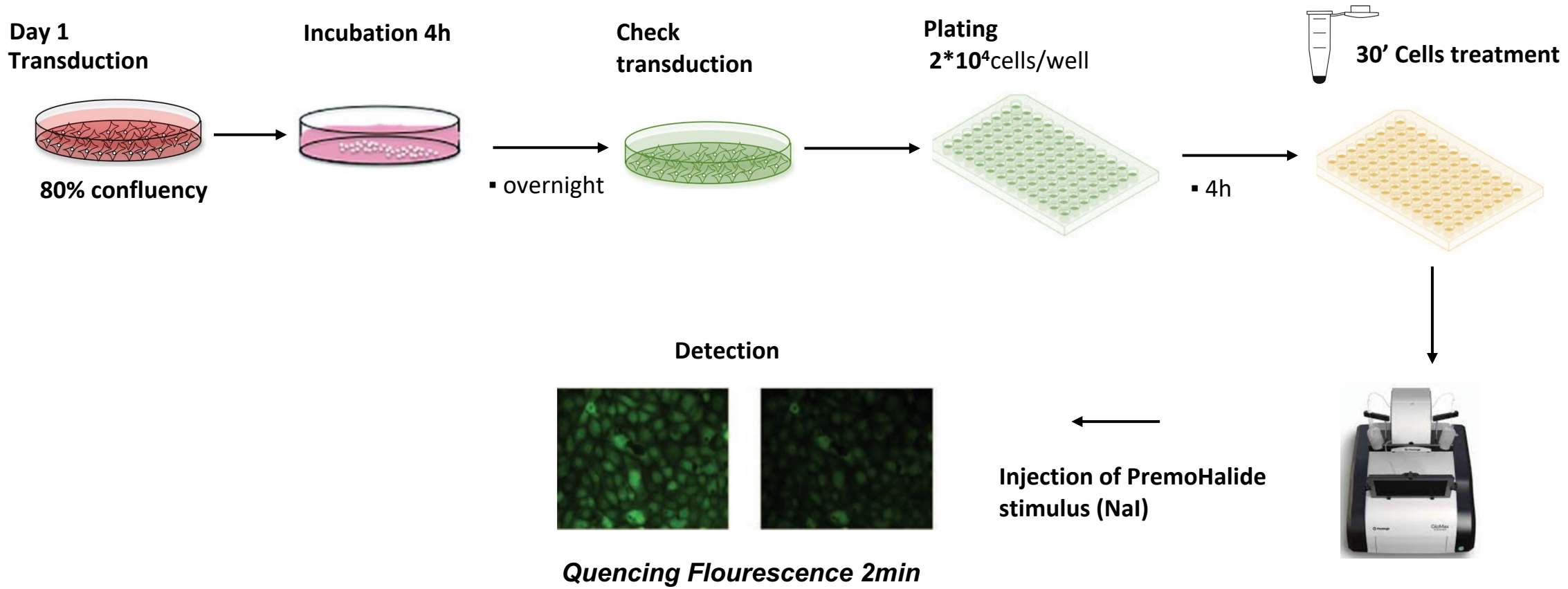
WORKFLOW



Saggio funzionale per CFTR basato sulla “proteina fluorescente gialla”

- PremoHalide sensor in 16HBE14o- cells line

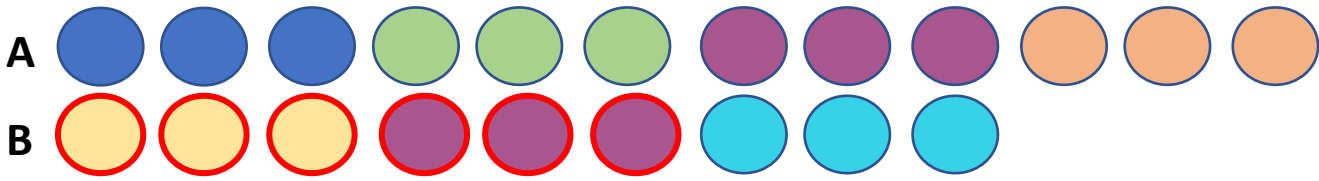
WORKFLOW



Saggio funzionale per CFTR basato sulla “proteina fluorescente gialla”

- PremoHalide sensor in 16HBE14o- cells line

Cells treatment 30':



- with no PremoHalide sensor
- with PremoHalide sensor
- UT
- KIT2014 10μM + CFTRinh-172 10μM
- Kaftrio
- KIT2014 10μM + Kaftrio
- KIT2014 10μM + Kaftrio + CFTRinh-172 10μM

Kaftrio:
VX-661 10μM
VX-445 3μM
VX-770 1μM

Saggio funzionale per CFTR basato sulla “proteina fluorescente gialla”

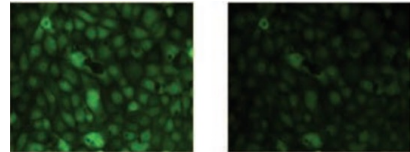
- PremoHalide sensor in 16HBE14o- cells line



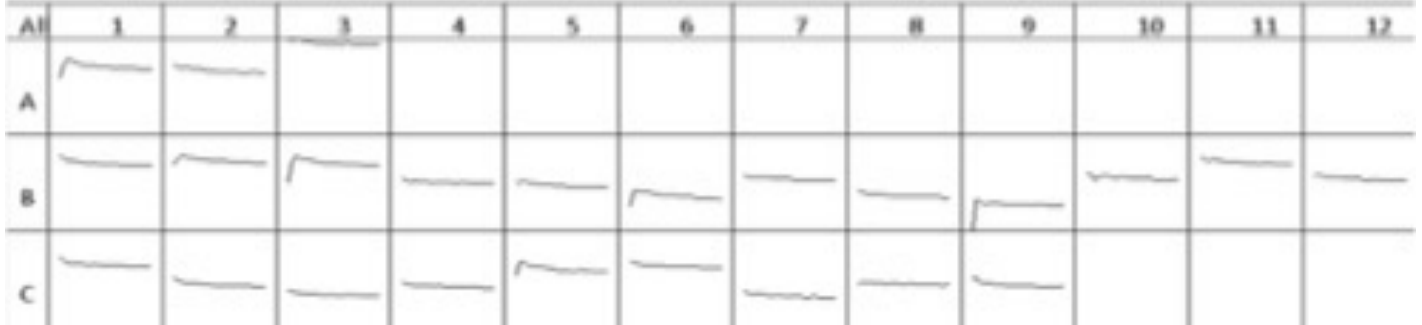
Injection of PremoHalide stimulus
(NaI 75μM)



Detection



*Quencing Flourescence
2min*



Saggio funzionale per CFTR basato sulla “proteina fluorescente gialla”

Read Type	Read Number	Row	Row	no probe			PROBE			FSK 10uM			FSK 1 uM			P1 25uM			P1 10uM			KIT 25uM			KIT 10uM			
		time (sec)	time (min)	A:1	A:2	A:3	A:10	A:11	A:12	B:1	B:2	B:3	B:4	B:5	B:6	B:7	B:8	B:9	B:10	B:11	B:12	C:1	C:2	C:3	C:4	C:5	C:6	
Fluorescence		1	0,000	0,000	3,56E+04	3,48E+04	3,50E+04	3,52E+04	3,50E+04	3,43E+04	3,42E+04	3,29E+04	3,38E+04	3,51E+04	3,49E+04	3,62E+04	3,59E+04	3,68E+04	3,55E+04	3,61E+04	3,49E+04	3,44E+04	3,39E+04	3,40E+04	3,41E+04	3,64E+04	3,59E+04	3,65E+04
Fluorescence		2	4,002	0,067	3,56E+04	3,48E+04	3,49E+04	3,52E+04	3,48E+04	3,42E+04	3,41E+04	3,28E+04	3,36E+04	3,49E+04	3,46E+04	3,56E+04	3,56E+04	3,63E+04	3,53E+04	3,57E+04	3,48E+04	3,45E+04	3,38E+04	3,38E+04	3,40E+04	3,41E+04	3,55E+04	3,63E+04
Fluorescence		3	8,004	0,133	3,56E+04	3,48E+04	3,49E+04	3,51E+04	3,48E+04	3,42E+04	3,40E+04	3,27E+04	3,36E+04	3,49E+04	3,43E+04	3,54E+04	3,55E+04	3,61E+04	3,52E+04	3,56E+04	3,47E+04	3,44E+04	3,38E+04	3,38E+04	3,39E+04	3,37E+04	3,53E+04	3,62E+04
Fluorescence		4	12,006	0,200	3,56E+04	3,47E+04	3,49E+04	3,51E+04	3,47E+04	3,43E+04	3,40E+04	3,27E+04	3,35E+04	3,48E+04	3,42E+04	3,52E+04	3,53E+04	3,59E+04	3,51E+04	3,56E+04	3,47E+04	3,44E+04	3,37E+04	3,38E+04	3,39E+04	3,36E+04	3,53E+04	3,61E+04
Fluorescence		5	16,008	0,267	3,56E+04	3,47E+04	3,48E+04	3,50E+04	3,46E+04	3,42E+04	3,39E+04	3,27E+04	3,35E+04	3,48E+04	3,41E+04	3,51E+04	3,53E+04	3,58E+04	3,50E+04	3,55E+04	3,46E+04	3,44E+04	3,37E+04	3,37E+04	3,38E+04	3,36E+04	3,52E+04	3,60E+04
Fluorescence		6	20,010	0,334	3,56E+04	3,46E+04	3,48E+04	3,49E+04	3,46E+04	3,42E+04	3,39E+04	3,27E+04	3,35E+04	3,47E+04	3,41E+04	3,51E+04	3,52E+04	3,58E+04	3,50E+04	3,54E+04	3,46E+04	3,43E+04	3,37E+04	3,37E+04	3,38E+04	3,36E+04	3,51E+04	3,59E+04
Fluorescence		7	24,012	0,400	3,56E+04	3,46E+04	3,47E+04	3,49E+04	3,46E+04	3,42E+04	3,39E+04	3,27E+04	3,34E+04	3,47E+04	3,40E+04	3,50E+04	3,52E+04	3,57E+04	3,49E+04	3,54E+04	3,46E+04	3,43E+04	3,36E+04	3,37E+04	3,38E+04	3,36E+04	3,51E+04	3,59E+04
Fluorescence		8	28,014	0,467	3,56E+04	3,47E+04	3,47E+04	3,49E+04	3,45E+04	3,41E+04	3,38E+04	3,26E+04	3,34E+04	3,47E+04	3,40E+04	3,50E+04	3,51E+04	3,57E+04	3,49E+04	3,54E+04	3,46E+04	3,43E+04	3,36E+04	3,37E+04	3,38E+04	3,36E+04	3,50E+04	3,58E+04
Fluorescence		9	32,016	0,534	3,56E+04	3,46E+04	3,47E+04	3,48E+04	3,45E+04	3,41E+04	3,38E+04	3,26E+04	3,34E+04	3,46E+04	3,40E+04	3,50E+04	3,51E+04	3,56E+04	3,49E+04	3,54E+04	3,45E+04	3,43E+04	3,36E+04	3,36E+04	3,37E+04	3,36E+04	3,50E+04	3,58E+04
Fluorescence		10	36,018	0,600	3,55E+04	3,47E+04	3,46E+04	3,48E+04	3,45E+04	3,41E+04	3,38E+04	3,26E+04	3,34E+04	3,46E+04	3,39E+04	3,49E+04	3,51E+04	3,56E+04	3,48E+04	3,53E+04	3,45E+04	3,43E+04	3,36E+04	3,36E+04	3,37E+04	3,36E+04	3,50E+04	3,58E+04
Fluorescence		11	40,020	0,667	3,55E+04	3,46E+04	3,46E+04	3,48E+04	3,45E+04	3,41E+04	3,38E+04	3,26E+04	3,34E+04	3,46E+04	3,39E+04	3,49E+04	3,51E+04	3,56E+04	3,48E+04	3,53E+04	3,45E+04	3,43E+04	3,36E+04	3,36E+04	3,37E+04	3,35E+04	3,49E+04	3,58E+04
Fluorescence		12	44,022	0,734	3,55E+04	3,46E+04	3,46E+04	3,48E+04	3,44E+04	3,41E+04	3,38E+04	3,26E+04	3,34E+04	3,46E+04	3,39E+04	3,49E+04	3,50E+04	3,56E+04	3,48E+04	3,53E+04	3,45E+04	3,43E+04	3,36E+04	3,36E+04	3,37E+04	3,35E+04	3,49E+04	3,57E+04
Fluorescence		13	48,024	0,800	3,55E+04	3,46E+04	3,46E+04	3,48E+04	3,44E+04	3,40E+04	3,37E+04	3,26E+04	3,33E+04	3,46E+04	3,39E+04	3,49E+04	3,50E+04	3,55E+04	3,48E+04	3,53E+04	3,45E+04	3,43E+04	3,36E+04	3,36E+04	3,37E+04	3,35E+04	3,49E+04	3,57E+04
Fluorescence		14	52,026	0,867	3,55E+04	3,46E+04	3,46E+04	3,48E+04	3,44E+04	3,40E+04	3,37E+04	3,26E+04	3,33E+04	3,46E+04	3,39E+04	3,48E+04	3,50E+04	3,55E+04	3,48E+04	3,53E+04	3,45E+04	3,42E+04	3,35E+04	3,36E+04	3,37E+04	3,35E+04	3,49E+04	3,57E+04
Fluorescence		15	56,028	0,934	3,55E+04	3,45E+04	3,46E+04	3,48E+04	3,44E+04	3,40E+04	3,37E+04	3,26E+04	3,33E+04	3,45E+04	3,39E+04	3,48E+04	3,50E+04	3,55E+04	3,48E+04	3,53E+04	3,45E+04	3,42E+04	3,35E+04	3,36E+04	3,36E+04	3,35E+04	3,49E+04	3,57E+04
Fluorescence		16	60,030	1,001	3,55E+04	3,45E+04	3,47E+04	3,48E+04	3,44E+04	3,40E+04	3,37E+04	3,26E+04	3,33E+04	3,46E+04	3,39E+04	3,48E+04	3,50E+04	3,55E+04	3,47E+04	3,52E+04	3,44E+04	3,42E+04	3,35E+04	3,36E+04	3,36E+04	3,35E+04	3,49E+04	3,56E+04
Fluorescence		17	64,032	1,067	3,55E+04	3,45E+04	3,46E+04	3,48E+04	3,44E+04	3,40E+04	3,37E+04	3,26E+04	3,33E+04	3,45E+04	3,38E+04	3,48E+04	3,50E+04	3,55E+04	3,48E+04	3,52E+04	3,44E+04	3,42E+04	3,35E+04	3,36E+04	3,36E+04	3,35E+04	3,48E+04	3,56E+04
Fluorescence		18	68,034	1,134	3,55E+04	3,46E+04	3,46E+04	3,47E+04	3,44E+04	3,40E+04	3,37E+04	3,26E+04	3,33E+04	3,45E+04	3,38E+04	3,48E+04	3,49E+04	3,55E+04	3,47E+04	3,52E+04	3,44E+04	3,42E+04	3,35E+04	3,35E+04	3,36E+04	3,35E+04	3,48E+04	3,56E+04
Fluorescence		19	72,036	1,201	3,55E+04	3,45E+04	3,46E+04	3,47E+04	3,44E+04	3,40E+04	3,37E+04	3,28E+04	3,33E+04	3,45E+04	3,38E+04	3,48E+04	3,49E+04	3,55E+04	3,47E+04	3,52E+04	3,44E+04	3,42E+04	3,35E+04	3,35E+04	3,36E+04	3,35E+04	3,48E+04	3,56E+04
Fluorescence		20	76,038	1,267	3,55E+04	3,45E+04	3,46E+04	3,47E+04	3,44E+04	3,40E+04	3,37E+04	3,28E+04	3,33E+04	3,45E+04	3,38E+04	3,48E+04	3,49E+04	3,55E+04	3,47E+04	3,52E+04	3,44E+04	3,42E+04	3,35E+04	3,35E+04	3,36E+04	3,35E+04	3,48E+04	3,56E+04
Fluorescence		21	80,040	1,334	3,55E+04	3,45E+04	3,46E+04	3,47E+04	3,43E+04	3,40E+04	3,37E+04	3,28E+04	3,33E+04	3,45E+04	3,38E+04	3,47E+04	3,49E+04	3,55E+04	3,47E+04	3,52E+04	3,44E+04	3,42E+04	3,35E+04	3,35E+04	3,36E+04	3,35E+04	3,48E+04	3,56E+04
Fluorescence		22	84,042	1,401	3,55E+04	3,45E+04	3,46E+04	3,47E+04	3,43E+04	3,40E+04	3,37E+04	3,28E+04	3,33E+04	3,45E+04	3,38E+04	3,47E+04	3,49E+04	3,54E+04	3,47E+04	3,52E+04	3,44E+04	3,42E+04	3,35E+04	3,35E+04	3,36E+04	3,35E+04	3,48E+04	3,56E+04
Fluorescence		23	88,044	1,467	3,55E+04	3,45E+04	3,46E+04	3,47E+04	3,43E+04	3,39E+04	3,37E+04	3,28E+04	3,33E+04	3,45E+04	3,38E+04	3,47E+04	3,49E+04	3,54E+04	3,47E+04	3,52E+04	3,44E+04	3,42E+04	3,35E+04	3,35E+04	3,36E+04	3,35E+04	3,48E+04	3,55E+04
Fluorescence		24	92,046	1,534	3,55E+04	3,45E+04	3,46E+04	3,47E+04	3,43E+04	3,39E+04	3,36E+04	3,27E+04	3,33E+04	3,45E+04	3,38E+04	3,47E+04	3,49E+04	3,54E+04	3,47E+04	3,52E+04	3,44E+04	3,42E+04	3,35E+04	3,35E+04	3,36E+04	3,35E+04	3,48E+04	3,55E+04
Fluorescence		25	96,048	1,601	3,55E+04	3,45E+04	3,46E+04	3,47E+04	3,43E+04	3,39E+04	3,36E+04	3,27E+04	3,33E+04	3,45E+04	3,38E+04	3,47E+04	3,48E+04	3,54E+04	3,47E+04	3,52E+04	3,44E+04	3,42E+04	3,35E+04	3,35E+04	3,36E+04	3,35E+04	3,48E+04	3,55E+04
Fluorescence		26	100,050	1,668	3,55E+04	3,45E+04	3,46E+04	3,47E+04	3,43E+04	3,39E+04	3,36E+04	3,27E+04	3,32E+04	3,45E+04	3,38E+04	3,47E+04	3,48E+04	3,54E+04	3,47E+04	3,51E+04	3,44E+04	3,42E+04	3,35E+04	3,35E+04	3,36E+04	3,35E+04	3,47E+04	3,55E+04
Fluorescence		27	104,052	1,734	3,55E+04	3,45E+04	3,46E+04	3,46E+04	3,43E+04	3,39E+04	3,36E+04	3,27E+04	3,32E+04	3,44E+04	3,38E+04	3,47E+04	3,48E+04	3,54E+04	3,46E+04	3,51E+04	3,44E+04	3,42E+04	3,35E+04	3,35E+04	3,36E+04	3,35E+04	3,47E+04	3,55E+04
Fluorescence		28	108,054	1,801	3,55E+04	3,45E+04	3,46E+04	3,46E+04	3,43E+04	3,39E+04	3,36E+04	3,27E+04	3,32E+04	3,45E+04	3,38E+04	3,47E+04	3,48E+04	3,54E+04	3,46E+04	3,51E+04	3,44E+04	3,42E+04	3,35E+04	3,35E+04	3,36E+04	3,35E+04	3,47E+04	3,55E+04
Fluorescence		29	112,056	1,868	3,55E+04	3,45E+04	3,46E+04	3,46E+04	3,43E+04	3,39E+04	3,36E+04	3,27E+04	3,32E+04	3,44E+04	3,38E+04	3,47E+04	3,48E+04	3,54E+04	3,46E+04	3,51E+04	3,43E+04	3,42E+04	3,34E+04	3,35E+04	3,36E+04	3,35E+04	3,47E+04	3,55E+04
Fluorescence		30	116,058	1,934	3,55E+04	3,45E+04	3,45E+04	3,46E+04	3,43E+04	3,39E+04	3,36E+04	3,27E+04	3,32E+04	3,44E+04	3,38E+04	3,47E+04	3,48E+04	3,54E+04	3,46E+04	3,51E+04	3,44E+04	3,42E+04	3,34E+04	3,35E+04	3,36E+04	3,35E+04	3,47E+04	3,55E+04

DRI-Pep #20 increases the Activity of the cAMP- Dependent Chloride Channel CFTR

