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

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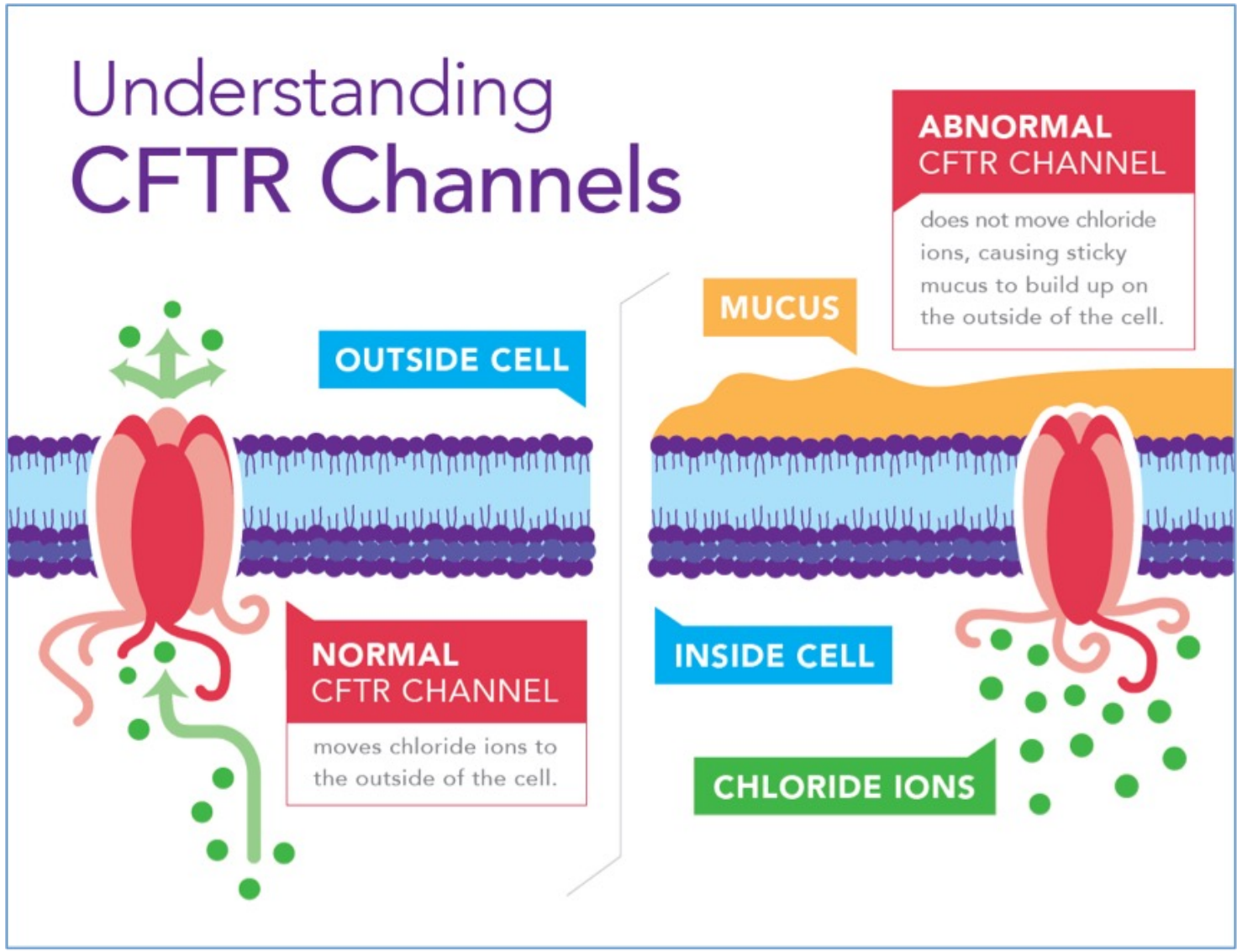
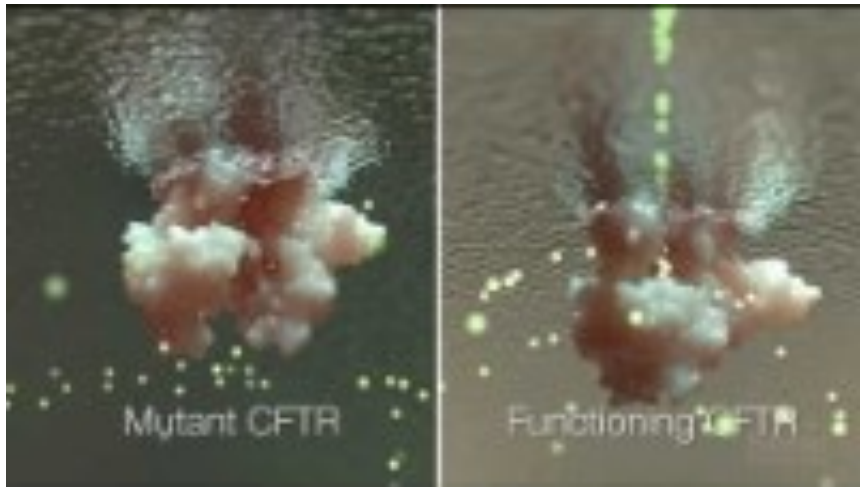
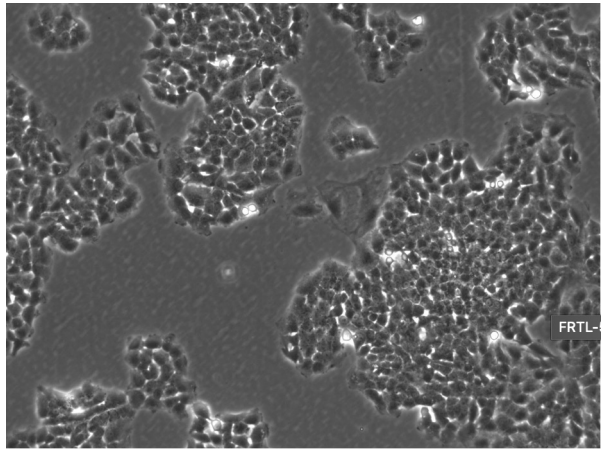
Cystic Fibrosis Conductance Regulator





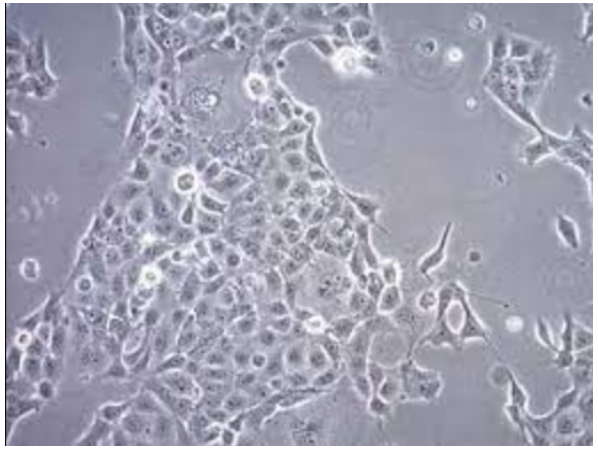
I Modelli *in-vitro*

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



FRT 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

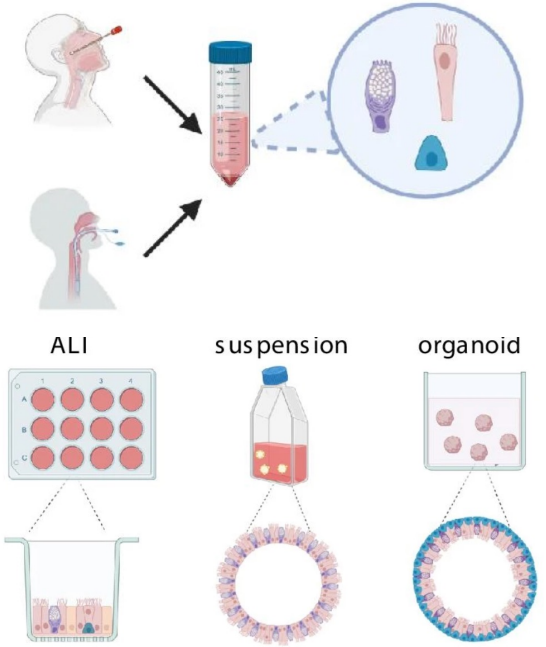
- **Immortalized Human Respiratory Cell Lines**
  CF and Non-CF Cells



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

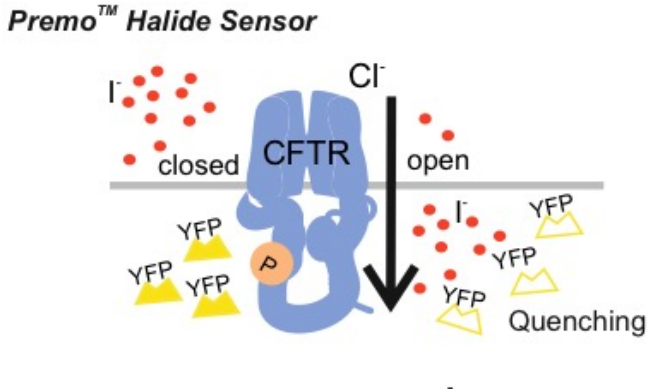
- **Primary Respiratory Cell Lines**
  Bronchial and Nasal Cells



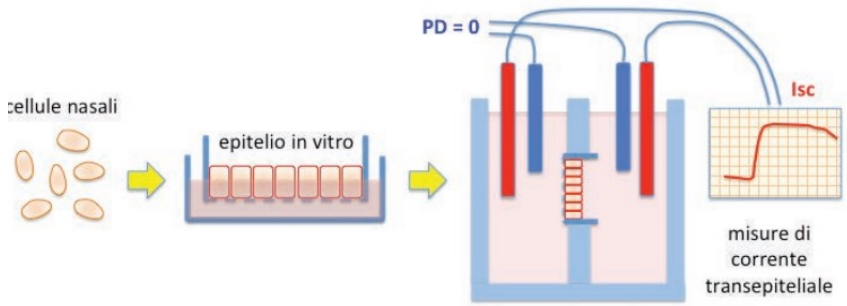
Primary Respiratory cells are commonly used in:
•Short-circuit current (Isc)
•Studying CFTR mutations in the native gene context
•Western blot
•Analysis of mRNA abundance

Test Cellulari per Valutare la Funzione del CFTR

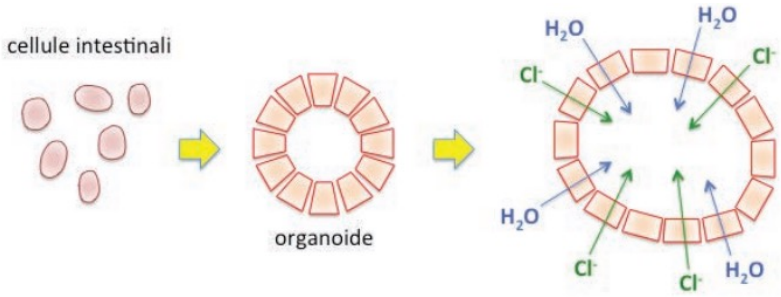
- **YFP test in FRT cells by PremoHalide sensor**



- **Cl- conductance in primary cells by ISC measurements**

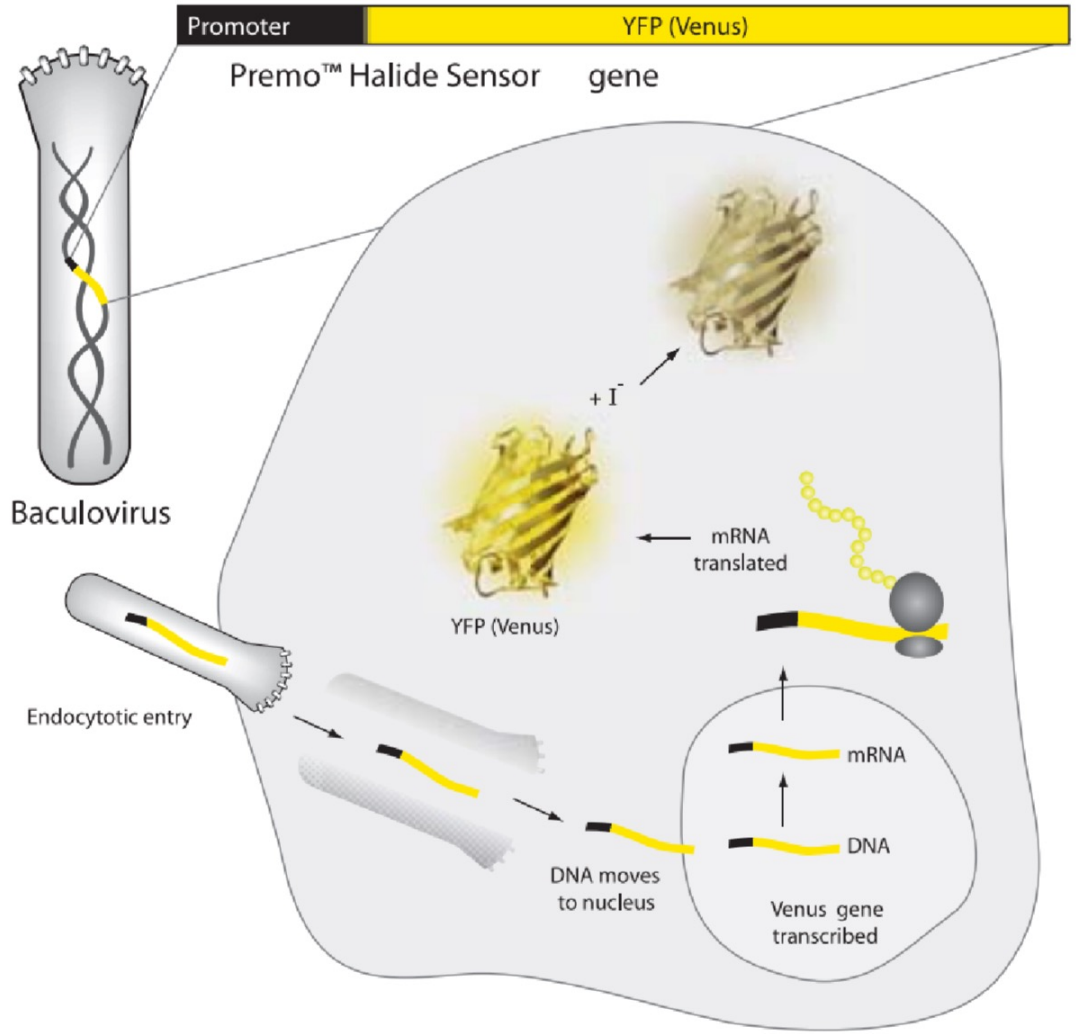


- **Forskolin-induced swelling Assay in Patients derived Organoids**



Saggio funzionale per CFTR basato sulla “proteina fluorescente gialla”

- **PremoHalide sensor expression**

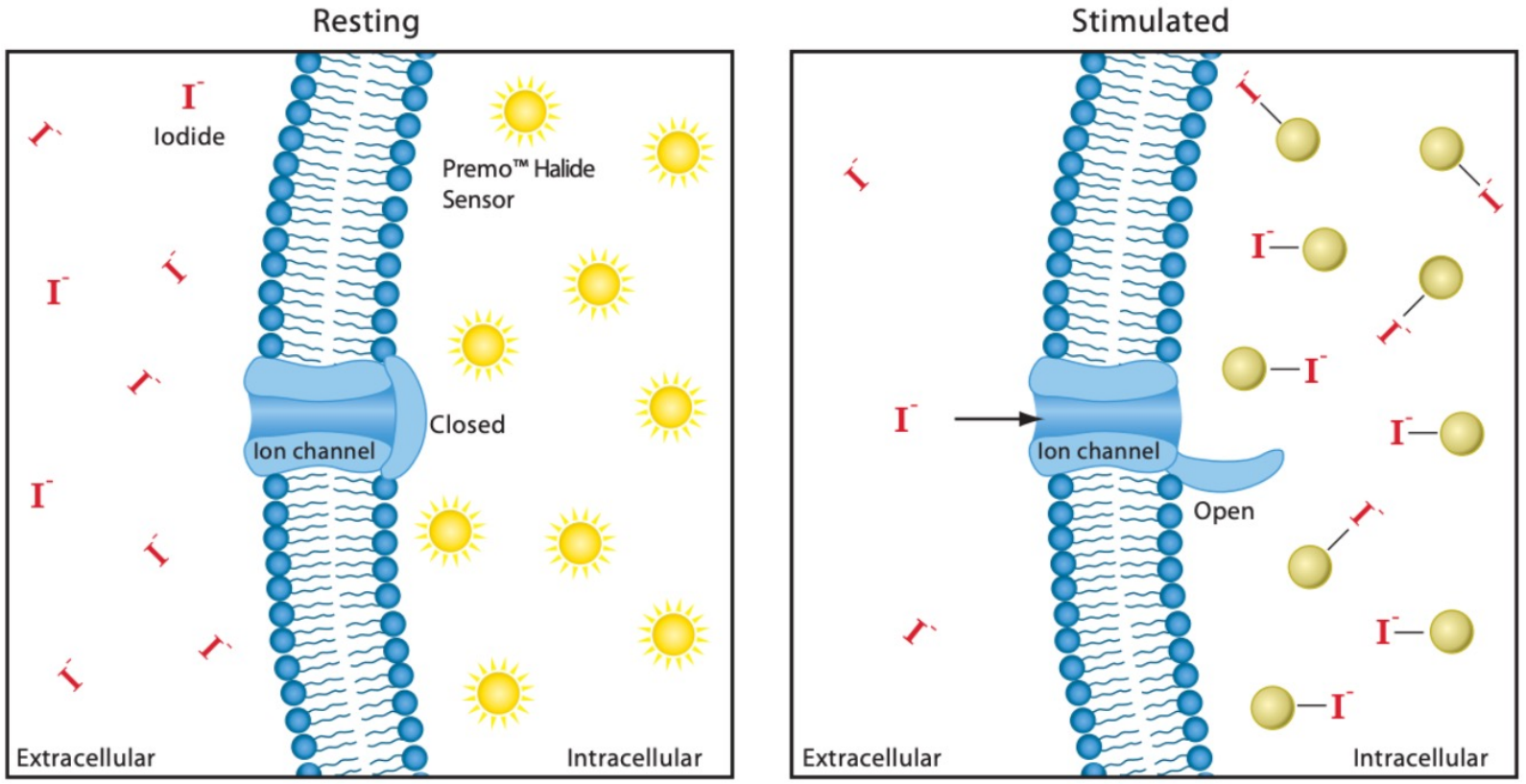


Baculoviral particles encoding Premo™ Halide sensor enter the cells via an endocytotic pathway. Following cellular entry, the baculovirus moves to the nucleus where the Premo™ Halide sensor gene is expressed.

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”

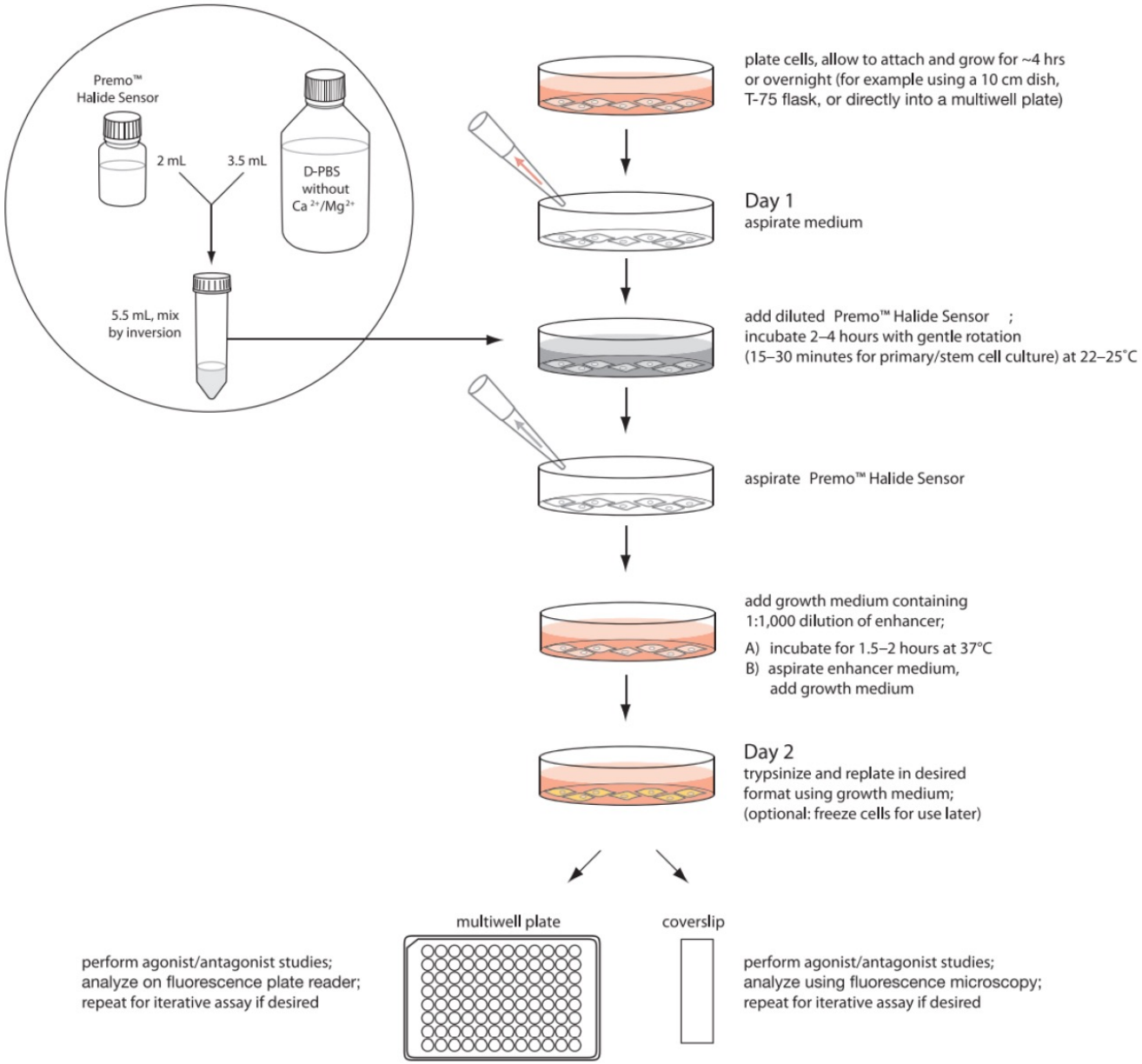
- **Principle of PremoHalide sensor**



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**

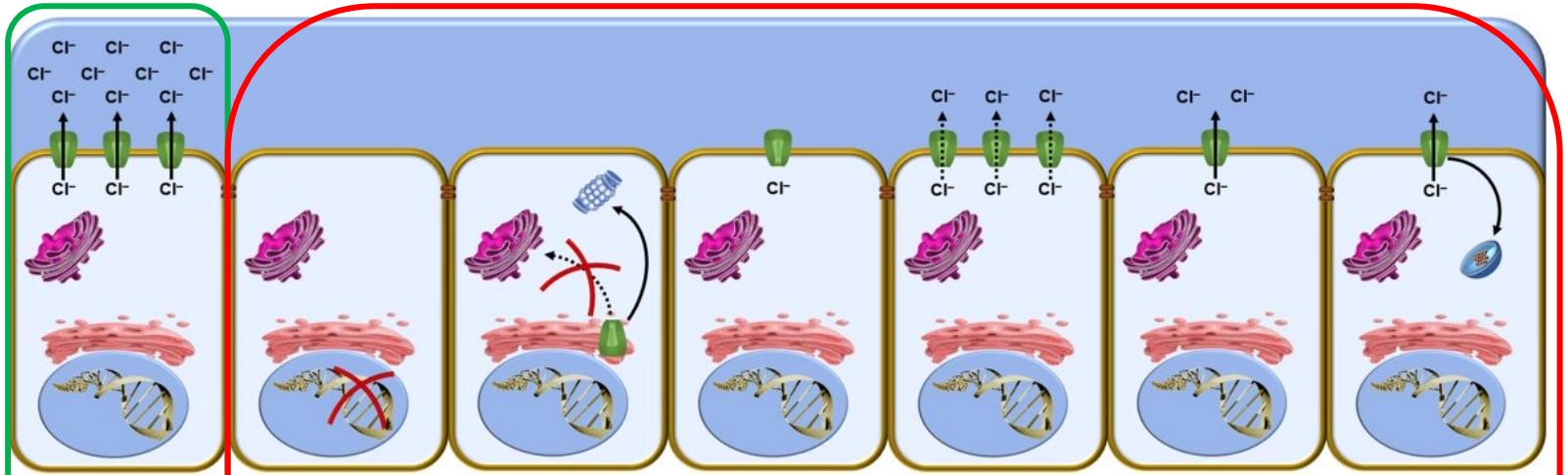


Le Mutazioni Responsabili della Fibrosi Cistica sono piu' di 2000

Sano

90% of FC

FC



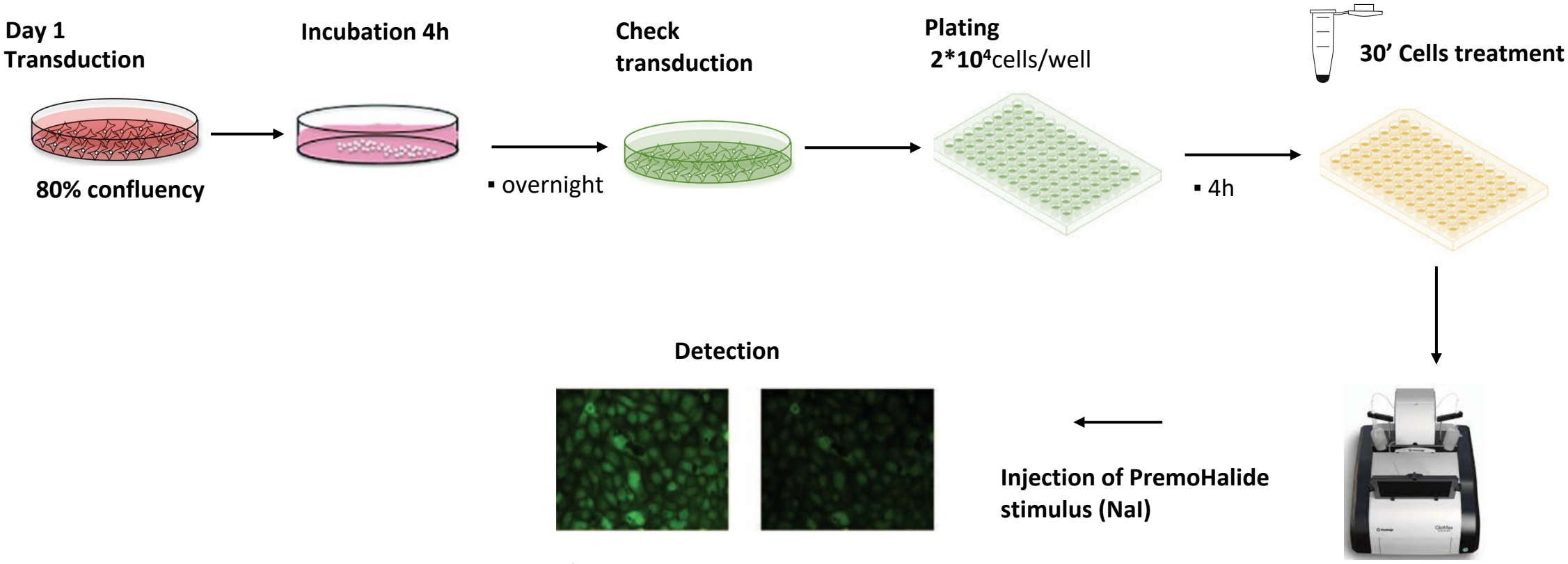
	wt-CFTR	I	II	III	IV	V	VI
Defect types		**No protein**	**No traffic**	**No function**	**Less function**	**Less protein**	**Less stable**
Mutation examples		G542X R553X W1282X	G85E ΔI507 **ΔF508** N1303K	V520F S549R G551D	R117H R334W S1235R	A455E 1680-886A>G 2657+5G>A	**rΔF508** Q1412X
Required approaches		Rescue protein synthesis	Correct protein folding	Restore channel conductance	Restore channel conductance	Maturation / Correct misplicing	Promote protein stability

Disease severity

Saggio funzionale per CFTR basato sulla “proteina fluorescente gialla”

- **PremoHalide sensor in FRT cells line expressing the Class IV (impaired Conductance): R334W mutant**

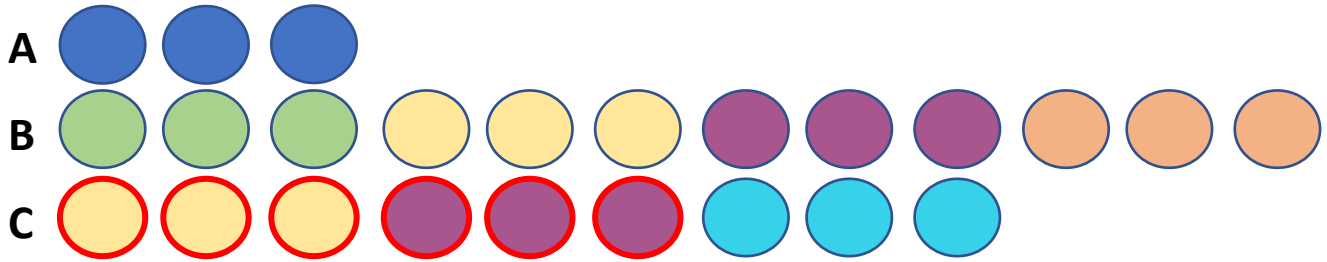
WORKFLOW



Saggio funzionale per CFTR basato sulla “proteina fluorescente gialla”

- **PremoHalide sensor in FRT cells line expressing the Class IV (impaired Conductance): R334W mutant**

Cells treatment 30’:



A FRT with no PremoHalide sensor

B-C FRT with PremoHalide sensor

UT

KIT2014 10µM + CFTRinh-172 10µM

KIT2014 3µM + CFTRinh-172 10µM

VX-770 1µM

Foskolin 1µM (positive control)

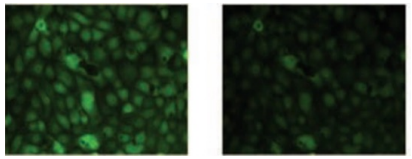
Saggio funzionale per CFTR basato sulla “proteina fluorescente gialla”

- **PremoHalide sensor in FRT cells line expressing the Class IV (impaired Conductance): R334W mutant**

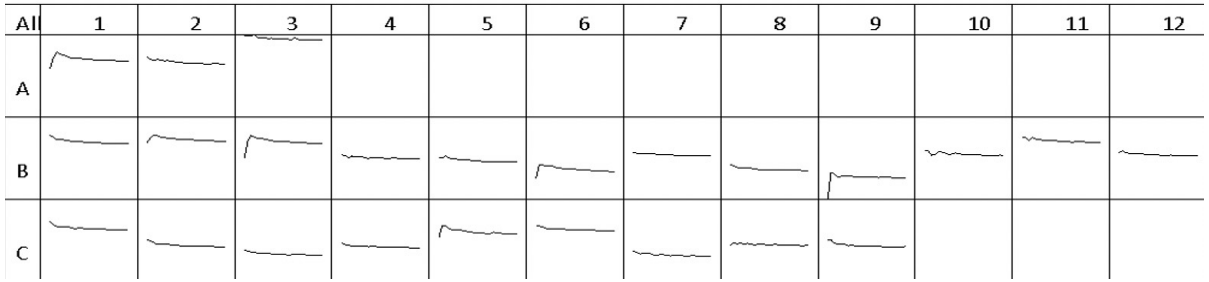
Injection of PremoHalide stimulus (NaI 75µM)

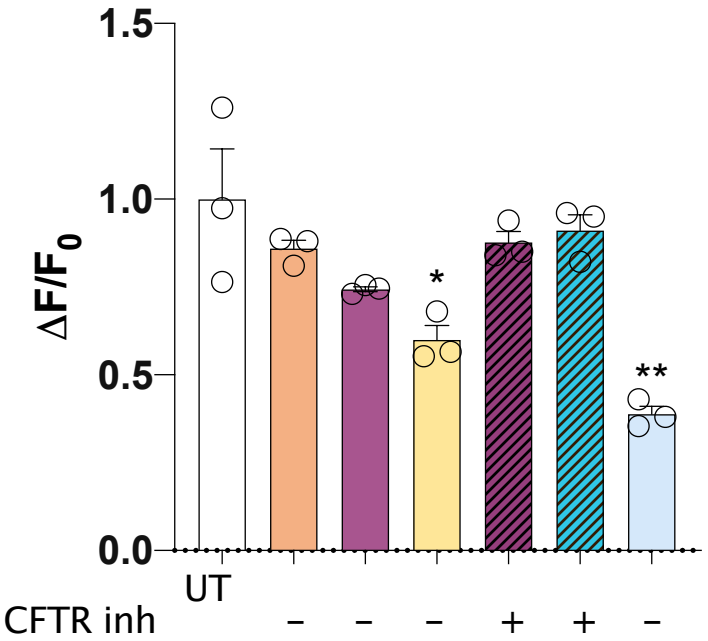
↓

Detection



Quencing Flourescence 2min





KIT2014 (10µM) can exert its therapeutic action in cells expressing the rare class IV R334W mutant, while the potentiator VX-770 shows no effects.