## CFBE cells expressing wild type CFTR, F508del CFTR, or EGFP

- CFBE (cystic fibrosis bronchial epithelial) parental cells are a gift of Dr. D. Gruenert (UCSF, CA)[1,2].
- CFBE cell lines expressing CFTR or EGFP are transduced by lentivirus based expression vector and the transgenes are expressed under the control of CMV promoter.
- The cells were selected in puromycin (2µg/ml) following transduction.

  The cells have been cultured since being generated, however we do not always keep puromycin in the media for our continuously cultured cells. We select the cell population with puromycin for minimum of 2 weeks when needed.
- These are a pool of selected cells, not clonal cell lines.
  Expression level of transgene in this cell population is not homogeneous.

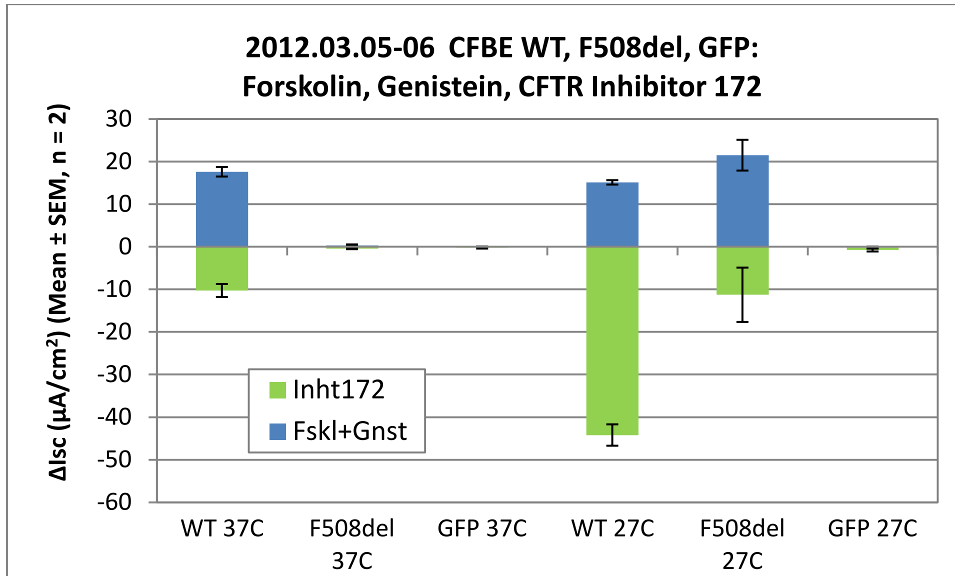
  Data from the CFTR functional and western blot analysis is shown on the second page of this document. **Note:** The CFBE-wt or F508del CFTR cells may change epithelial characteristics or lose expression following multiple passages. Because of this, it is important to maintain low passage frozen cell stocks.
- The protocols provided are adapted from the original protocol from the Gruenert laboratory and other open internet sources.
- Cells were tested mycoplasma free by a PCR based method (MycoProbe Mycoplasma detection kit, R&D Sytems).
- It is important to split the cells in a timely fashion and not to wait until cells are over confluent. This will maintain expression and phenotype of the cell lines.
- We culture the CFBE cells on a collagen coated surface.
- We ship the lowest passage cells available.

Reference:

1. Kunzelmann K, Schwiebert EM, Zeitlin PL, Kuo WL, Stanton BA,Gruenert DC. An immortalized cystic fibrosis tracheal epithelial cell line homozygous for the delta F508 CFTR mutation. Am J Respir Cell Mol Biol. 1993 May;8(5):522-9.
2. Gruenert DC, Willems M, Cassiman JJ, Frizzell RA. Established cell lines used in cystic fibrosis research. J Cyst Fibros. 2004 Aug;3 Suppl 2:191-6.

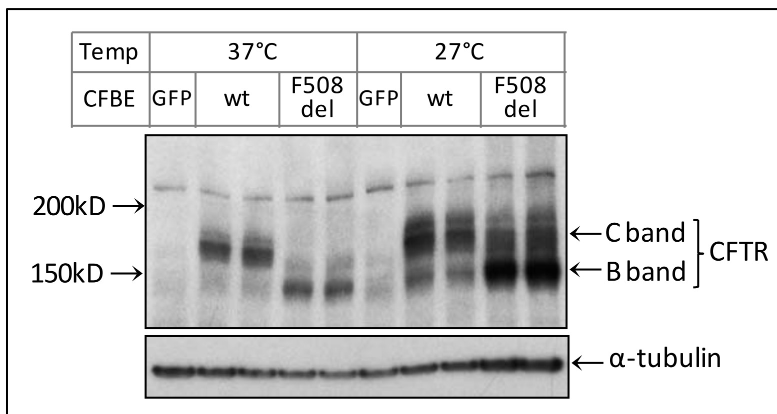
**Short circuit current measurement on CFBE cell monolayers**

1. Cells were cultured on coated 6.5mm transwell filters (Costar 3470) for 4 days.
2. Transepithelial resistance reaches >3000 Ω.cm$^2$.
3. One set of cells was moved to 27°C and incubated for 24 additional hours prior to short circuit current measurement.




**Western blot using CFBE-wt and F508del CFTR cell lysates**

1. Cells were cultured on coated plastic surface in 6-well plates.
2. Cells lysed in RIPA with Halt (Pierce 78429).
3. 30µg total lysates loaded per lane on 4-12% gradient gel (Invitrogen).
4. CFTR probed with 3G11 antibody (1.7µg/ml; http://cftrfolding.org/reagentrequests.htm) and anti rat-HRP secondary antibody (1:20,000; Zymed), then developed using West femto chemiluminescent substrate (Pierce 34095).
5. Alpha tubulin was probed with DM1A antibody (1:10,000; Genetex GTX11302) and anti mouse-HRP secondary antibody (1:20,000, Dako P0447), then developed using west pico chemiluminescent substrate (Pierce 34077).



## Upon receiving cells

Cells in frozen vials

1. Each vial contains approximately $5x10^5$ cells in 0.5-1 ml freezing solution.
2. Warm MEM complete medium to 37°C.
3. Thaw the cryovial in a 37°C water bath.
4. As soon as the cell supension has thawed, remove the cryovial and wipe off the outside with alcohol.
5. Transfer cells to a 15 mL centrifuge tube.
6. Dilute the cell suspension by slowly adding an equal volume of warm MEM medium. Wait 1 min.

   (**Note**: Warm medium must be added in a step-wise manner so that the DMSO concentration gradient is not so steep that DMSO exits the cells too quickly.)
7. Dilute the volume another 1:2 and wait 1 min.
8. Add more MEM medium to fill the tube.
9. Spin at 600g for 5 minutes, 4°C.
10. **Gently** resuspend cells in the appropriate plating medium and transfer to one T-25 coated flask.
11. Incubate at 37°C in a $CO_2$ incubator.
12. Passage the cells when 80-90% confluent.

Cells in flask

1. Cells are shipped at least ~70% confluent and attached to the bottom of the flask (collagen coated).
2. The flask is filled with complete MEM culture medium.
3. Remove wrapping and spray the flask with 70% ethanol.
4. Transfer the excess medium to sterile conical tubes and save at 4°C.
5. This media can be mixed with your own for the first few passages as a precaution while cells are adapting to your media. (The shipping medium contains puromycin for selection.)
6. Passage the cells when 80-90% confluent.

## Solutions for CFBE cells

Medium:

MEM (Minimum Essential Medium, 1x) with Earle's salt and L-glutamine (Life Technology 11095-080)

| | |
|---|---|
| 10% | Fetal Bovine Serum (Life Technology 26140) |
| 1% | L-Glutamine (200mM) (Life Technology 25030-149)(optional) |

If needed, add Penicillin/Streptomycin (100x) (Life Technology 15140-122 or Sigma P4333).
Add puromycin (2µg/ml; Sigma P8833), if needed to select transduced cell population.

Freezing Solution:

| | |
|---|---|
| 50% | Fetal Bovine Serum |
| 40% | MEM complete medium |
| 10% | DMSO (Sigma D2650) |

Fibronectin Coating Solution*:

| | |
|---|---|
| 100 ml | LHC basal medium (Biosource Int'l/Biofluids 118-500 or Life Technology #12677019) |
| 10 ml | BSA (Bovine serum Albumin, 1 mg/ml) (Biosource Int'l/Biofluids 343-020) |
| 1 ml | Collagen I, bovine (2.9 mg/ml) (Fisher CB-40231) |
| 1 ml | Human fibronectin (1 mg/ml) (Fisher CB-40008A) |

*Note: We substituted the fibronectin coating solution with PureCol (protocol on page 5).
(Fisher 50-360-230; Advanced Biomatrix cat# 5005-B).
We culture CFBE cells on a collagen coated surface.

Hepes Buffered Saline (HBS): (1 liter recipe, make final pH7.4-7.6)

| | |
|---|---|
| 5.2g | Hepes Sodium Salt (Sigma H3784, MW 260.29), 20mM |
| 7.13g | NaCl (Sigma S5886, MW 58.44), 122mM |
| 0.2g | Glucose (Sigma G7021), 0.02% |
| 1.7g | $Na_2HPO_4.7H_2O$ (dibasic) (Sigma S9390, MW268.07), 6.34mM |
| 0.25 ml | 0.5% Phenol Red (Sigma P0290) |

Note: This HBS recipe is slightly different from conventional HBS.
HBS can be purchased from Athena Enzyme Systems.*
(0408, http://www.athenaes.com/HepesBufferedSaline.php)

PET: (stock of 50ml trypsin solution)

| | |
|---|---|
| 36 ml | Hepes Buffered Saline (HBS) |
| 5 ml | 10% Polyvinylpyrrolidone (Biosource int'l/Biofluids 345-020 or Sigma P0930) in HBS |
| 5 ml | 0.2% EGTA in HBS |
| 4 ml | Trypsin, 0.25% with 0.02% EDTA (Sigma T4049 or Life Technology 25200056) |

Premade PET can be purchased from Athena Enzyme Systems.*
(0405, http://www.athenaes.com/PET.php)

*Note: We make our own solutions and have not tried the Athena Enzyme Systems.

## Splitting T25 Flasks Containing CFBE cells.

1. Wash the cells with 4 mls HBS (no Ca or Mg).

   Do a second wash with HBS to remove most of the serum.
2. Wash cells with 1 ml of PET. Rotate the flask and aspirate the trypsin immediately.
3. Add 1ml of PET making sure that it covers the bottom of the flask.
4. Incubate the cells at 37°C.

   After five minutes, check the cells to determine whether they have detached.
   (Briskly tapping the side of the flask can help dislodge cells.)
5. If the trypsinization process requires longer than 5 minutes, remove the first 1 ml of PET (containing some detached cells) and put this into a sterile conical tube with at least 2 ml of MEM complete medium.
6. Then add another 1 ml of PET to the cells remaining in the T-25 flask and continue incubating for an additional five minutes.
7. Continue this process until all the cells are detached.
8. Combine all cells; pellet by spinning at 600g for 5 minutes.
9. Remove the supernatant and gently resuspend the cells in complete MEM medium.
10. Split the cells 1:5 - 1:6.
11. Coated flasks or plates can be re-used 2-3 times. (In our experience twice is better.)

# Collagen Coating Plates for Human Airway Epithelial Cells using PureCol

(Ref. http://www.med.unc.edu/cfpulmcenter/core-facilities/files)

Materials

- PureCol® (Fisher 50-360-230; Advanced Biomatrix Cat# 5005-B), which contains approximately 3 mg/mL of Bovine Collagen Type I/III
- Sterile Deionized Water
- Tissue Culture Dishes or Flasks

The protocol is adapted from one available from the referenced web site. (http://www.med.unc.edu/cfpulmcenter/core-facilities/files)

1. Make a dilution of PureCol®to 56 µg/3 ml in sterile $dH_2O$.
2. Add 3 to 4 mL of the collagen solution to each 100 mm dish.
3. Swirl collagen around so the entire plate is coated.
4. Incubate at 37°C for 2-17 hours. Swirl the plates occasionally to cover the entire surface area during incubation.
5. Transfer plates to a laminar flow hood.
6. Aspirate any excess liquid.
7. Continue to dry plates by resting the plate bottoms tilted to one side.
8. UV sterilize 30 minutes to 1 hour while drying.
9. Aspirate off any remaining liquid.
10. Store plates at 4°C for up to 6 weeks.

Note: This procedure will expose plates to 1 µg collagen/$cm^2$
Use 1 mL for 35mm plate, 1.5 mL for 60mm plate

**IMPORTANT; UV treat plates for 10 minutes prior to use.**

## Coating Plates using Fibronectin/Collagen/BSA coating Solution

(This method was provided with the CFBE parental cells)

Material

- 100 ml LHC basal medium (Biosource Int'l/Biofluids 118-500 or Life Technology #12677019)
- 10 ml BSA (Bovine serum Albumin, 1 mg/ml) (Biosource Int'l/Biofluids 343-020)
- 1 ml Collagen I, bovine (2.9 mg/ml) (Fisher CB-40231)
- 1 ml Human fibronectin (1 mg/ml) (Fisher CB-40008A)

To coat T-25 flasks with fibronectin/collagen/BSA coating solution,

1. Add 2 mls of coating solution to a T-25 flask.
2. Stack your supply of coated flasks on a flat tray.
3. Transfer the tray to an incubator and allow the coating solution to sit for a minimum of two hours or overnight.
4. Take the tray out of the incubator and remove the excessive coating solution from each flask.
5. Flasks should be allowed to dry flat in a tissue culture hood for at least one hour.
6. Coated flasks are stored at room temperature in their original bags.
7. Coated plasticwares are generally stored for less than 1 month.
8. A similar procedure is followed for preparing all culture flasks and culture dishes for growing our epithelial cell lines.