Trachea and bronchial epithelial cells

From: Duke/UNC/UT/EBI ENCODE group
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1. **Source of cells:** National Disease Research Interchange-provided fresh lung tissue, designated DD009C in-house. Primary tracheal and bronchial epithelial cells were harvested using protease XIV dissociation for culture on collagen coated plastic in BEGM medium as described in detail (ML Fulcher et al., Well-Differentiated Human Airway Epithelial Cell Cultures. Methods in Molecular Medicine. J Picot Ed. Humana Press, 2005.). At 70-90% confluence the cells were trypsinized and cryopreserved as P1 cells. For the current experiments, cells were thawed and replated on plastic in BEGM medium and again grown to 70-90% confluence, at which point they were trypsinized and passaged to porous 30 mm diameter collagen IV coated Millicell CM tissue culture supports in ALI media as passage 2 cells. The staff of the UNC CF Center Tissue Procurement and Cell Culture Core maintained cultures until they were turned over to Scott Randell on day 5 (2/3 of the culture wells) and on day 40 (1/3 of the wells). Cells were subjected to RNA, protein harvest as well as an adaptation of the FAIRE protocol as specified in S. Randell lab notebook.

2. **Lineage of cells:** Passage 2 primary, non-immortalized, human trachea and bronchial epithelial cells

3. **Donor age/sex:** 21 y.o. male

4. **Karyotype:** unknown, presumed normal diploid

5. **Media for cell lines:** Passage 0 and 1 cells grown in bronchial epithelial growth media (BEGM) on collagen 1/3 coated tissue culture plastic and P2 cells were grown in air-liquid interface (ALI) differentiation media on type 4 collagen coated 30 mm Millicell CM inserts for 5 or 40 days, to poorly- and well-differentiated phenotypes, respectively.

6. **Growth conditions:** 37°C, 5% CO_2, humidified

7. **Protocol of cell growth:** see detailed protocols in Book Chapter below

8. **Cell passages:** Cells from LN2 storage at passage 1 and grown to passage 2

Cell growth protocol

Note: for detailed protocol, see: ML Fulcher, SE Gabriel, KA Burns, JR Yankaskas and SH Randell. Well-Differentiated Human Airway Epithelial Cell Cultures. Methods in Molecular Medicine. J Picot Ed. Humana Press, 2005.

Materials:
- in-house made BEGM (growth) and ALI (differentiation) medias
- 0.1% Trypsin with 1mM EDTA in PBS (1X)
- Soybean Trypsin Inhibitor 1mg/mL in F12 (1X)
- Sterile 1X PBS
- Collagen coated tissue culture dishes
- F12 media
- Human placental collagen coated Millicell inserts (Millipore PICM03050)

1. Thaw frozen vial of cells by gradual addition of F12 media to dilute DMSO. Spin cells at 1500g and resuspend in F12 for counting.
2. Count cells and plate at 1-3M viable cells in a collagen coated p100 dish in 10mL BEGM.
3. At 24 hours, rinse plate with PBS and add fresh media. Change media every other day until ready to passage.
4. When ~90% confluent, trypsinize cells using 0.1% Trypsin with 1mM EDTA and collect into a centrifuge tube; add an equal volume of Soybean Trypsin Inhibitor.
5. Spin cells at 1500g and resuspend in F12; count and seed at 1.3M per Millicell insert in ALI. Use 1-2mL media inside insert (apical side) and 10mL outside insert (basolateral side) in a containing p100 dish.
6. Rinse apical side with PBS and change media every day until confluent.
7. At confluence, aspirate apical side of insert and continue changing media on just basolateral side every day until harvest.