Primary Human Tracheal Epithelial Cells (pHTE)

From: Duke ENCODE group
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1) Source of cells: Primary cells were harvested from human donor trachea post-mortem by Dr. Cal Cotton at Case Western Reserve University, using previously published protocols (Davis et al., Am J Phys, 1990). At the end of protocol cells were shipped and passaged no more than one time before harvesting.

2) Lineage of cells: Primary

3) Donor information: Organ donors, male and female.

4) Protocol for harvesting

<u>Day 1</u>
Reagents:

- HBSS: Hank's Balanced Salt Solution, Ca2+- and Mg2+-free
- Tissue collection: 150-ml container + HBSS containing AM/AB, keep at 4°C.
- Protease solution: 1mg/ml Sigma protease XIV (P5147) in Ca2+- and Mg2+-free HBSS. Store at -20°C. Add 1X amphotericin B/antibiotic (AM/AB) before use.
- EDTA solution: 10mM EDTA in HBSS, pH to 7.2. Filter sterilize. Store at -20°C.
- BEGM complete medium with supplement from Lonza (CC-3171)

1. Place trachea in Petri dish, and use scissors and forceps to clean off fat, nodes, vessels.
2. Use scissors to cut open the trachea cartilage side (this will leave a section of trachea with the smooth muscle in the center with the cartilage on either side).
3. In a clean container, use cold HBSS (~ 50 ml each time) to clean the trachea few times until it is clear of debris.
4. Aspirate the last rinse of HBSS, and add 50 ml of protease solution with AM/AB.
5. Replace cover and incubate at 4°C for 16 hr.

<u>Day 2</u>
Reagents

- Warm EDTA solution.
- Filter cup with 100µm mesh.

- FBS
- Rinsing medium: DMEM + 10% FCS in autoclavable squeeze bottle.

1. Squirt 5-6 ml EDTA solution to moisten the filter mesh.
2. Hold onto the trachea with forceps and pour the protease solution through the mesh filter.
3. Place the trachea in a 50-ml tube with 40 ml EDTA solution. Replace lid and incubate the tube in 37°C water bath for 15 mins.
4. Collect the filtered protease solution from the filter cup to one 50-ml conical tube. Add 1 ml FBS to neutralize the enzyme activity.
5. Collect the EDTA solution incubated with the trachea. Add 1 ml FCS to the solution, and pour through mesh filter.
6. Place the trachea in a Petri dish with epithelial side up. Gently run metal spatula's flat side on epithelium (perpendicular and parallel). Repeat scraping in opposite direction. After each series of scrapes, pick trachea up with forceps and rinse with stream of rinsing medium from squeeze bottle. Pour the medium with scraped cells through mesh filter.
7. Repeat scraping and rinsing until no more cells are coming off. Pour the medium with scraped cells through mesh filter.
8. Transfer the filtered medium in filter cup to 50-ml conical tube(s).
9. Centrifuge at 1200 rpm (200×g).
10. Aspirate the medium but 5ml from each tube. Combine all the cells from the same donor into one tube. Wash again at 1200 rpm (200×g).
11. Count the cells and check the viability (only count epithelial cells, RBCs).
12. Resuspend the cells in DMEM + 10% FCS, and seed at ≥ 5×104 cell/ml.
13. In the next day, gently wash the culture with warm PBS to remove RBCs. Replace with 10 ml BEGM complete medium (with supplement) per 10-cm Petri dish.

5) Protocol for Passaging. Growth conditions: cells are grown at 5% CO2 on Primaria primary cell culture dishes; media changed every 2-3 days. Passaging/subculture: Cells are scraped off trachea and plated – this is passage 0. To subculture we use HEPES Saline solution (Lonza CC-5022) to media off cells, trypsinization with Lonza trypsin/EDTA (CC-5012). After typsinization, cells are rinsed with Trypsin Neutralizing Solution (Lonza CC-5002) and detached from plate. Cells can be passaged up to 3-4 times before they change morphology.