PREPARATION OF PRIMARY EPITHELIAL CELLS FROM HUMAN LUNG AIRWAY

- By Mary Brummet

1. Coat flasks/dishes with collagen. Vitrogen (diluted 1:15 in HBSS)
2. Clean Specimen:
    a. Using forceps and scissors, cut away remaining parenchyma attached to the outside of the bronchi. Specimen can be cleaned in sterile Petri dishes with HBSS. Perform all steps in the sterile hood and use universal precautions when handling human tissue.
    b. Cut all bronchi longwise to expose inner surfaces of the airway
    c. Place all pieces of airway into a 50ml conical tube containing 20ml of HBSS. Shake to rinse away extraneous cells and debris.
3. Pronase Digestion:
    a. To 50ml of F12 media add: 1mg/ml of Pronase, 0.5ml Pen-Strep, and 0.1ml fungizone. Sterile filter to a clean 50ml conical tube.
    b. Place pieces of cleaned airway into 40ml of the media. Incubate $4^{o}C$ for 24 hours.
4. Isolate cells:
    a. Add 8ml of FBS to the pronase tube. Transfer pieces of airway to a Petri dish. Rinse the inside of the airway many times with F12media+20%FBS using a 5ml pipet.
    b. Transfer suspension to a 50ml conical tube and repeat the washing process again with fresh media.
    c. Pellet the cells at 1200rmp, 8min, $4^{o}C$
    d. Resuspend the cells in F12/DMEM (1:1 ration) +20% FBS, +1%pen-strep, + .002% fungizone.
5. Culture:
    a. Count cells suspension.
    b. Seed approx. $2x10^{6}$ per T75 flask
    c. Incubate $37^{o}C$+ 5% CO_2. Change media every other day.

d. Incubate cells approx. 1-2 weeks or until confluent.

6. ALI Culture:
    a. Collagen coat PET membrane inserts (Fisher 35-3090) in 6 well plates (Fisher 35-3502). Add 1ml vitrogen (1:15), coat membrane, remove excess. Allow the wells to dry for 1hr before using.
    b. Once cells are confluent, harvest with 0.25% trypsin/EDTA. Resuspend in DMEM/BEGM (1:1) plus singleQuots (Lonza CC-3170). Count cells.
    c. Seed cells $2x10^5$ on collagen coated PET membrane inserts. Seed cells with 2ml of media in apical chamber and 3ml of media in the basal chamber. Continue incubating 37^oC+ 5% CO_2. Next day remove media from apical chamber. Change basal media every other day until cells are confluent, usually about 14 days.