Transfection of IMEC cells using SuperFect Transfection Reagent

Experimental Design Considerations:

- IMEC (Immortalized Mammary Epithelial Cells) were immortalized in Dr. Myles Brown's laboratory at the Dana Farber Cancer Institute by stably transducing them with a telomerase gene expression vector. Therefore, these cells are not transformed (they presumably have not accumulated associated genetic alterations, although p16 is likely to be hypermethylated) and should be treated like primary cells. See "Culturing IMEC cells".
- Qiagen (the SuperFect vendor) recommends that when transfecting primary cells, the amount of DNA and SuperFect should be cut in half relative to the amounts typically used with tumor cells. We have previously reported that the simple introduction of fairly significant amounts of plasmid DNA into cells (as occurs during transfection) causes specific signaling to cell cycle and apoptotic pathways in tumor cells (see Rodriguez A, and Flemington EK. Transfection mediated cell cycle signaling. Anal Bio 1999; 272:171-181). This signaling is likely to be more significant in primary cells since apoptotic signaling has not been altered by genetic lesions (as are frequently observed in tumor cells). Therefore, the lower amounts of DNA (and the associated amount of SuperFect) used to transfect primary (and IMECs, in our case) probably reflects a need to decrease DNA dependent cell cycle and apoptotic signaling by decreasing the concentration of DNA that enters each cell.

Protocol

- Split 70-80% confluent IMECs 1:5 onto 100mm plates (See **Note, below)
- Two days later, change media (AM, ca. 4-6 hrs prior to SuperFection).
- Dilute 5ug (total) DNA in 300ul DME (Dulbecco's Modified Eagles) medium (although IMECs are cultured in MEGM**, MEGM should not be used here because it contains proteins – lipid/DNA encapsidation must be carried out in the absence of proteins or antibiotics) and transfer to 5 or 15ml sterile tubes.
- Add 30ul SuperFect Reagent (Qiagen, Cat# 301305) to each DNA sample. Mix each tube immediately after adding SuperFect Reagent.
- Let stand at room temperature for 10 minutes.
- While complex is forming, replace media on plates with 8ml 1X PBS.
- Take off 1X PBS from plates one at a time. As soon as 1X PBS is taken off, add 3 ml MEGM [+Bovine Pituitary Extract + 10% Fetal Bovine Serum (FBS) (note: although cells are not cultured using FBS, FBS greatly stabilizes the lipid/DNA complex and increases transfection efficiency so we include it during transfection only)] to the DNA samples, pipette this solution up and down 3-4 times times (try not to generate bubbles) and then transfer immediately to the plate without PBS.
- Take 1X PBS off plates one at a time and immediately replace with the lipid/DNA solution.
- Incubate for 2-3 hrs at 37^{o}C in 5% CO_2 tissue culture incubator.
- Take off transfection solution and wash 1X with 1X PBS.
- Add 10ml MEGM (with bovine pituitary extract) and culture at 37^{o}C, 5% CO_2 for desired time.

**Note IMECs must be cultured in MEGM media (Bio*Whittaker, Cat# CC-3051)[supplemented with bovine pituitary extract (supplied by vendor)]. In addition, cells must be split using the trypsin pack from Bio*Whittaker (Cat# CC-5034) as per vendor's instructions (the trypsin we use for tumor cells will kill these cells in no time).