

Protocol for Growing NHBE cells:

1. Seed P1 passage cells in 6, T-75 flasks. Change media every other day (BEBM + aliquots = BEGM).
2. Split to P2 on the 6 day after seeding. Can split each flask 1:6 or more as per requirements.
3. When they are 75 – 80 % confluent (may take 5-6 or even 7 days), seed NHBE on transwells this passage will be P3.
4. Seeding density for 12 well transwell plates 150,000 per well for the 24 well transwells use 50,000 per well.
5. Media for seeding NHBE is very important, use 50:50 media + 50nM RA to seed them. Generally any volume from 100 – 400 μ l is ok for seeding as per my experience in the top compartment and add 1.5 ml of 50:50 media alone in the bottom compartment. Count the cells quickly, don't leave them in the hood but keep the tube in the incubator whilst counting. Use the p1000 pipettors to mix up/ break up the cells avoid the harsh shaking or the tapping along the stand. This may have worked for others but the pipettor method worked for me. I can show you how I did it when I seed this batch.
6. Change media (i.e. 50:50 + RA) the next day after seeding. 0.5 ml in the top and 1.5 ml in the bottom compartment.
7. Change media every other day till cells are confluent, this takes 2 days only, but I can confirm this better once the next batch works (keeping our fingers crossed).
8. After the cells look confluent enough remove the apical or top surface media and change media every other day in the bottom compartment (1 ml each time). Setting the time you did air-liquid interface as day 0 continue this till day 14 and you should be able to see mucus on the top surface. I can show you how the mucus looks with this batch and hopefully with the next too.

Glossary:

1. BEBM= Bronchial epithelium basal media alone.
2. BEGM= Bronchial epithelium basal media + aliquots.
3. RA= Retenoic Acid
4. DME low glucose = DMEM (cat #-12-707f from cambrex + glutamax from gibco) I will let you know how much of glutamax to add to each 500 ml bottle once it comes in. Glutamax is a more stable form of glutamine.
5. 50:50 media = First take a bottle of BEBM (without aliquots) and make a bottle of DME low glucose + glutamax. Then take half (250 ml approx of BEBM + 250 ml of the DME low glucose + glutamax) and to this full bottle of 500 ml add a full set of aliquots to make 1 full bottle of 50:50. KINDLY DONOT use any other method to make 50:50 as this saves aliquots and provides flexibility to add them fresh as required.

Note: Dr gray thought if the media is used with a few days there is no need to add fresh RA.