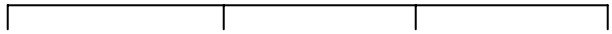
dsRNA Soaking

Materials:

1) T25 or T75 tissue culture flasks
2) 6-well tissue culture plates
3) 15-ml Corning conical tubes
4) Normal S_2 complete medium (S_2 containing 10% serum)
5) S_2 medium containing 2%, and15% serum
6) SFM medium
7) Trypan Blue (GIBCO, catalog number: 15250-061)

Time Line:



Day-1: Harvest cells from 3-day culture, and reseed $20x10^6$ in T75 flasks in 10ml S2 containing 2% or 5% serum, 25^0C overnight;
Day 0: Soaking
Day 1: Check viability of the cells
Day 2: Check viability of the cells

Protocol:

Day –1:

1) Harvest cells into using plastic pipette and pipette 5-6 times gently to remove the cells from the bottom of the flask. Visually check the flask to make sure proper removal of the cells. Transfer each flask of cells into two 15-ml conical tubes (equally distributed) and take 10μl of cells from one of the tubes using sterile tip for counting.
2) Spin the cells at 1000 rpm for 5 min. During the time of centrifugation, you can dilute the cells in trypan blue and count total number of the cells. (Each tube has half of the total number of cells)
3) Remove the supernatant; flick the tube gently to loose up the pellet. Resuspend one tube of cells in S_2 medium containing 2% serum (S_2/2%) and the other tube in S_2/10% to have final $10x\ 10^6$ cells/ml.
4) Label two T75 flasks with S_2/2% or S_2/10%, and add 8ml of S2/2% medium or S2/10% medium respectively. Then transfer 2ml of cell suspension to each of the flask (2% to 2%, and 10% to 10%, do not mix it up). Now you have two flasks, each contains 20×10^6 cells in 10ml of medium. You will keep the cells in 10% serum as your stock flask for future experiment, and the cells in 2% serum as your experimental flask for tomorrow's dsRNA soaking.

Note: Generally, you should have enough cells for 10 wells (in 6-well plate) for your soaking experiment from each flask you are seeding today.

Day 0: dsRNA Soaking

Note: Before setting up the experiment, take SFM and S_2 containing15% serum (S2/15%) out of the refrigerate and warm up to room temperature in the water bath. Dilute dsRNA to 2.5µg/µl in DEPC-H2O and keep on ice.

1) Harvest cells from S_2/2% flask into 15-ml conical tube as described above, spin down at 1000rpm for 5min.
2) Remove the supernatant, and flick the tube. Add 10ml of SFM and pipette up and down twice to resuspend the cells. Spin down again and remove the supernatant.
3) Resuspend the cells in 5ml of SFM, and take 10µl of cell suspension using sterile tip and count the cells. After you have the total cell number, re-adjust your cell suspension using SFM to have final 2 x 10^6 cells/ml.
4) Using **P1000** and sterile pipette tips, seed 1ml of the adjusted cell suspension into each well of the 6-well plate. Therefore, each well contains 2x10^6 cells in 1ml.
5) Add dsRNA to each well directly in the cell suspension -- use P20 to dispend 2µl to have final 5µg of dsRNA. (You may change the amount of RNA you want to treat your cells with.) After finish with one plate, rock the plate sideways at least 5 times each side to mix and do NOT swirl.
6) Return the cells (plate) to the incubator for 1hr.
7) Take the plate out of the incubator, add 2ml of S_2 containing 15% serum to each well using sterile 10-ml pipette. Again, rock the plate to mix, and the final serum concentration in each well is 10%. Incubate overnight.

Note: You want to count the cells after one wash in SFM. The reason is that cells stick to the plastic when there is no serum/protein around and you may loose some cells during SFM washing. Therefore counting cells before washing will not be accurate.

Day1 and Day 2: Check the viability of the cells

Note: Make sure you look at your cells under microscope before harvesting them and write down your observations. Generally this visual check gives good idea how your experiment worked.

1) After staring at your cells under microscope ☺, harvest cells by pipetting up and down for at least 6 times using 5-ml plastic pipette. The cells stick to the bottom of the plate a little bit so you want to either look under the microscope or just hold the bottom of the plate against light to see if you have harvested all the cells.

Note: When you hold the plate against light, the cells still attached to the plate will show up as some sort of cloudy stuff. Check with your TAs if you are not sure.

2) Spin the cells down at 1000rpm for 5min, remove the supernatant, and resuspend in 1 or 2ml of complete S_2 medium. Count live cells using trypan blue exclusion.