MPC Cell Culture

C. To Passage Plated Cells:

Once the cells become ~80% confluent, they should be passaged, or split. I passage once every 7 days and feed every other day. No need for collagen coated plates of plastic coverslips. If using glass coverslips, see either collagen coated of Alkonox-treated coverslips.

- Heat complete cell media, Hank's Ca/Mg free saline, and trypsin in a 37 °C water bath. After all solutions are warmed (~45 min) and the hood area is sterile.
- Remove all of the complete media from the cells, add 5-10 ml of Hank's Ca/Mg free saline (for a P10 or T75 flask) for 2-3 min, room temperature (RT).
- Remove all of the Hank's saline, add 4 ml Trypsin, swirl plate gently and let sit ~1 min until the cells begin to lift. During this incubation add 10ml of complete media to a 15ml Falcon conical tube.
- Lightly "blow-off" the cells using the Trypsin drawn up into a pipette and squirted back onto the cells to dislodge them from the surface of the plate. Remove all of the cells and solution with a pipette to the waiting 10 mls of complete media in the 15ml conical tube.
- Spin at ~75 xg for 5 min.
- Remove the supernatant carefully from the cell pellet.
- (If freezing, skip this section and go to step 9.) If plating, add 5 mls of fresh media, triturate 5-10x to loosen and disperse the pellet. Dilute the cells to desired concentration, either counting on a hematocrit (see below) or making 2-3 various dilutions of cells (1/3 dilution usually works well, make the dilutions with complete media and bring the volume to 15 mls for a T75 and to 10 mls for P10's (2.5 mls for 35 mm dishes).
- Place flasks into a 5% CO_2 incubator at 37 °C.
- If freezing, add 1-2 mls of freezing solution (see *Reagents, Solutions, and Media*) and triturate gently to loosen the pellet. If the cells are grown on a P10 and not too confluent, I use 1 ml and transfer the entire 1 ml to a 2 ml cryotube to freeze. If the cells are grown on a T75 and are quite confluent, I use 2 mls of freezing media and transfer 1 ml to each of 2 cryotubes. Freeze the vials in a quick chill unit for 1-4 hours in a -20 °C freezer, transfer to a -70 °C freezer overnight, and transfer to a liquid nitrogen freezer the next day. If you do not have a quick chill unit for freezing cells, you can use dry ice in a cooler to transfer the cells to the subsequent freezer. Any thawing in the freezing media may result in cell death when trying to thaw the cells in the future.
- Note: all other thaw/freeze reagents, see *Reagents, Solutions, and Media*)

To Replate Cells:

The following guidelines can be used to plate the cells:

T75 flask: Plate 0.75 - 1.8 x 10^6 cells/flask
T25 flask: Plate 0.25 - 0.625 x 10^6 cells/flask
35 mm dish: Plate 0.1 x 10^6 cells/dish

* Note: to collagen coat plates, see *Mixing Collagen* and *Collagen Coating Plates* protocols under *General Protocols for Cell Culture*