GD-O Cell Culture

B. To Passage Plated Cells:

Once the cells become ~80% confluent, they should be passaged, or split. These cells do not grow well if they are passaged too dilutely, and they easily lift and die if they are left to become too dense. Although I can only provide guidelines for confluency, you will have to determine how the cells work for you. These cells are also a bit clumpy and do not lend themselves well to counting, however, I recommend that you attempt to count them until you have a feel for the dilutions and the timing for passages. I always passaged once every 7 days and fed every other day. For electrophysiological experiments, all cells were replated the day before use onto collagen-coated coverslips and used within 24 hours of plating (due to the long processes that give a poor space-clamp). Cells were also differentiated with NGF (See *General Protocols: Mixing mNGF*) and replated (with trituration) onto collagen-coated coverslips the night before recording. (See Reagents, Solutions, and Media) Note: Dishes do not need to be treated for culture-only for recording and replating).

- Heat complete PC12 cell media and Hank's Ca/Mg free HBSS (see *Reagents, Solutions, and Media*) in a 37 °C water bath. Heat 10 ml of RPMI-1640 in a 50 ml Falcon conical tube for each flask/plate of cells to passage. After all solutions are warmed (~45 min) and the hood area is sterile
- Remove all of the complete media from the cells, add ~8 ml of Hank's Ca/Mg free saline (for a T75 flask) for 2-3 min, room temperature (RT).
- Remove all of the Hank's saline, add 5 ml of fresh Hank's Ca/Mg free saline to each flask and lightly "blow-off" the cells using the media drawn up into a pipette and squirted back onto the cells to dislodge them from the surface of the plate. **DO NOT USE TRYPSIN.**
- Remove all of the cells and solution with a pipette to the waiting 10 mls of warmed RPMI in the 50 ml conical tube. Triturate to mix gently a couple of times.
- Spin at 75 x*g* for 4 min.
- Remove the supernatant carefully from the pelleted cells.
- (If freezing, skip this section and go to step 9.) If plating, add 5 mls of fresh media down the side of the tube, triturate 5-10x to loosen and disperse the pellet. Transfer a small volume (about 0.1 ml from a 1 ml pipette) to a hematocrit to count the cells (see below). Calculate the amount of cells needed, make the dilutions with complete media and bring the volume to 15 mls for a T75 and to 10 mls for P10's (2 mls for 35 mm dishes). If plating throughout the week for electrophysiological recording, plate to T25's and add complete media to 5 mls. In general, when using a new line of cells, I make a number of different cell dilutions to plate at first: 0.1 ml of 5 mls cell suspension, 0.5 ml of 5 mls, 1ml of 5mls, 1.5 mls of 5 mls. This ensures that I don't lose a line due to over/under plating the cells.
- Place flasks into a 5% CO_2 incubator at 37 °C.

- If freezing, add 1-2 mls of freezing solution (see reagents) and triturate 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.

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