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

To Replate Cells For Recording:

The afternoon prior to the day of recording, remove coverslips in tissue culture dishes from refrigeration (plastic or glass). I remove all of the NGF-complete media from the T25 flask, and replace with 5 mls of NGF-complete media and gently blow-off the cells and lightly triturate to remove the long processes from the PC12 cells. I place about 1 ml of cell suspension on each of 5 treated coverslips (that have been pre-scored). Leave the dishes in the hood for about 15 min at RT to settle and attach onto the coverslips. Then, add 1.5 ml of complete media to each of the 35 mm tissue culture dishes and return to the incubator. (Note: I use a 150 mm plate to transport between the hood and incubator to reduce the likelihood of infection; once in the incubator, I move the 150 mm plate lid off to the side a bit to allow the air to equilibrate.) Try different dilutions of cells depending on how densely the cells are growing in the flasks when replating to give the optimal density for recording. Within a single day, there should be cells that can be located visually with the microscope that do not have processes more than 2 times the diameter of the cell body, and that remain completely isolated from any other cells. Greater trituration of the cell suspension results in better separated clumps of cells into individual cells and cells with shorter processes. I choose cells to record that are isolated and that are well-attached to the coverslip and have short processes leaving the cell body. Avoid any fibroblast-appearing cells as the PC12 cells are not a pure culture. Initially, I always try about 2-3 different dilutions of cells on coverslips treated with collagen.