Care and Passage of MCF-10A Cells In Monolayer Culture

1) Aspirate the Growth Medium (see Table 1), and rinse with 10.0 mls of Phosphate Buffered Saline (PBS).
2) Aspirate the PBS and add 2.0 mls of 1X Trypsin Solution (0.05% Trypsin: 0.53mM EDTA, Cellgro#25-052-C1). It is not advisable to use high concentration trypsin solutions when passaging cells.
3) Aspirate the trypsin leaving a thin film behind. The cells will tend to clump if the excess trypsin is not removed, making it difficult to obtain a single cell suspension.
4) Incubate in a 5% CO_2 humidified incubator at 37^{o}C for 15-25 minutes. Check the extent of trypsinization after 10 minutes, gently tapping the plate to dislodge the cells. Continue every few minutes. The cells should be completely dissociated from the plate to avoid clonal selection of adherent cells. Trypsinization may take up to 30-45 minutes.
5) Once cells are dislodged, add 1.0-2.0 ml. of Resuspension Medium to the plate (Table 1) and pipette to break up cell clumps.
6) Transfer the cells to a 15 ml conical tube and rinse the plate with another 1.0ml of Resuspension Medium. Add an additional 1.0ml of Resuspension Medium to the conical tube. Ultimately the cells are resuspended in 3-4 mls. If you are dealing with multiple plates, it is very important to process only 1 or 2 plates at a time. The cells will reattach if they are not resuspended in a timely manner after the serum is added.
7) Spin down the cells at 150Xg in a tissue culture centrifuge for 3-5 minutes.
8) Aspirate the medium and resuspend the cells in 1.0 ml of MCF-10A Growth Medium. Add 4.0-5.0 mls to the tube mix the cells and plate 1.0 ml cells per 10 cm dish in a total of 10 ml of MCF-10A Growth Medium (1:5 to 1:6 dilution). A 1:5 passage ratio will become confluent in 2.5 to 3 days and a 1:6 passage ratio will be confluent in 3.5 to 4 days.
9) Keep track of the passage number; cells may start behaving aberrantly in 3-D morphogenesis assays starting at passage 35.