Regular culture of ES cells

- Add 1 ml 0.2% gelatin solution in T25 flasks at room temperature for 5 minutes, remove gelatin solution, and add ~ 106 EF cells (irradiated) in DMEM + 10% FCS.

- 6-48 hours later, feeders should be confluent. (If not, more feeders can be added and ES cells thawed onto the feeders 6 hours later). Remove old medium and add 1-1.5 x 10^6 ES cells per flask in ES cell medium (day 0). Feed ES cells daily until colonies are large and dense, but not dying (Dying cells are bright cells on the surface of colonies visible under phase contrast microscopy). Cells will be ready to split 1 to 5 at day 3 or so.

- To pass cells, change medium, then 2 hours later wash cells once with HBSS, add 1 ml trypsin-EDTA solution, incubate at 37°C for 5 minutes, add 3 ml FCS-containing medium, pipette up and down 10 times. Spin down cells in a 15 ml tube at 150Xg and split cells 1 to 5 to fresh feeders.

  Or, to freeze cells, resuspend cells in 2.5 ml medium, add 0.5 ml to 5 cryotubes containing 0.5 ml freezing solution, freeze down cells at -70°C overnight then transfer into liquid N2. Each vial can be thawed onto a T25. If desired, cells can resuspended in a smaller volume and 3 times as many cells frozen in each vial. Vials can then be thawed onto a T75 and used for electroporations as described below.