MAKING FREEZEDOWNS OF CELLS

1. Thaw Trypsin at 37°C.
2. Aspirate off media and wash cells with PBS and aspirate off.
3. Add 1-2mls of 1X trypsin to each plate. Take off almost immediately for 293's. Leave on for about a minute for 3T3-L1cells.
4. Aspirate off the trypsin.
5. Blast the cells off the bottom of the plate with the correct amount of the 10%FBS
6. Break the cells up by pipeting up and then forcing them against the bottom of the plate a few times. This will prevent clumping of the cells when plated again.
7. Add enough DMSO to the cells to reach 10% total volume. This will prevent the cells from crystallizing when freezing.
8. Add 1.0-1.5mls of cell suspension to a cryovial.
9. Place the cryovials in a Styrofoam tray in a cardboard box and place in the -80°C for 24 hrs.
10. After 24 hrs, the cells are taken out and placed in the liquid nitrogen dewar of for long-term storage.
11. Log placement of cells on the Dewar log book.