STABLE CELL LINE PREPARATION
($CaCl_2$ transfection method)

1. Seed the cells at 1 x 10^6 cells per 10 cm petri dish in 10 ml DME/5% CS.

2. 24 hours later prepare the following (all should be fresh).
   - 2.5 M $CaCl_2$
   - 2X Hepes buffer saline
     - 280 mM NaCl
     - 50 mM Hepes
     - 1.5 mM Na_2HPO_4 (pH 7)
   - pH to 7.1-7.2
   - 0.1X TE pH 8.0

3. In an Eppendorf tube add 20 μg of the plasmid. Bring the volume to 450 μl with 0.1X TE.

4. Add 50 μl 2.5 M $CaCl_2$ and vortex. Let it stand in the Laminar flow hood for 5 min.

5. During this period prepare a 15 ml plastic centrifuge tube with 500 μl Hepes buffered saline.

6. While vortexing the 2X Hepes solution, add the plasmid solution drop by drop, but fast (in less than 30 seconds).

7. Let the mix stand for 30 min in the Laminar flow hood.

8. Add the mixture (1 ml final volume) to a 10 cm petri dish containing 9-10 ml DME/5% CS, drop by drop, covering the entire surface of the plate.

9. After 4 hours incubation in the CO_2 incubator it is possible to enhance the plasmid incorporation into the cells using a glycerol shock.

10. After 24 hours replace the media.

11. 24 hours later (48 hours after the time of transfection), trypsinize the cells and seed 1/10 or 1/5 of the total cells in 10 cm petri dishes. Add 400-1000 μg G-418/ml medium (the concentration of G-418 depends on the cell type).

12. Replace the media six days after transfection. If the selection is poor add a higher concentration of G-418.

13. The colonies should be ready 17-18 days after the time of transfection.

14. Trypsinize the individual colonies using sterilized plastic rings and transfer the cells to polypetri dishes. Feed with 1 ml DME/5% CS containing 400 μg G-418/ml.

15. When you have at least two confluent 10 cm petri dishes, store the cells in liquid nitrogen at a density 5 x 10^6 cells / ml DME/90% FCS.