SOFT AGAR CLONING TECHNIQUE

1. Prepare 0.5% agar medium:

For 500 ml:		
10x EBSS	5.5 ml	Mix and warm to 45°
Horse serum	11 ml	for 20 min before adding
20% HoS, DME	434 ml*	5% agar.
5% agar	50 ml	Heat to 100° before adding.

Incubate in 45° waterbath.
Allow for 18 ml 0.5% agar/plate.

2. Hybrid cell dilutions: for each line use 4 tubes labeled with line's name and marked 1, 8^{-1}, 8^{-2}, and 8^{-3}. To tube 1 add 0 ml, to others 0.7 ml of 20% HoS-DME. Hybrid cells should be healthy and in log growth. Suspend them and transfer 0.7 ml to tube 1 and 0.1 ml to tube 8^{-1}. Mix and do 8-fold serial dilutions by diluting 0.1 ml from the tube 8^{-1} into tubes 8^{-2}, 8^{-3} and discard 0.1 ml from 8^{-3} tube, so all contain 0.7 ml. Place in 37° bath. (It is best to use all these dilutions for first cloning. For subcloning, the '1' dilution may usually be omitted).

3. Label 100 x 15 mm petri dishes and pour with a 25 ml dispo pipette 15 ml of 0.5% Bacto-agar medium into dishes and allow to harden for 5-20 min before adding the soft agar overlay containing cells in 0.33% agar medium. Do not tilt plates during this period. Keep the remainder of the 0.5% Bacto-agar medium at 45° C.

4. Soft agar overlay: Pour an aliquot of 0.5% agar into a 50 ml plastic tube and place in a hot-cold styrofoam disposable cup containing 45° water in the hood. Remove tubes containing cells individually from the 37° bath. Add 1.4 ml agar with a 5 or 10 ml disposable pipette, pipette up and down to mix, and distribute most of the mixture (avoiding bubbles) onto the 0.5% agar base. It is essential during this step that neither the agar nor cells have cooled, otherwise a homogeneous 0.33% agar mixture will not be obtained.

5. Allow plates to harden for 30 min without moving (except for sliding in hood) and incubate at 37°C - 10% CO_2 for 7-14 days.

6. When discrete colonies appear, 'pick' clones with sterile pasteur pipettes (i.e., place tip over colony, suck up, using a clean pipette for each colony), and place cells in 150 µl of 20% FCS or HoS in DME (96 well Costar plates) or in 500 µl (24 well plates).

7. Incubate plates at 37°C - 10% CO_2 for 7-14 days.

*Estimate the volume by pouring into a medium bottle with graduations, then add other components. Can also use RPMI 1640, and 5% CO_2.