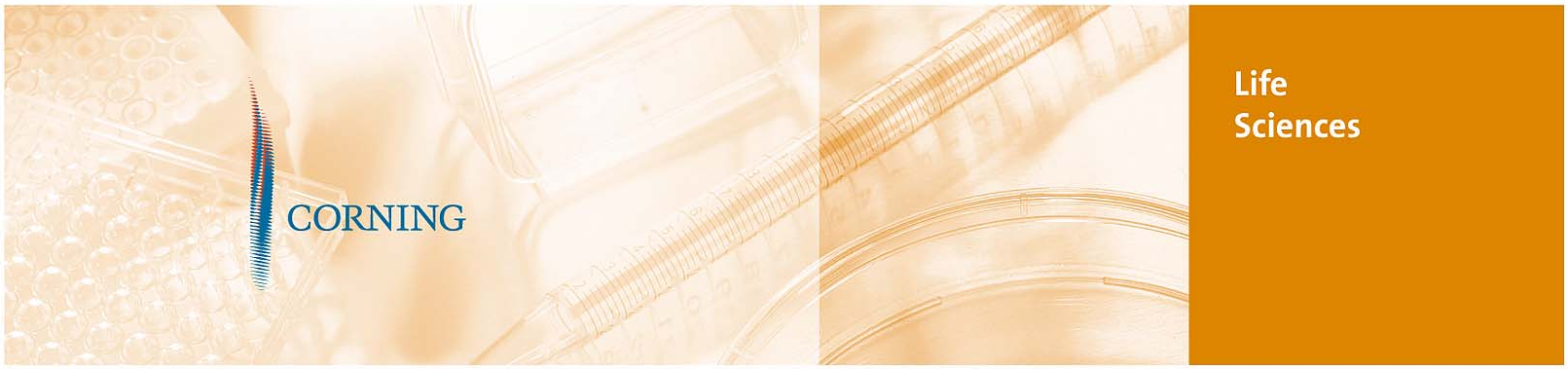
Single Cell Cloning by Serial Dilution



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Introduction

This technique is widely used for clonal isolation of hybridomas and other cell lines that are not attachment dependent. However, it is also very useful for cloning attachment dependent cells when the cell plating efficiency is very low, unknown or unpredictable. This method is fast and easy; however, like most clonal isolation methods, there is no guarantee that the colonies arose from single cells. Recloning a second time is advised to increase the likelihood that the cells originated from a single cell.

Supplies

Nonsterile

1. Pipetting aids - Corning Cat. # 4910 (1)
2. Disposal tray or bucket for used pipettes (1)
3. Marking pen (1)
4. 200µL pipettor - Corning Cat. # 4963 (1)
5. 8-channel multichannel 200µL pipettor- Corning Cat. # 4888 (1)

Sterile

1. Cell culture medium – (Appropriate culture medium for the cells that will be cloned) (30mL)
2. Cell suspension at $5x10^4$ to $1x10^5$ cells/mL (200µL)
3. 96 well cell culture plate - Corning Cat. # 3585 (1)

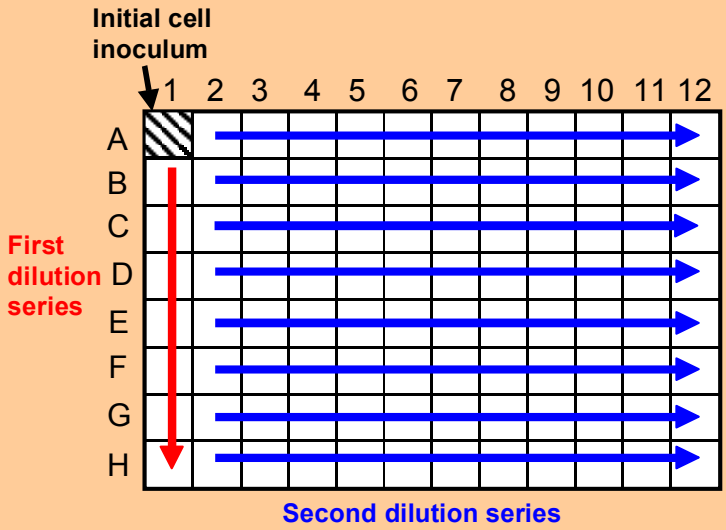
Corning offers a variety of multichannel pipettors for use in 96 well plates.

4. Sterile pipettor tips
5. Reagent dispensing reservoir/tray - Corning Cat. # 4870 or 4871 (1)
6. 1, 5, and 10mL pipettes - Corning Cat. # 4485, 4487 and 4488

Procedure

1. Fill the reagent dispensing tray with 12mL of the appropriate culture medium, then using the 8 channel pipettor add l00µL medium to all the wells in the 96 well plate except well Al (see diagram below) which is left empty.

Figure 1. Initial plate setup.



Adding 1000 to 2000 cells in well A1 ($5x10^3$ to $1x10^4$ cells/mL) is a good starting cell concentration. Increase this concentration for more difficult to grow cell lines.

2. Add 200µL of the cell suspension to well A1. (See Figure 1.) Then using the single channel pipettor quickly transfer 100µL from the first well to well B1 and mix by gently pipetting. Avoid bubbles. Using the same tip, repeat these 1:2 dilutions down the entire column, discarding 100µL from H1 so that it ends up with the same volume as the wells above it.

3. With the 8-channel pipettor add an additional l00µL of medium to each well in column 1 (giving a final volume of cells and medium of 200µL/well). Then using the same pipettor quickly transfer l00µL from the wells in the first column (Al through H1) to those in the second column (A2 through H2) and mix by gently pipetting. Avoid bubbles.

4. Using the same tips, repeat these 1:2 dilutions across the entire plate, discarding l00µL from each of the wells in the last column (A12 through H12) so that all the wells end up with 100µL of cell suspension.

Adding filtered conditioned medium (medium in which cells have been previously grown) to the wells can increase the cloning efficiency for difficult to grow cells.

5. Bring the final volume of all the wells to 200µL by adding 100µL medium to each well. Then label the plate with the date and cell type. Incubate plate undisturbed at 37°C in a humidified CO_2 incubator.

Transferring clones directly from a well in a 96 well plate into a T-25 flask is not recommended. The cells may be unable to grow due to their inability to condition the larger volume of medium in the flask. Using some conditioned medium when subculturing the cells for the first time will also help them survive and grow.

6. Clones should be detectable by microscopy after 4 to 5 days and be ready to score after 7 to 10 days, depending on the growth rate of the cells. Check each well and mark all wells that contain just a single colony. These colonies can then be subcultured from the wells into larger vessels. Usually each clone is transferred into a single well in a 12 well or 24 well plate.

Figure 2. Example of a stained (1% crystal violet) plate containing a dilution plating of CHO-K1 cells. The highest cell densities occur in the wells immediately surrounding the A1 position. Wells A10, D10, E6, E11, F7, G6 and H4 appear to contain single colonies.

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Acknowledgements

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