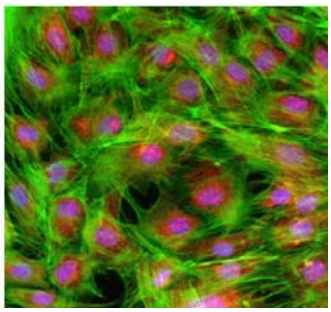
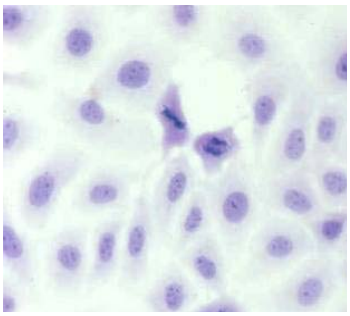
Use of Corning® Cloning Cylinders for Harvesting Cell Colonies - Protocol





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Introduction

Several approaches are available to isolate and develop a population of cells derived from the descendants of a single cell. The choice of method depends upon the type of cell to be isolated and the manner in which the clonal cultures were established. One of the simplest procedures is to encircle a colony with a small cloning cylinder and to harvest the cells within the cylinder in the same way as would be done in a flask or plate. These cylinders (glass, porcelain or stainless steel) are sealed to the plate with sterile silicone high vacuum grease to prevent leakage. The main advantage of this method is that the cells being picked have already demonstrated their ability to undergo cell division. The disadvantage is that there is no guarantee that the colony originated from only one cell.

Materials

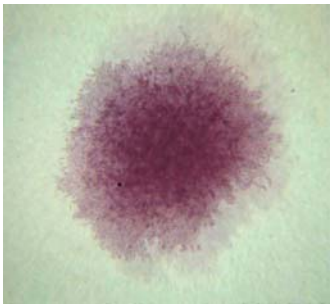
Sterile Supplies

1. Medium Forceps
2. Canister of short Pasteur pipettes or 200 µL micropipettor with tips
3. 1 mL pipettes (Corning Cat. #4485 or 4012)
4. 6 well microplates (Corning Cat. #3516 or 3506) or T-25 Flasks (Corning Cat. #430639)
5. 60 mm glass Petri dish (Corning® Cat. #3160-60) containing 10 cloning cylinders (Corning Cat. # 3166-8 or 3166-10)
6. 60 mm glass Petri dish (Corning Cat. #3160-60) containing Dow Corning® 976V silicone high vacuum grease (sterilize by placing a small amount in the bottom of a glass petri dish and autoclaving under standard conditions)

Medium and Solutions

1. Calcium- and magnesium-free phosphate buffered saline (CMF-PBS)
2. 0.25% Trypsin in CMF-PBS (or other dissociating solution suitable for the cells being picked)
3. Growth medium appropriate for cells being picked
4. 100 mm dish containing 20 to 40 colonies suitable for picking

Procedure



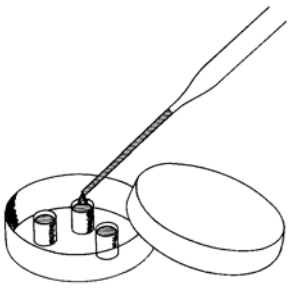
A colony of CHO-K1 cells suitable for picking with a cloning cylinder. This colony was fixed with methanol and then stained with 1% crystal violet in water.

1. Examine the culture dish containing well-isolated clones with an inverted or dissecting microscope. By manipulation of the intensity and angle of illumination it is possible to visualize the living colonies with a dissecting microscope, however, an inverted phase contrast microscope with a 4x or 10x objective is better.

2. Once satisfactory colonies have been located, draw a circle around them on the bottom of the dish with a marking pen. Select colonies that are of average size (very large colonies probably started from a clump of cells) and well isolated from other colonies.

3. Remove and discard the growth medium. Rinse the plate twice with CMF-PBS to remove any floating cells.

4. Using sterile medium forceps pick up a cloning cylinder. Gently press the flat bottom of the cylinder into the smooth silicone grease and remove with a sudden vertical motion. If done properly, this will give even distribution of grease on the bottom of the cylinder. Set the cylinder over a colony. Gently press the cylinder down evenly with the forceps. Uneven pressure will cause the grease seal to leak. Be very careful not to slide the cylinder across the colony. This will smear the silicone grease over the cells and prevent the trypsin from contacting them.



Trypsin is added to a cloning cylinder that has been sealed around a colony using sterile silicone high vacuum grease.

5. Verify positioning of the cylinder over the colony with the microscope. Make sure there are no other colonies within the sealed area in the cylinder.

6. Add about 0.2 mL of the 0.25% trypsin to the cloning cylinder.

7. Incubate the dish at 36.5°C for 5 minutes. Then examine cells under the microscope every two to three minutes until the cells have begun to round up and come off the dish bottom. Add a few drops of growth medium to the cylinder and gently aspirate the cells with a Pasteur pipette or micropipettor.

8. Transfer the cells to a suitable culture vessel and add the appropriate volume of medium. For large colonies, a T-25 flask or well in a 6 well plate can be used. For smaller colonies a well from a 24 or 12 well plate should be used. Diluting the cells too much will often result in slow or no growth. It may be necessary to rinse the cylinder with additional medium to remove cells left after the initial transfer.

9. Incubate cells as usual.

Acknowledgments

This protocol evolved from protocols developed for cell culture training courses at the former W. Alton Jones Cell Science Center in Lake Placid, New York; Manhattan College, New York City; and the University of Connecticut, Storrs, Connecticut. I would like to thank all of my colleagues and students who, over the years, have contributed ideas and suggestions to its development.

References

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2. Reid, L. (1979). **Cloning.** In: Methods in Enzymology, Vol. 58: Cell Culture. W. B. Jakoby and I. H. Pastan, Eds. Academic Press, New York, pages 152 – 164.

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