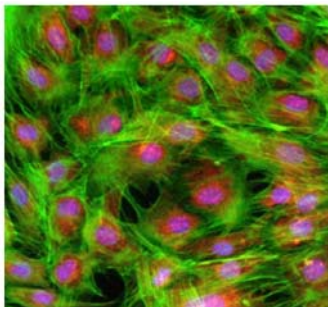
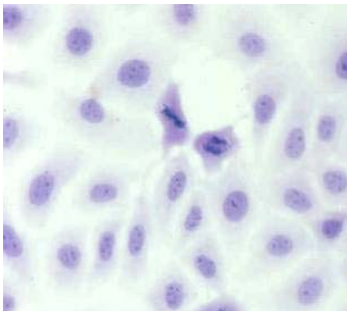
Single Cell Isolation Using Glass Chips

Protocol





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Introduction

Glass chips have two advantages over other clonal isolation techniques. First, visual observation is generally easier; the presence of a single cell can be easily verified. Second, if you have a steady hand, a fairly large number of chips can be picked in a short time period. A disadvantage of the method is that when working with primary cultures or cell lines that have a low cloning efficiency, many chips must be picked before one will be found that will develop into a colony. Another disadvantage is that preparing the chips is tedious.

Materials

Supplies

1. Watchmaker forceps
2. Stainless steel U.S. Standard soil sieves (1 mm and .45 mm diameters)
3. 24 well microplates (Corning® Cat. #3526 or 3524)
4. 60 mm culture dishes (Corning Cat. #430166)
5. Glass coverslips (Corning Cat. #2875-25 or similar)
6. Mortar and pestle (must be very clean)
7. Concentrated nitric acid
8. Glass beaker (1 L)
9. Glass tubes with metal caps

Medium and Solutions

1. Calcium-and magnesium-free phosphate buffered saline (CMF-PBS) for dissociating cultures

2. 0.25% Trypsin in CMF-PBS (or other enzyme solution suitable for cells being picked)
3. Growth medium appropriate for cells being picked.
4. Cell culture for harvesting

Procedure

1. Break coverslips into 1 mm^2 to 2 mm^2 fragments. (This can be done by a variety of methods. One suggestion is using a mortar and pestle.)
2. The fragments are then sifted through two stainless steel U.S. Standard soil sieves (1 mm and .45 mm diameters). The fragments retained on the .45 mm screen are then washed. The larger fragments can be collected and broken into smaller fragments and rescreened.

   SAFETY PRECAUTION: THE FOLLOWING STEP MUST BE DONE UNDER A FUME HOOD.

3. Put the coverslip fragments into a large PYREX® beaker and add equal volumes of water and nitric acid (add acid to water). Then bring to a fuming point (60 to 70°C) on an electric hot plate in a fume hood for 30 minutes. Remove beaker from hot plate to cool. When cool, decant the acid into the hood's cup sink with water running; rinse the chips thoroughly under cold running water for no less than three hours, then rinse six times in distilled water.
4. Transfer the sized coverslip fragments to glass tubes with metal closures and dry heat sterilize at 180°C for 3 hours.
5. Add 50 to 200 coverslip fragments to each of six 60 mm plastic tissue culture dishes and distribute evenly across bottoms.
6. Carefully add 3 ml of growth medium to each plate. Avoid floating the glass fragments.
7. Prepare cell suspension by the gentlest means possible.
8. Inoculate (using 1 mL of medium) duplicate dishes containing glass fragments with 1, 2, and 5 x 10^3 cells per dish. It is important to do a range of cell concentrations. If the initial cell concentration is too high most of the chips will have more than one cell. If it is too low then very few if any chips will have a cell.
9. After cells have had sufficient time to attach, (1 to two hours) look for coverslip fragments with a single adherent cell. Start with the cell concentration that has the best ratio of chips with single cells.
10. Using watchmaker forceps transfer those fragments with a single cell to separate wells of a 24 well plate containing 1 mL medium/well (Figure 1). Use conditioned medium if possible; it will increase the cloning efficiency as well as help the cells grow faster. Make sure the cell is on the top side of the glass chip after it is placed into the well or else the cell may be unable to divide.
11. Once the surface of the glass fragments is overgrown with cells (usually 5-10 days), it is best to disperse the colony by enzymatic treatment. The cells can then be replated in a similar culture vessel. When left alone on the glass

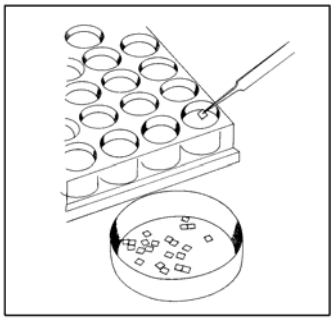

Figure 1. It takes a steady hand to pick up the glass chips and is initially quite challenging. But with practice it is possible to find and remove chips quickly and efficiently.

fragments, colonies will migrate onto the dish surface, but growth is usually slower in this manner.

Acknowledgments

This protocol has 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. Freshney, R. I. (2000). **Culture of Animal Cells: A Manual of Basic Technique.** Fourth edition, Wiley-Liss, Inc. New York, pages 194 – 213.
3. 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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