in case B after 48 h of digestion. Additional proteinase K treatment, up to 4 days, for cases A, C and D did not eliminate the artifact of inconsistent multiple bands in SSCP analysis. This suggests that factors other than cross-linked proteins may also contribute to the mis-incorporation. It has been reported that DNA from formalin-fixed, paraffin-embedded tissue tends to be fragmented (4,10). The “jump” of Taq DNA polymerase from one template to another has been observed when amplifying highly degraded DNA (9). Multiple extra bands detected in the amplification of the 256-bp fragments, but not the 172-bp products, suggests that such a “jump” may occur, in addition to mis-incorporation, in the amplification of fragments as large as 256 bp.

In conclusion, a high frequency of DNA polymerase infidelity was observed during amplification of poor DNA template from archived tissue. The fidelity can be markedly improved by longer proteinase K treatment in DNA extraction and by designing a study to minimize the size of the fragment to be amplified.

REFERENCES


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Easy Method for the Cloning of Mammalian Cell Colonies


Recent advances in immunology have led to a renewed interest in the development of tumor vaccines. Frequently, the patient’s own tumor cells are considered a source of antigens for the vaccine. One approach for obtaining sufficient tumor cells from limited clinical material is culturing cells from individual patients. In addition, the test-

Figure 1. Phase-contrast photomicrograph of B16 colony after isolation. A monolayer of B16 melanoma cells was irradiated by UV light for 1 min. A small area of cells was protected from the UV light by the black ink dot in the picture. After 48 h, there is some outgrowth of the “colony” past the confines of the spot.
Benchmarks

ing of a clinical response to vaccines often requires sufficient tumor to be used for skin testing or in vitro cytotoxicity assays. These tests require that the tumor cells be well-characterized and free of contaminating normal cells, such as fibroblasts. Too often in culture, potential tumor cells are contaminated by fibroblasts or other normal cells. These fast-growing normal cells can overgrow and cause loss of the tumor cell line. There are methods that can be used to rid cultures of fibroblasts (2). However, these methods are time-consuming, potentially damaging to tumor cells and not always effective. It is often easier to eliminate unwanted cells early in the culture when cells are growing as colonies.

Many methods used to isolate colonies in culture have also been described. Often these techniques require individuals skilled in the art of cell culture (1,3). Manipulation of colonies with cloning rings can frequently result in culture contamination. Alternatively, the procedures may require specialized equipment not always available to most laboratories.

Our laboratory routinely isolates tumor cell lines from clinical material with good success, using an easy method of colony isolation that is versatile and uses equipment that is readily available in most laboratories. In addition to its use in tumor-cell isolation, this method can be used for subcloning of colonies with a particular phenotype or selection of colonies that express a specific gene product during expression cloning.

This method uses selective killing of unwanted cell colonies by UV light. Adherent cell colonies grown in any culture medium and attached to either flasks or culture dishes can be used. The clones to be isolated are protected from UV irradiation by shadowing the colony with a black ink spot, placed directly under the colony by use of an ink marker. A spot of any size or shape can be placed, and there is no limit to the number of colonies to be isolated. The culture vessel complete with medium is placed upright on a UV transilluminator. The same transilluminator often used for DNA gels is used. The culture is irradiated between 1 and 2 min. The exact time may have to be determined experimentally for your particular cell type, but these times are more than sufficient to kill most cells including pigmented B16 melanoma cells. In addition, transilluminators have considerable variation in UV light intensity. After irradiation, the culture is returned to the 37°C CO₂ incubator. Noticeable cell death will begin 48 h after irradiation. Dead cells round up and detach, leaving the UV-protected colonies intact and viable (Figures 1 and 2). The isolated colonies can be allowed to grow out or can be trypsinized and placed in another culture vessel.

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Figure 2. Low-power digital image of crystal violet-stained B16 melanoma cells. A monolayer of B16 cells was protected from the UV light irradiation by drawing the patterns shown here with ink underneath the vessel. After 48 h, the dead cells were washed away, and the remaining cells were stained with crystal violet/methanol. The black ink was removed with ethanol before the cellular pattern was reproduced by scanning the multi-well plate on a flatbed digital scanner equipped with transillumination.