of the plasmid DNA prepared by this method is high enough for restriction mapping, polymerase chain reaction, nucleotide sequencing and transfection of eukaryotic cells. Moreover, our novel procedure should be particularly useful for the preparation of plasmid DNA from large numbers of samples.

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Color Coding the Cell Death Status of Plant Suspension Cells

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Evans Blue is a non-permeating dye with low toxicity in plant cells. It is a commonly used chemical to selectively stain dead cells (1,3). This technique has been used to determine the relative number of dead cells in a plant cell culture population. To quantify the ratio of dead cells accurately, it is necessary to count blue (dead) cells and clear (live) cells individually. However, staining of individual cells can be faint and fades with time. Recent research interest in apoptosis in plants necessitates an unambiguous method to determine the cell death status in cultured plant cells.

We have developed a technique to visualize the cell death status in color by utilizing epifluorescence. Soybean (*Glycine max* L., cultivar Williams 82) suspension cells were grown in a 12-well tissue culture plate with shaking. At appropriate times, the cells were stained with 0.05% Evans Blue (Sigma, St. Louis, MO, USA) for 15 min. After washing with phosphate-buffered saline to remove excess dye, cells were transferred to a glass slide and gently pressed with a cover slip. When the cells were observed with an Optiphot®-2 Fluorescence Microscope (Nikon, Melville, NY, USA) with the BV-2A filter combination (excitation filter 400–440 nm, barrier filter 470 nm, dichroic 455 nm), autofluorescent live cells appeared green, whereas Evans Blue-stained dead cells showed bright red fluorescence from the dye (Figure 1). There was a good correlation between blue/clear cells seen under natural light (Figure 1A) and red/green cells seen with fluorescence (Figure 1B). However, many Evans Blue-stained dead cells are only slightly blue (Figure 1C, arrows), which makes them diffi-

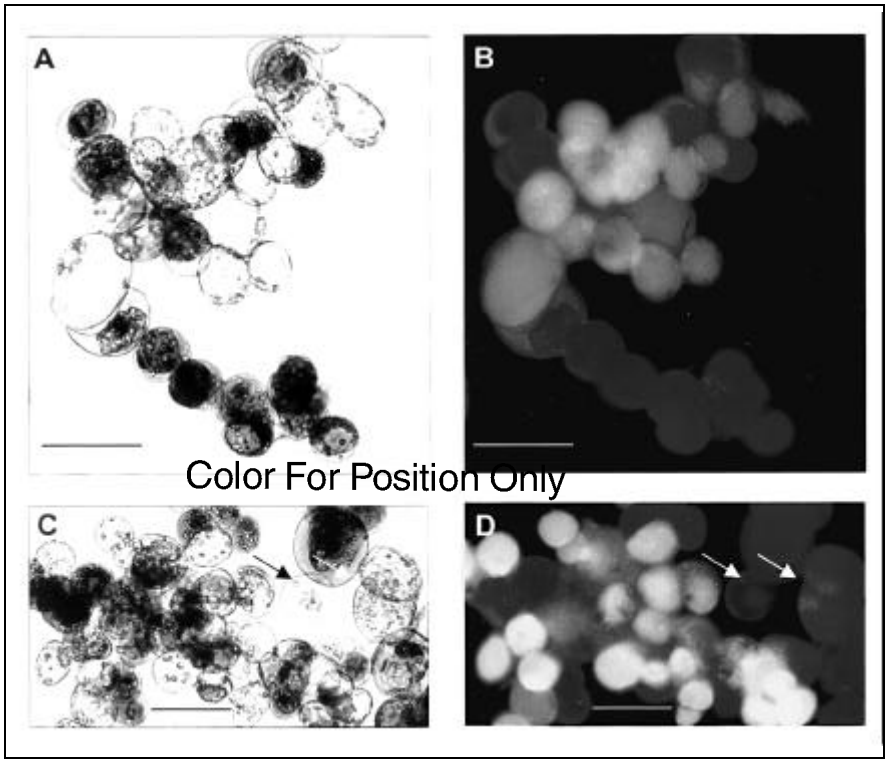

Figure 1. Soybean (*Glycine max* L., cultivar Williams 82) suspension cells were observed with a Nikon Optiphot-2 fluorescence microscope. Cells were treated with a phosphoinositide-specific phospholipase C inhibitor U-73122 (100 μM) for 1 h to induce cell death. (A and C) Cells stained with 0.05% Evans Blue for 15 min, observed with natural light. (B and D) Same samples observed with a fluorescence microscope equipped with the BV-2A filter combination. The cell death status of the cells indicated with arrows becomes clear with fluorescence. Scale bar = 50 μm.

cult to detect. In contrast, with fluorescence, these cells appear bright red (Figure 1D, arrows).

We have successfully used this technique on cultured suspension cells of two additional plant species, *Medicago truncatula* and *Nicotiana tabacum*. Unlike in soybean and *M. truncatula* cells, the green autofluorescence in *N. tabacum* was mainly from nuclei rather than the whole cells. However, the dead cells appeared clearly red as observed in other species tested.

Fluorescein diacetate (FDA), a compound commonly used in determining cell viability, was tested in soybean cell suspensions. In this method, intracellular esterases cleave FDA, producing fluorescein in live cells (2). We observed that the strong fluorescence from live cells could spill over to non-fluorescent dead cells, making it difficult to clearly distinguish dead cells from live cells, especially when cells are aggregated. Evans Blue does not have this problem. In addition, unlike in the FDA staining technique our method allows us to count dead and live cells at the same time with fluorescence. Therefore, this technique is especially useful when accurate and quick counts of both dead and live cells are necessary.

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