FIXATION OPTIMIZATION OF HEPATOMA CELLS FOR COMBINED β-GAL AND GFP EXPRESSION ANALYSIS

H. Oakley and G.A. Bulla. Department of Biological Sciences, Eastern Illinois University, 600 Lincoln Avenue, Charleston, IL 61920

ABSTRACT

Cells transfected with the green fluorescent protein (GFP) can readily be identified by fluorescent microscopy, but standard staining techniques for β-gal detection yield high background auto-fluorescence, making identification of GFP-transfected cells difficult. The goal of this research project was to determine an optimal staining technique that would allow identification of individually transfected mammalian cells (by monitoring GFP production by fluorescent microscopy) as well as allow the monitoring of β-galactosidase (β-gal) gene expression (using light microscopy of X-gal stained cells). Of several methods tested (including glutaraldehyde, acetone, methanol, formaldehyde and DMSO fixation), we found that acetone fixation for 10 minutes resulted in the lowest levels of cell auto-fluorescence. Thus, using this technique, we now have the ability to observe activation of β-gal expression upon transfection of transcription factors into cells. This technique should prove valuable in our ongoing studies examining the role of transcription factors in regulating liver-specific gene expression.

INTRODUCTION

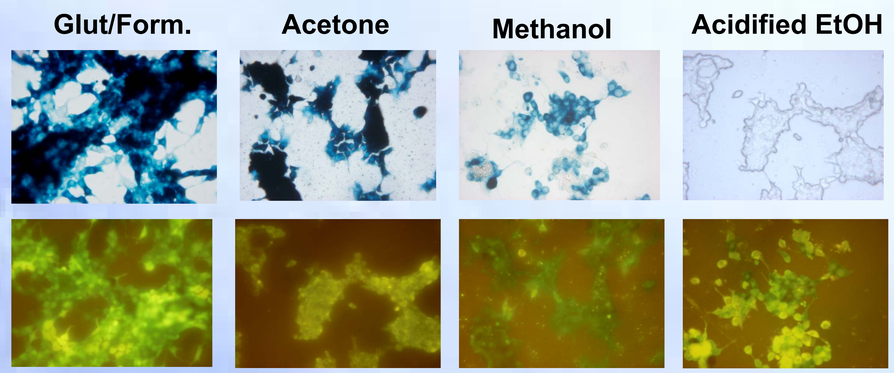
Standard cell fixation techniques result in high background fluorescence, depending on the cell type used. Techniques have been developed in which cells can be examined on a cell-by-cell basis. However, in order to carry out studies designed to understand the influence of gene expression on other cellular genes, a technique is needed to simultaneously identify cells that have "picked up" introduced DNA and also be able to monitor expression of other introduced genes in those cells. We focused our studies on detection of both GFP and β-gal in individual cells. Although β-gal staining of transfected cells works very well, it results in high levels of auto-fluorescence, making it difficult to detect GFP expression. The purpose of this research project is to determine the optimal staining technique for transfected cells. Our hypothesis is that one or more staining techniques can be developed which will allow for identification of cells that have taken up foreign DNA (by fluorescent microscopy) *and* for quantitation of expression of co-introduced DNA (by light microscopy).

METHODS

Cultured FTO2B hepatoma cells that contain a constitutively active β-gal expression cassette (called FT6.8-15 cells) were plated onto glass cover slips in 24-well plates. Once cells were attached, they were fixed for 5 minutes in the solutions described below, then rinsed two times with phosphate-buffered saline (PBS). Cells were then incubated with X-gal stain solution (4μM potassium ferrocyanide, 4μM potassium ferricyanide, 2μM $MgCl_2$, 0.4mg/ml X-gal in PBS) overnight at 37°C. Several fixing solutions were tested, including acetone, methanol, acidified ethanol, glutaraldehyde+ formaldyehyde, DMSO and Glacial Acetic Acid. The latter two fixatives resulted in severe cell damage and were not considered further. Cells were photographed using both fluorescent and light microscopy.

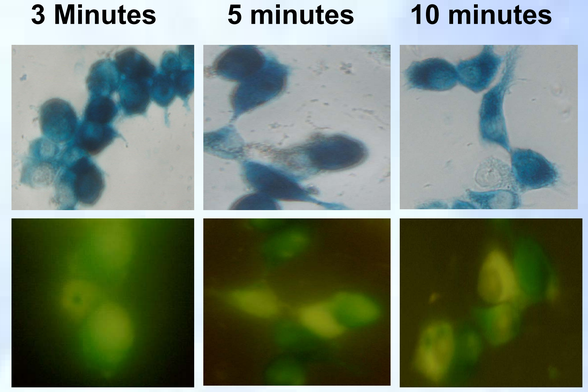
RESULTS

Fig 1. Comparison of cell fixation techniques



Cells attached to glass cover slips were rinsed with PBS and fixed for five minutes in 100% acetone, 100% methanol (4°C), or acidified ethanol. The 0.2% glutaraldehyde –1% formaldehyde method (left panel) is the standard staining technique used to detect β-gal. Cells were stained overnight at 37°C in the X-gal staining solution. Cells were then photographed using both fluorescent and light microscopy.

Fig 2. Optimization of fixation time using acetone



Cells attached to glass cover slips were rinsed with PBS and fixed for 3, 5, 10, 15 and 25 minutes in 100% acetone. The results obtained from the 15 minute and 25 minute time points (results not shown) were not significantly different from the 10 minute time point. Cells were stained overnight at 37°C in the X-gal staining solution. Cells were then photographed using both fluorescent and light microscopy.

CONCLUSIONS

Using fluorescent microscopy, we have employed several fixation methods in order to identify a technique that results in minimal background signal, while still allowing identification of β-gal positive cells. Of all of the methods tested, acetone fixation was found to produce the least amount of auto-fluorescence, yet still allowed a strong β-gal signal. Other methods tested, for example DMSO and glacial acetic acid, produced high background and also resulted in damaged cells. The time course of fixation was also tested and acetone fixation for ten minutes produced the best results. These conditions will next be applied to cells transiently transfected with both GFP and β-gal expression plasmids to allow both identification of transfected cells as well as levels of β-gal expression.

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