High efficiency gene transfer into mammalian cells by a double transfection protocol

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INTRODUCTION

The CMT expression system is a potent way of obtaining high expression in a protein of interest (1). The CMT cell is a derivative of COS cells, in which expression of SV40 large T-antigen is under the control of a metallothionein promoter, thus the accumulation of T-antigen in the cell is further enhanced by the addition of heavy metal ions in the medium. Obtaining the maximum transfection efficiency is one of the keys to successful expression. The protocols employing DEAE−dextran constitute one of the most commonly employed methods for transient expression in COS cells (2). We have obtained a several-fold increase in the efficiency of transfection simply by transfecting the cells twice.

MATERIALS AND METHODS

CMT cells were seeded in 10 cm-diameter petridishes to the confluence of 70% in DMEM containing 10% FBS. After rinsing with 10 ml of PBS, the cells were treated with 0.2 ml of trypsin solution (3) for 10 minutes at room temperature. 2 ml of the complete medium containing 200 μg of DEAE dextran, 100 μM chloroquine, and various amounts of the plasmid, RSV β-GAL (4), were added and incubated at 37°C for 4 hours, followed by DMSO-shock (10%) for 2 minutes. After the shock the cells were washed with 10 ml of PBS once and incubated in 10 ml of the complete medium. The next day the same transfection procedure was repeated. The cells were then incubated in the complete media containing 1 μM $CdSO_4$ and 100 μM $ZnCl_2$. The cells were incubated for 72 hours before staining for β-galactosidase expression (5). The percentage of cells expressing β-galactosidase activity was used as the measure of transfection efficiency.

RESULTS AND DISCUSSION

Transfection efficiency estimated by X-gal staining was increased several-fold over that obtained with the conventional, single transfection method. These data suggest that transfection efficiency is significantly increased simply by transfecting the cells twice. Similar results were obtained with COS cells (data not shown).

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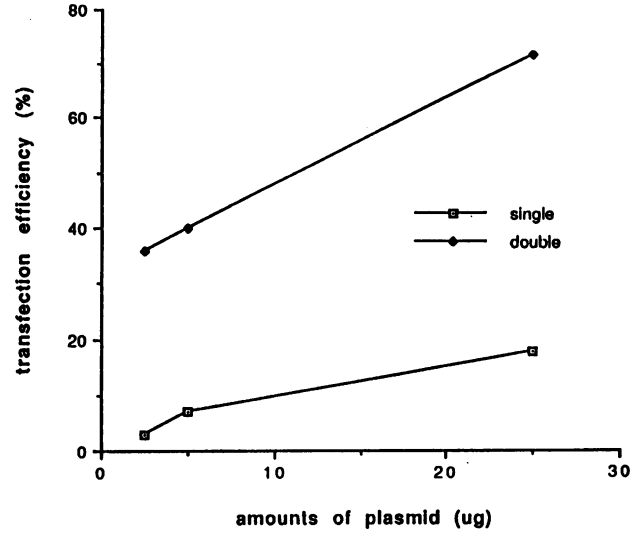

Figure 1. Transfection efficiency estimated with X-gal staining. Different amounts of plasmid were used per each transfection by the DEAE−dextran method. The protocol employed either single (single) or double transfection (double) steps. Two hundred cells were examined at six distinct locations in each plate for X-gal staining. Each transfection was performed in duplicate. Similar results were obtained in two independent experiments.