Volume 17 Number 12 1989 Nucleic Acids Research

Transfection of DNA into adherent cells by DEAE-dextran/DMSO method increases drastically if the cells are removed from surface and treated in suspension

Efim I.Golub, Helen Kim and David J.Volsky

Molecular Virology Laboratory, St Luke's/Roosevelt Hospital Center and College of Physicians and Surgeons, Columbia University, 432 West 58th Street, New York, NY 10019, USA
Submitted April 26, 1989

DEAE-dextran treatment followed by DMSO shock is one of the most effective procedures for introducing DNA into mammalian cells(1). The procedure is applicable to adherent cells and to cells grown in suspension(2). As a rule, adherent cells are treated without removing from surface. We show here that efficiency of DNA transfection into adherent cells can be dramatically increased if such cells are transfected in suspension.

We compared efficiency of transfection for different adherent cells treated with DEAE-dextran/DMSO either in a monolayer culture or in suspension. $2x10^6$ cells were transfected with plasmid pU3R-III (0.2ug) or pSV2-cat (0.5ug) which carry cat gene under the control of HIV-1 or SV-40 virus promoter, respectively (3,4). Transfection efficiency was estimated by measuring CAT enzyme activity in cell extracts(4) obtained 48h after transfection. **Treatment of HTB-139 cells with DEAE-dextran/DMSO in suspension lead to over 40-fold increase in transfection efficiency as compared to identical treatment in monolayer (Figure). Similar results were obtained with HeLa, NIH-3T3, HTB-148 and U251MG cells (data not shown).**

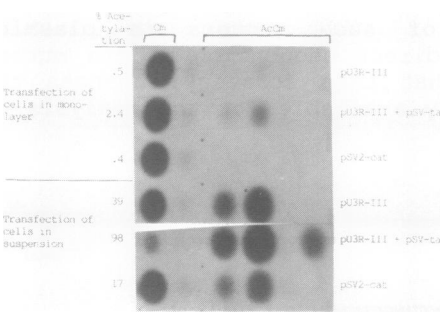

FIGURE: CAT activity in neuroblastoma HTB-139 cell extracts. For transfection in suspension, cells were removed by incubation with trypsin/EDTA. DEAE-dextran (100ug/ml) was applied for 1h at 37°C followed by DMSO shock(2). pSV-tat is an expression vector for HIV-1 tat gene whose product transactivates cat gene in plasmid pU3R-III(5).

SUPPORTED by grant AI 25902.

REFERENCES: 1) Lopata, M.A., et al. (1984) Nucleic Acids Res., 12,5707-5017; 2) Current Protocols in Molecular Biology (1989) J. Wiley and Sons, Inc., New York 3) Sodroski, J., et al. (1985) Science 227,171-173 4) Gorman, C.M., et al. (1982) Mol. Cell Biol. 2,1044-1051 5) Arya, S.K., et al. (1985) Science 229, 69-77.