Transfection Efficiency of Metafectene Pro in Murine M2-10B4 Cells and Comparison With Commonly Used Transfection Reagents

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Introduction:

M2-10B4 cells are a murine bone marrow stromal cell line which support MCMV replication to high titers *in vitro*[1]. Our studies of the replication of this virus have led us to investigate the effects of MCMV-specific siRNA on virus replication *in vitro*. In order to properly assess this, it is important that cells are transfected to high efficiency with the siRNAs under study. Therefore, we have compared a number of commercially available, lipid-based transfection reagents, including Metafectene Pro, for their ability to facilitate the transfection of a GFP-expressing reporter plasmid into M2-10B4 cells. These include the popular reagents Lipofectamine 2000, TransIT-TKO, Effectene, Super Fect and Lipofection.

Materials and methods:

Plasmids: pmaxGFP was obtained from Amaxa Inc.

Cell Culture: M2-10B4 cells were obtained from American Type Culture Collection and cultured in RPMI1640 medium supplemented with 10% fetal bovine serum, 4.5 gm/liter glucose, 1mM sodium pyruvate, 10mM Hepes and 1.5gm/liter sodium bicarbonate and penicillin/streptomycin

Experimental procedures / transfection protocol:

M2-10B4 cells were seeded into 24-well tissue culture plates and grown overnight to 80-90% confluency. Transfection of 2µg of pmaxGFP/well in duplicate wells, was performed using Metafectene-Pro, Lipofectamine 2000 (Invitrogen), TransIT-TKO (Mirus), Effectene and Super Fect (Qiagen) and Lipofectin (Gibco BRL) according to

the manufacturers' instructions. Uptake of the GFP-expressing plasmid was measured by flow cytommetry using a FACS Calibur flow cytometer (Beckton Dickinson) 48 hours following initiation of transfection and relative levels of fluorescence were compared for the various transfection reagents.

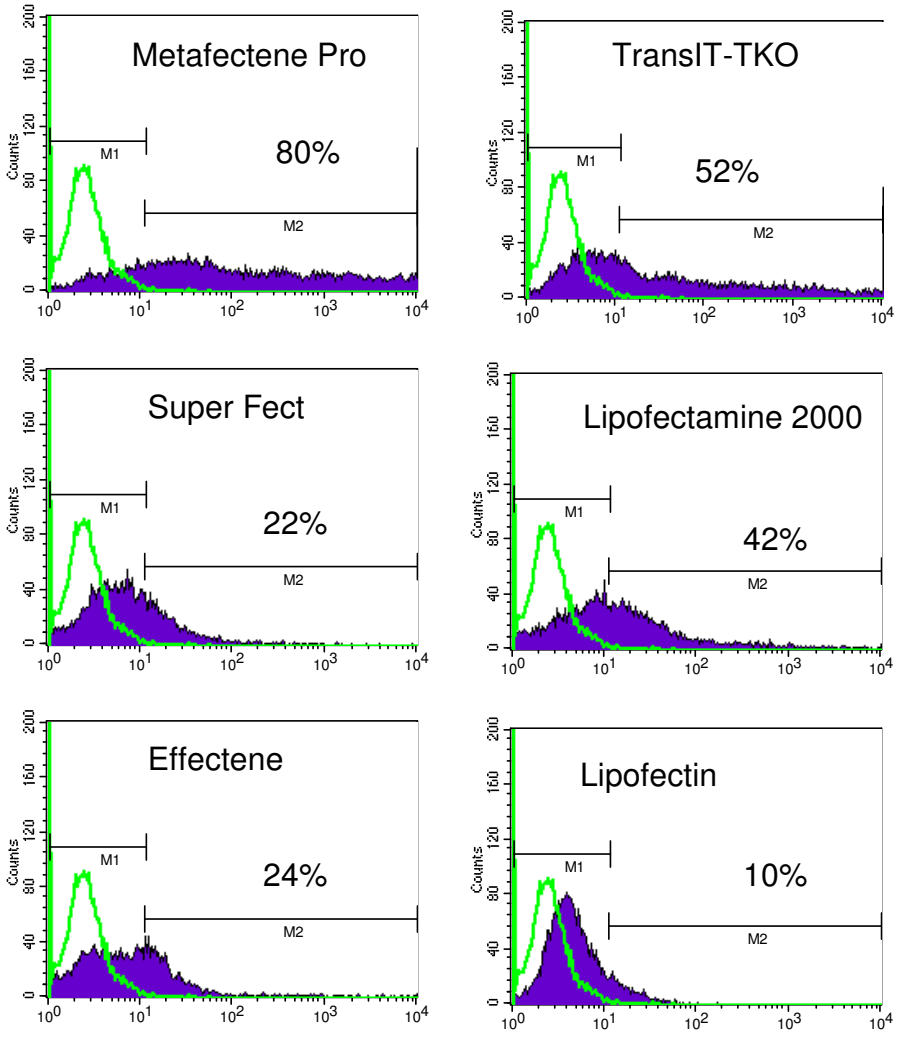
Results and discussion:

The highest levels of GFP fluorescence were obtained using Metafectene Pro (Fig 1) which routinely produced transfection efficiencies between 65-90% in M2-10B4 cells. Viability was also substantially increased over the other reagents, with viabilities normally over 90%. TransIT-TKO produced transfection efficiencies in the region of 40-60%, while Lipofectamine 2000 routinely gave transfection efficiencies between 25-50%. Neither Effectene, Super Fect, nor Lipofectin produced transfection efficiencies consistently above 30%.

Thus Metafectene Pro was the superior transfection reagent for our purposes and has been used in subsequent studies involving siRNA.

Figure 1

Representative flow cytommetry plots of GFP fluorescence (blue trace) in M2-10B4 cells, 48 hours following transfection of pmaxGFP using the indicated transfection reagents. Percentage values refer to the relative numbers of cells within the M2 window.



Conclusion / summary:

Metafectene-Pro efficiently transfects the murine bone marrow stromal cell line M2-10 B4 while producing minimal toxicity.

References:

1. Lutarewytch, M.A., Quirk, M.R., Kringstad, B.A., Li, W., Verfaille, C.M., and Jordan, M.C. “Propagation and Titration of murine cytomegalovirus in a continuous bone-marrow derived stromal cell line.“ *J. Virol. Meth.* 68: 193-198 (1997)