Comparative testing of METAFECTENE PRO (Biontex) versus Fugene 6 (Roche) for transfection of HeLa cells

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Experimental approach:

HeLa cells were transiently transfected with an expression plasmid carrying an internally truncated version of the human 7SK small nuclear RNA (snRNA) gene. Expression of the endogenous and mutant 7SK snRNAs was compared by Northern blot analyses. Localisation of the transiently expressed mutant 7SK snRNA and possible effects of the transfection reagents were tested by fluorescent *in situ* hybridization (FISH) and DAPI staining.

Transfection protocol:

About 4 x 10^5 HeLa cells were plated into each well of 6-well plates. After 4-6 hours, cells were transfected with either METAFECTENE PRO or Fugene 6 according to the manufacturers' instructions. For both METAFECTENE PRO and Fugene 6 transfections, various amounts of transfection reagent and plasmid were tested:

A: 1 µg plasmid, 2 µl METAFECTENE PRO
B: 1 µg plasmid, 3 µl METAFECTENE PRO
C: 2 µg plasmid, 4 µl METAFECTENE PRO
D: 2 µg plasmid, 6 µl METAFECTENE PRO
E: 1 µg plasmid, 3 µl Fugene 6
F: 2 µg plasmid, 6 µl Fugene 6

RNA was extracted from the cells 48 hours after transfection.

For fluorescent microscopy, transfection was performed identically, except that the cells were plated on gelatine-treated coverslips.

Results:

Transfection efficiency was determined by measuring the expression levels of the mutant 7SK snRNA by Northern blot analysis and PhosphorImager quantification. The obtained expression levels were corrected by the expression levels of the endogenous 7SK snRNA. At all concentrations, METAFECTENE PRO was at least two times more efficient than Fugene 6. While it was not possible to reach higher transfection rates by increasing the amount of plasmid and Fugene 6, the transfection efficiency of METAFECTENE PRO depended on the amount of both plasmid and reagent. When using 2 µg plasmid and 6 µl transfection reagent per well, the transfection efficiency of METAFECTENE PRO was almost 6 times higher than the transfection efficiency of Fugene 6.

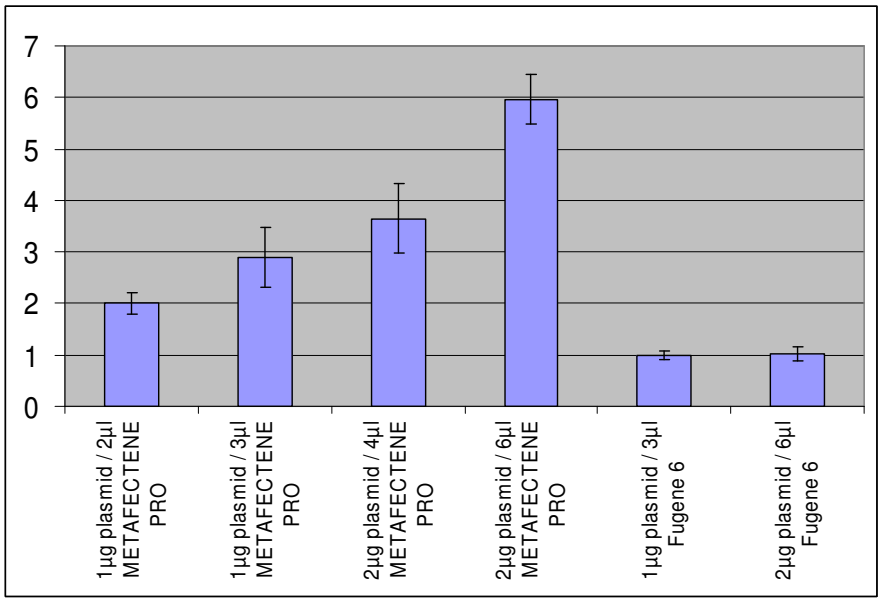

Figure 1. Comparison of transfection efficiencies of METAFECTENE PRO and Fugene 6. Transfection rates were determined by Northern blot analysis of the expression levels of a transiently expressed nuclear RNA (a mutant version of 7SK RNA). The expression levels were normalized to the expression level of the transiently expressed mutant 7SK RNA after transfection of 1 µg plasmid with 3 µl Fugene 6.

Analysis by fluorescent microscopy: DAPI staining revealed no significant toxic effect either for METAFECTENE PRO or for Fugene 6. Localisation of the transiently expressed mutant 7SK RNA corresponded to the localisation of the endogenous 7SK snRNA in all cases. The number of transfected cells was significantly higher after transfection with METAFECTENE PRO. The disadvantage of METAFECTENE PRO compared to Fugene 6 is that the transfection reagent-DNA complex is easily detectable around the cells as autoflourescent spots.