Use of Metafectene for the Transfection of Human Diploid Fibroblasts

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Optimization of Metafectene Transfection

Primary human diploid fibroblasts (HDF) were transfected with Metafectene under twelve different conditions in order to optimize the transfection efficiency. Both total micrograms of DNA and ration of Metafectene to DNA were varied. The most successful condition is described below for both 6-well plates and 60 mm dishes.

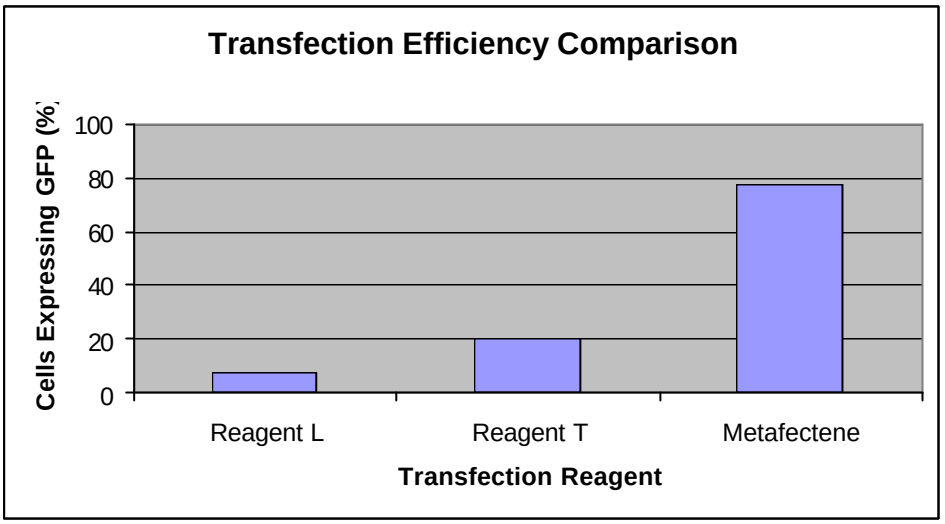
	6-well plate (per well)	60mm dish
cells plated	$1.5x10^5$	$4.5x10^5$
DNA	1 µg	3 µg
Metafectene	5 µl	15 µl
total volume	0.7 mL	2.0 mL

Transfection Protocol

The appropriate number of HDF cells were plated 24 hours prior to transfection and cultured in complete growth medium (DMEM + 10% fetal bovine serum + 1% penicillin + 1% glutamine (Gibco)) at 37°C. For transfections in 60 mm dishes, 15µl of Metafectene were diluted into a total of 250µl of OptiMEM (Gibco), and 3µg of DNA were diluted into a total of 250µl of OptiMEM. The dilutions were mixed and incubated at room temperature for 15 minutes. Cells were washed with PBS, and 1.5mL of OptiMEM were added to each plate. The Metafectene + DNA mixture was then added, for a total volume of 2mL. The transfections were incubated 37°C. After 5 hours, the transfection mixture was aspirated, and 5mL of complete growth medium was added to each plate. Cells were assayed 48 hours post-transfection.

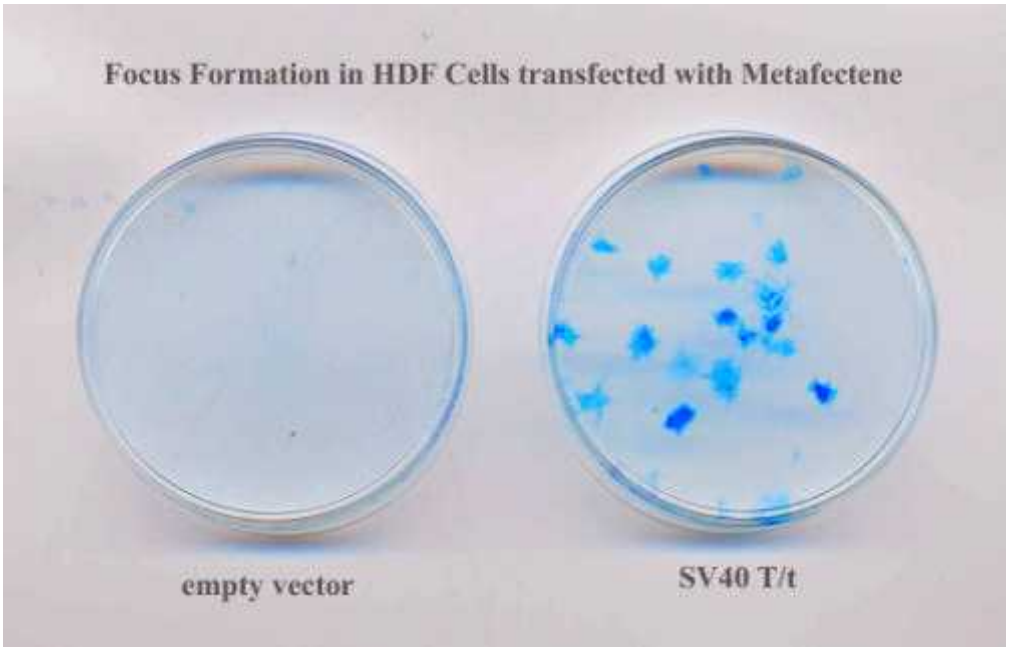
Comparison of Transfection Reagents

HDF cells were transfected with pEGFP using the conditions described above. Cells were also transfected using two other lipid-based transfection reagents (*reagent L* and *reagent T*) using the optimal conditions for each. 48 hours post-transfection, cells were collected and GFP-positive cells were counted using FACS. Below is a bar graph of the results.



Long-Term Viability of Metafectene-Transfected Cells

In order to determine if HDF cells transfected with Metafectene were viable over long periods of time, a focus-formation assay was performed. Occasionally, transfection reagents that produce results transiently fail to perform in focus formation or other long-term transformation assays. HDFs were transfected with either and empty vector or a plasmid encoding both large T and small t antigens of the SV40 virus. Metafectene-transfected HDFs did form foci after two weeks in culture. The results are shown below.



Conclusion

Metafectene can be used to transiently transfect primary human diploid fibroblasts. In a side-by-side comparison, the reagent performed better than others tested. In addition, Metafectene-transfected cells expressing SV40 large T and small t antigens are viable enough to produce foci after several weeks in culture.