Transfection of human HeLa cells

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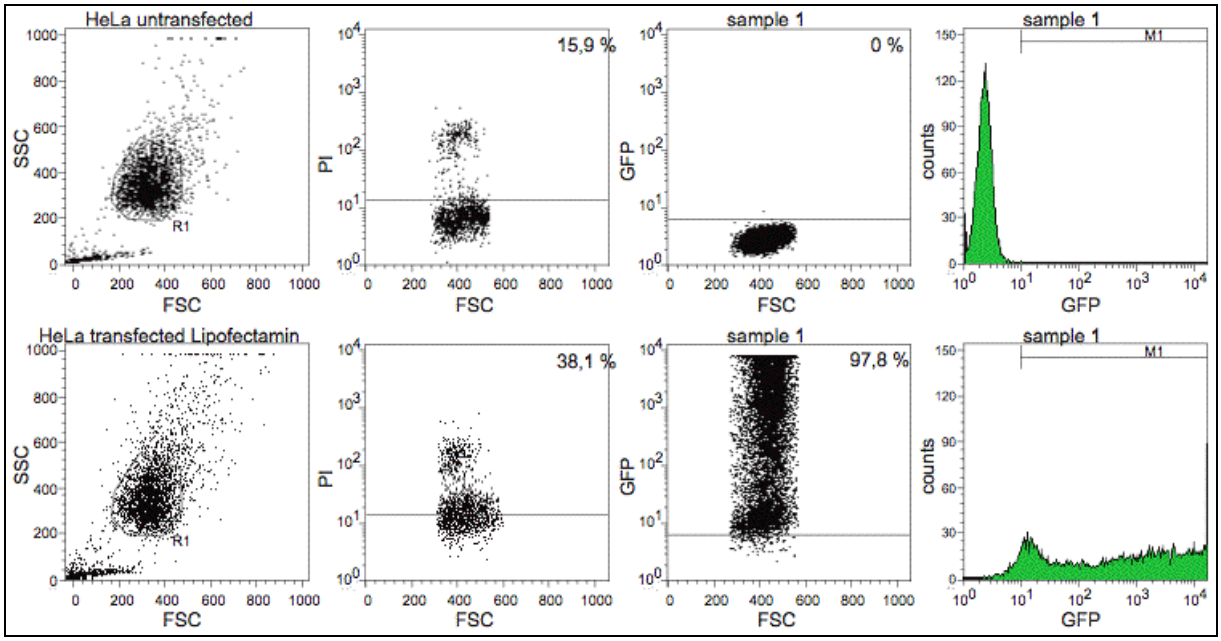
Introduction

From successful transfections, we await in our laboratory high transfection efficiency and low rates of apoptosis.

For HeLA cells we have already achieved a transfection efficiency of 87, 8 - 97,8 % with Lipofectamine. But unfortunately the survival rate of cells has only been 46,9 - 61,9 %. Please refer to Figure 1.



1 Transfection of human HeLa cells using Lipofectamine-transfection-protocol

Our target with METAFECTENE EASY has been to find another way to transfect the HeLa cells and possibly achieve higher survival rates, while having a comparable high transfection efficiency. Additionally we wanted to find a cheaper and faster method of transfection, because electroporation proved to be laborious and Lipofectamin to be expensive.

Materials and Methods

HeLa cells: Human epithelial cells derived from a zervix-carcinoma. Medium: 10 % FCS (PAA), 1 % Glutamin (100x), (Gibco BRL Paisley, Scottland), 1 % Penicillin/Streptavidin (10000 Units/ml / 10000 µl/ml, Gibco), 88 % DMEM (Dulbecos modified Eagel medium with Glutamax, Gibco).

Transfection

For the transfection we used the plasmid pmax-GFP (transfection control plasmid from Amaxa).

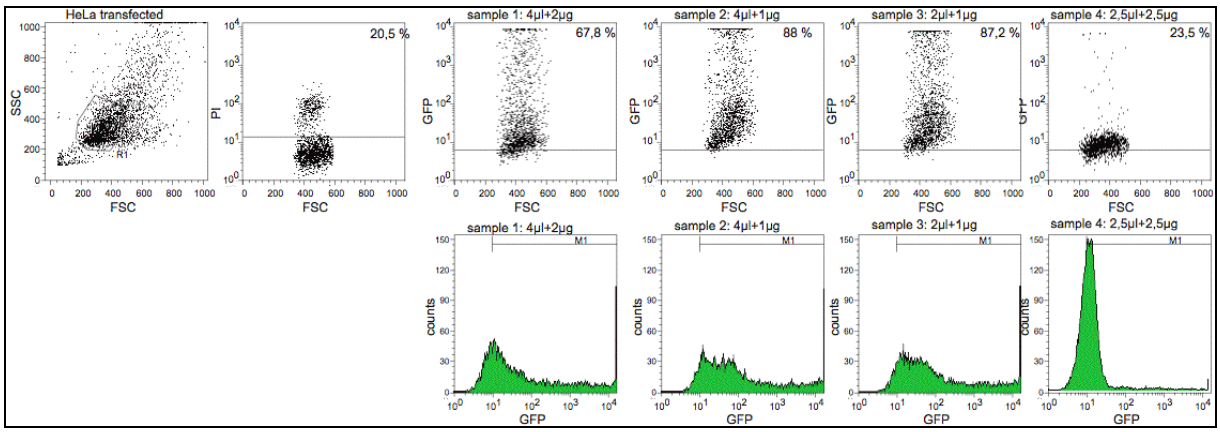
- Transfection with Liopfectamin:
  On the day before transfection the cells were plated predefined (6 x 10^5 per 48-well). Right before transfection the old medium was extracted and 2 ml DMEM with 1 % FCS was added. 5 µl of the DNA to be transfected were dissolved in 250 µl OptiMEM and 5 µl Lipofectamine 2000 from Invitrogen in another 250 µl OptiMEM. These mixtures were mixed after 5 minutes at room temperature and incubated for 20 minutes. Afterwards the mixture was pipetted on the cells. The next day the transfection medium was replaced by normal medium. Harvesting of the transfected cells took place after 48 hours.
- Transfection with METAFECTENE EASY:
  On the day before transfection the cells were plated predefined (6 x 10^5 per 48-well). Right before transfection the old medium was extracted, the cells were trypsinized and counted. 6 x 10^5 cells in a volume of 250 µl full medium was adjusted for the transfection. The ratios 2.5 µl : 2.5 µg, 4 µl : 2 µg, 4 µl : 1 µg and 2 µg : 1 µg between METAFECTENE EASY and DNA were used. METAFECTENE EASY was mixed with 50 µl 1 x EASY buffer and then with the DNA. After 15 minutes of incubation time at room temperature, the mixture was mixed with the cell suspension and pipetted into 12-well plates. The next day the transfection medium was replaced by normal medium. Harvesting of the transfected cells took place after 48 hours.

Transfection analysis

The transfected cells were examined after 48 h by FACS-analysis. In order to colour dead cells, propidium-iodid was added. All analysis were done with a FACSCaliburTM from BD-Biosciences and the results were evaluated with the CELLQuestProTM Software. For each colour 1 x 10^4 cells were pictured.

Results and discussion

With METAFECTENE EASY a transfection efficiency of 88 % (ratio 4 µl : 1 µg) could be achieved, while rate of apoptosis was 20.5 % ±0.5%. Concluding METAFECTENE EASY seems to be highly suitable for the transfection of HeLa cells, which can be improved further.



2 Transfection of human HeLa cells using METAFECTENE EASY transfection protocol

Conclusion / Summary

METAFECTENE EASY is a fast method of transfecting HeLa cells. It is easy to use and connected with little effort.