

Expression profile of transfected cells in relation with the cell doubling time

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SAINT-MIX is an efficient transfection device which transfects all kinds of cell-lines.

The cell doubling time of cells and the incubation time of the SAINT-MIX/ DNA complexes on the cells are factors which can influence the transfection efficiency. Previous studies of time courses showed that incubation of the cationic lipid/ DNA complexes is an important factor which affects the transfection efficiency^{1, 2, 3, 4}.

In this study we investigate the expression profile and the transfection efficiency in relation with the cell doubling time. Therefore we monitored the transfection efficiency after 24, 48, 72 and 96 hours in 4 different cell-lines (HEK-293, B16F10, COS-7 and FLF) and determined the doubling time of these cell-lines.

Methods

Cells were seeded in 6-wells plates 1 day before transfection. The total cell count was determined on the day before transfection and on the day of analysis of the transfection efficiency. With this data we calculate the doubling time of the different cell-lines (table 1). The transfections have been performed at a confluence of 50–80%.

The SAINT-MIX/DNA complex is prepared according to the standard protocol of 15nmol SAINT-MIX with 1µg of plasmid DNA (CMV-EGFP) and added within 5 minutes to the cells⁴. The transfection efficiency was measured after 24, 48, 72 and 96 hours by FACS analysis. The complexes are not removed from the cells!

Table 1: The doubling times of the cell-lines

Cell-line	Doubling time (hr)
HEK-293	10
B16F10	12
COS-7	18
FLF	48

Results

The doubling times from the different cell-lines are shown in table 1. For each cell-line the GFP (green fluorescent protein) expression is monitored after 24, 48, 72 and 96 hours of SAINT-MIX/ DNA complex incubation time (Figure 1). The HEK-293 and B16F10 cell-lines have an expression time optimum within 72 hours. In slower growing cell-lines (COS-7 and FLF) there is an increase of GFP expression

visible even after 96 hours. NIH-3T3 cells in our hands had a doubling time of 12 hours and the GFP expression is comparable with the B16F10 results (data not shown). The MFI (Mean Fluorescent Intensity) per cell is not dramatically changed during the time course, so the increased transfection percentage is not elucidated by cell division.

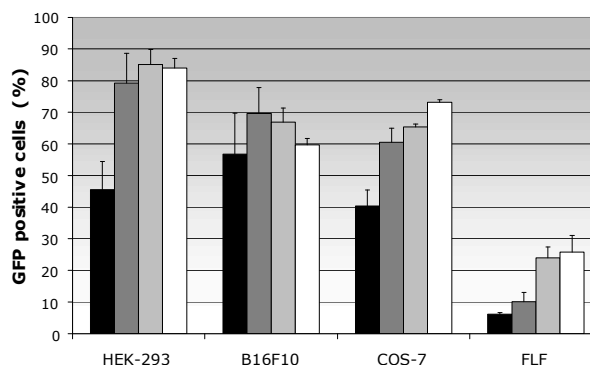


Figure 1: The expression of green fluorescent protein (GFP) in different cell-lines after 24 (black), 48 (dark grey), 72 (light grey) and 96 (white) hours of transfection.

Discussion

In general these results show that an increase of expression time of DNA delivered with SAINT-MIX, results in an increased protein expression over time, up to 96 hours. Cell-lines with a longer doubling time need more time to express the plasmid DNA encoded reporter protein maximally. In conclusion, slowly growing cells will reach the optimum expression profile at all later time points, so to determine the transfection efficiency, cell doubling time should be taken into account.

References:

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