Metafectene Technical Note

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Optimization of the transfection from HeLa cells with siRNA:

1. RNAi: Sequence-specific post-transcriptional gene-silencing

Using the method RNAi (RNA interference) cells are transfected with small, double-stranded RNAs (siRNA). With these siRNAs the translation of single genes, homologous to the siRNA, is inhibited in the cells. The mRNA of the corresponding gene is degraded and the corresponding protein can no longer be synthesised (Sayda et al., 2001). It is unconditional necessary that the used siRNA is 100% homologous to the corresponding mRNA.
We used siRNA homologous to the mRNA of the protein IDE (insulin degrading enzyme). We obtained the siRNA from Dharmacon Research (www.dharmacon.com).

2. Description of the related cell line

We used siRNA homologous to human IDE, because only the sequence of human IDE is known. We therefore worked with a human cell line. For the first experiments we toke HeLa cells. HeLa cells are epithelial cells and we transfected them adherent.

3. Description of the experiment

We cultured HeLa cells in 25mm^2- bottles in DMEM supplemented with 10% FCS without antibiotics. The cells were splitted twice a week. For that cells were washed with 0.05% EDTA/PBS for 5 minutes and than trypsinized. The day before transfection cells from a 25mm^2- bottles were splitted and transferred in a 6-well plate. On the day of transfection cells are 90-100% confluent (optical confluency, this means nearly 60% real confluency).

Transfection efficiencies were determined by cotransfection. The cells were transfected with IDE-siRNA and Aß plasmid-DNA. Aß is degraded by IDE. So Aß can't be degraded in cells transfected with IDE-siRNA.

The HeLa-cells are transfected as follows:
(Quantities statement relate on one well of a 6-well plate)
We put forward 100 µl 37°C warm DMEM without serum in a 1,5 ml Eppendorf-Cap. Than the indicated amount of METAFECTENE (see Table) was added (METAFECTENE was stored at 4°C). In a further cap we put forward 100 µl 37°C warm DMEM without serum. In this cap we added the indicated amount of plasmid-DNA and siRNA. Within 5 minutes we combined the diluted DNA/siRNA with the diluted METAFECTENE and mixed them by rocking. The solution was incubated for further 20 minutes to allow complexes to form.
During this time 2 ml new DMEM supplemented with 10% FCS was added to the cells. After this time the transfection solution was added to the cells, the 6-well was rocked and the cells were incubated at 37°C and 8.5% CO_2 for 24 hours. After this 24 hours the medium was changed and the cells were incubated for an additional day at 37°C and 8.5% CO_2.

48 hours after transfection the cells were rubbed in PBS, pelleted and lysed for 30 minutes on ice (0,5% Triton X-100, 5 mM EDTA, 1 µg/µl Aprotinin, 10 µM E-64, 1 µM Pepstatin, 50 µM Leupeptin, 1 mM PMSF). We isolated supernatant and pellet, analyzed the protein concentration and separated the proteins (25 µg) on a gel to detect Aß.

The transfection efficiencies were determined by the detection of Aß. The results are shown in Table I and in Figure I.

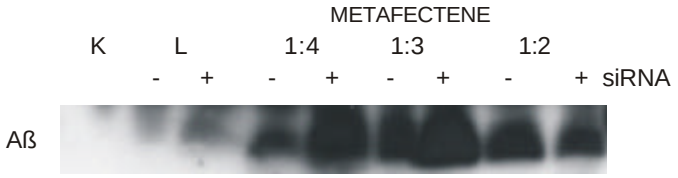
4. Comparison with other methodes

We compared the transfection efficiency from METAFECTENE with the transfection efficiency from another commercial transfection reagent (L2). Transfection with L2 was carried out as described by Sayda et al. (2001).

Table I: Transfection efficiency from HeLa cells transfected with METAFECTENE and siRNA

Amount of DNA and siRNA per well	Amount of METAFECTENE per well	Proportion of DNA/siRNA: METAFECTENE	Time of incubation	Efficiency of the transfection
2 und 0,84 µg	11 µl	1:4	24 h	++
2 und 0,84 µg	8,5 µl	1:3	24 h	++
2 und 0,84 µg	5,7 µl	1:2	24 h	-

Figure I: Transfection efficiency from HeLa cells transfected with METAFECTENE and siRNA; biochemical detection of Aß



5. Discussion

As you can see in Figure I the transfection efficiency from METAFECTENE is much higher than the efficiency from L (=L2).

For a optimal transfection from siRNA with METAFECTENE following points are important :

A. The proportion between siRNA and METAFECTENE must not be to high. That means, METAFECTENE should always exist in excess. Optimal conditiones are given if the siRNA/METAFECTENE proportion is 1:4 or 1:3.
B. One further important point for the transfection with METAFECTENE and siRNA is the confluence of the cells. The cells must reach a optical density from 90-100% for a transfection with siRNA . The amount of living cells and therefore the efficiency of the transfection decrease if the cell density is lower. Further on METAFECTENE has a toxic effect if the cell density is lower then 70% at the time of transfection. That means, no cells survive the transfection (The toxic effect from the transfection with a cell density about 70% or lower could also result from the used, high concentration of DNA/siRNA).

6. References

Elbashir, S. M., J. Harborth, et al. (2001). "Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells." Nature **411**(6836): 494-8.

Elbashir, S. M., W. Lendeckel, et al. (2001). "RNA interference is mediated by 21- and 22-nucleotide RNAs." Genes Dev **15**(2): 188-200.