# Optimization of reverse siRNA transfection of human HEK293 cells with Metafectene PRO

**Gunnar Jacobs, Philip Rosenstiel, Stefan Schreiber**

**Institute of Clinical Molecular Biology and Department of General Medicine, University Hospital Schleswig-Holstein, Kiel D-24105**

## Introduction:

RNA interference (RNAi) is a mechanism for RNA-guided regulation of gene expression. Conserved in most eukaryotic organisms, the RNAi pathway is thought to have evolved as a form of innate immune response against viruses and also plays a major role in regulating development and genome maintenance. RNAi was shown to occur in mammalian cells in response to double-stranded small interfering RNAs (siRNAs) of ~21 nt in length that serve as the effector molecules of sequence-specific gene silencing. An efficient delivery of siRNA into the cells is the critical step for the analysis of sequence-specific gene silencing. The aim of this work was to establish a protocol for efficient delivery of siRNA into human embryonic kidney HEK293 cells by reverse transfection as a prerequisite for genome-wide analysis of RNAi.

## Materials and methods:

### Cell culture

The human embryonic kidney cell line HEK293 was cultured in Dulbecco's modified Eagle's MEM (DMEM) (PAA, Pasching, Austria) supplemented with 10% FBS (Biochrom AG, Berlin, Germany), penicillin (100 U/ml), and streptomycin (100 µg/ml) (Biochrom AG, Berlin, Germany) and grown in humidified atmosphere (5 % $CO_2$) at 37°C.

### Materials

Metafectene PRO (Biontex Laboratories GmbH, Munich, Germany), a polycationic liposomal transfection reagent, was obtained from Biontex Laboratories GmbH (Munich, Germany). For first optimization of the transfection conditions, a control siRNA labeled with AlexaFluor647 (Qiagen, Hilden, Germany) was used. Further fine optimization of siRNA delivery was performed using with the KDalert GAPDH assay kit (Ambion), which provides the possibility to measure cell viability and GAPDH gene knockdown.

### Transfection protocol

Optimization of reverse transfection was done in 96-well format. Transfection reagent and siRNA were diluted in DMEM to the desired concentration. Ten microliter each of

diluted siRNA and transfection reagent were mixed in one well of the 96-well plate. For formation of transfection complexes, the mixture was incubated for 15 min at room temperature. Reverse transfection was carried out by addition of 100 µl cell suspension (14,000 cells/well) to the preincubated transfection complexes, followed by incubation at 37°C.

For optimization, siRNA was diluted to a final concentration of 0 nM, 2 nM, 5 nM, and 8 nM, respectively. At least three different amounts of transfection reagent were tested in accordance with the protocol provided by the manufacturer.

**Determination of transfection efficiency**

The fluorescence of AlexaFluor647 was measured in a GENios Pro plate reader (Tecan) with 612 nm excitation and 670 nm emission wavelength 24 h after transfection with media and after replacement of the media against 150 µl PBS. Equal amount of added siRNA was shown by equal fluorescence in the 96-well plates containing media. The remaining AlexaFluor647 fluorescence after media replacement is proportional to the uptake of the labeled siRNA by transfected cells.

**Determination of cell viability/cytotoxicity**

The activity of the housekeeping enzyme GAPDH was uesd as a marker for cell viability/cytotoxicity. 48 hours after reverse transfection, the GAPDH activity was measured with the KDalert GAPDH kit (Ambion).

# Results and discussion:

For a first screen, Metafectene PRO was complexed with the AlexaFluor647-labeled control siRNA at different reagent:siRNA ratios. For transfection of the human embryonic kidney cell line HEK293, we used a reverse transfection protocol. Transfection reagent and siRNA are allowed to form complexes at room temperature in a 96 well plate. After 15 min of complexation, the HEK293 cells were added. To check an equal content of siRNA, the fluorescence of AlexaFluor647 was measured in a GENios Pro plate reader 24 h after transfection. After measurement, the media was replaced by 150 µl PBS. The incorporated (labeled) siRNA is proportional to the remaining fluorescence in the 96 well plate.

Figure 1 shows the results of a concentration-dependent transfection of HEK293 cells with Metafectene PRO. In the range from 1 to 4 µl Metafectene PRO per well, the incorporation of labeled siRNA (transfection efficiency) depends on the siRNA concentration (Figure 1). Nevertheless, a tendency to a saturation of the transfection efficiency is seen in the range from 3 to 4 µl Metafectene PRO per well. A comparison of the fluorescence units of the applied labeled siRNA and the incorporated siRNA shows that nearly the total fluorescence is incorporated into HEK293 cells (Data not shown).

In a further analysis, the effect of Metafectene PRO transfection on cell viability/cytotoxicity was evaluated. Therefore, HEK293 cells were reverse

transfected with a negative control siRNA in combination with 1 to 3 µl Metafectene PRO. The activity of the housekeeping enzyme GAPDH was uesd as a marker for cell viability/cytotoxicity. 48 hours after transfection, the GAPDH activity was measured with the KDalert GAPDH kit (Ambion). A decrease in cell viability was detected in cells transfected with 2.5 µl or more Metafectene PRO per well. In the range from 1 to 2 µl Metafectene PRO per well, the cell viability was not affected.

## Conclusion / summary:

Metafectene Pro was used to transfect the human embryonic kidney cell line HEK293 with siRNAs. The delivery of the siRNA by Metafectene PRO was efficient without effecting the cell viability/cytotxicity using 5 nM siRNA and 2 µl Metafectene PRO.

## Appendix: Tables and/or figures:

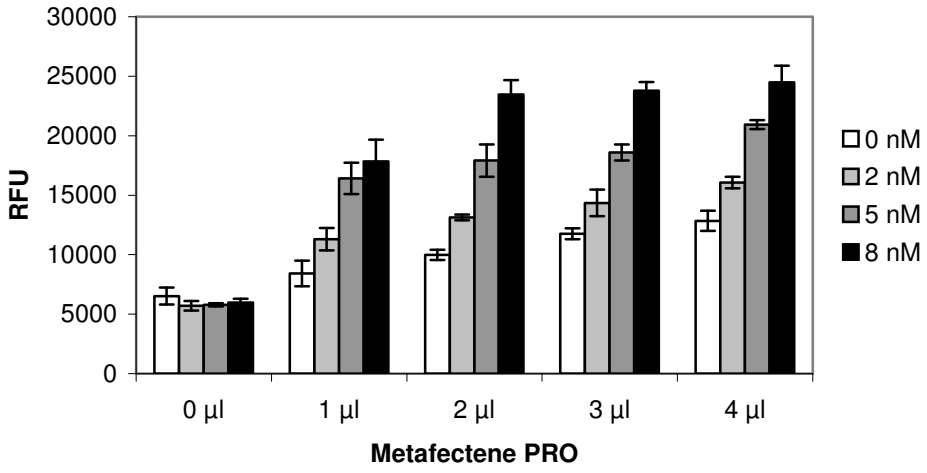


Figure 1: Incorporated AlexaFluor647 fluorescence after transfection of HEK293 cells with different concentration of siRNA and different amounts of Metafectene PRO per well (96 well plate). Incorporated fluorescence is proportional to transfection rate. RFU: relative fluorescence units of AlexaFluor647.

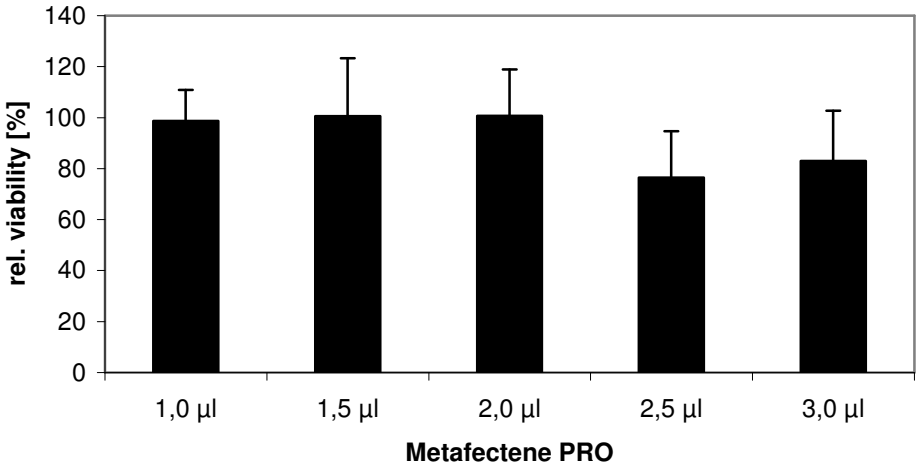


Figure 2: Relative cell viability of HEK293 cells transfected with Metafectene PRO. The cell viability was measured with the KDalert GAPDH kit (Ambion) 48 h after reverse transfection of a control siRNA into HEK293 cells.