Plasmid DNA transfection of CT26 mus musculus colon carcinoma cells with the Biontex K2® Transfection System

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Materials and methods

Cell culture

The mus musculus colon carcinoma cell line CT26 was obtained from the American Type Culture Collection (ATCC, Promochem, Germany). Cells were cultured in RPMI-1640 (Life Technologies, Darmstadt, Germany) supplemented with 10% fetal calf serum (FCS; Life Technologies), 50 U/ml penicillin and 50 µg/ml streptomycin (Sigma-Aldrich, Munich, Germany) at 37 °C in a humidified atmosphere containing 5 % CO2.

Transient transfection of cells and evaluation of transfection efficiency

For optimization of transfection conditions, 90.000 CT26 cells (confluency of approx. 80%) per well were plated in a 48-well plate (Greiner Bio-One, Frickenhausen, Germany) 24 h before transfection. Cells were transfected with K2® Transfection System (Biontex), according to the optimization protocol provided by the manufacturer using the pGL3-Control luciferase expressing plasmid (Promega, Mannheim, Germany). Transfection efficiency was evaluated 48 h after transfection using the Luciferase Assay System (Promega) and an ELISA reader (TECAN, Männedorf, Switzerland). For analysis of BLOCK-iT™ PolII miR RNAi GFP expression vectors (Invitrogen, Carlsbad, USA), cells were plated on coverslips in 6-well plates 24 h before transfection. On the day of transfection, cell confluency was approximately 50%. The best condition from the optimization results was used for transfection according to the upscaling protocol of Biontex. In brief, cells were incubated for 2 h with 40 µl K2® Multiplier in 2 ml complete medium prior to transfection. 4 µg of plasmid and 12.8 µl of K2® Transfection Reagent were each diluted to 130 µl in Opti-MEM Reduced Serum Medium (Life Technologies). Plasmid and K2® Transfection Reagent solutions were then mixed gently by pipetting up and down and incubated at room temperature for 20 min. Immediately after the incubation step, DNA-transfection reagent solution was slowly added dropwise and cells were gently agitated in the cell culture plates. Cells were fixed with 3.7% paraformaldehyde in PBS and counterstained with DAPI. Coverslips were mounted on slides with Vectashield antifade mounting medium (Vector Laboratories, Burlingame, USA) and transfection efficiency was analyzed 48 h after transfection using an Axiovert 40 CFL microscope (Carl Zeiss, Göttingen, Germany).

Results

Optimization of transfection conditions using a Luciferase reporter assay

Transfection of the difficult-to-transfect mus musculus colon carcinoma cell line CT26 was optimized using a Luciferase expression plasmid and the K2® Transfection System. Optimal transfection conditions were identified after analyzing Luciferase acitivity (Figure 1). The maximum transfection efficiency was achieved with 0.4 µg plasmid at a plasmid:Transfection Reagent ratio of 1:4 with 5 µl K2® Multiplier treatment (yellow bar).

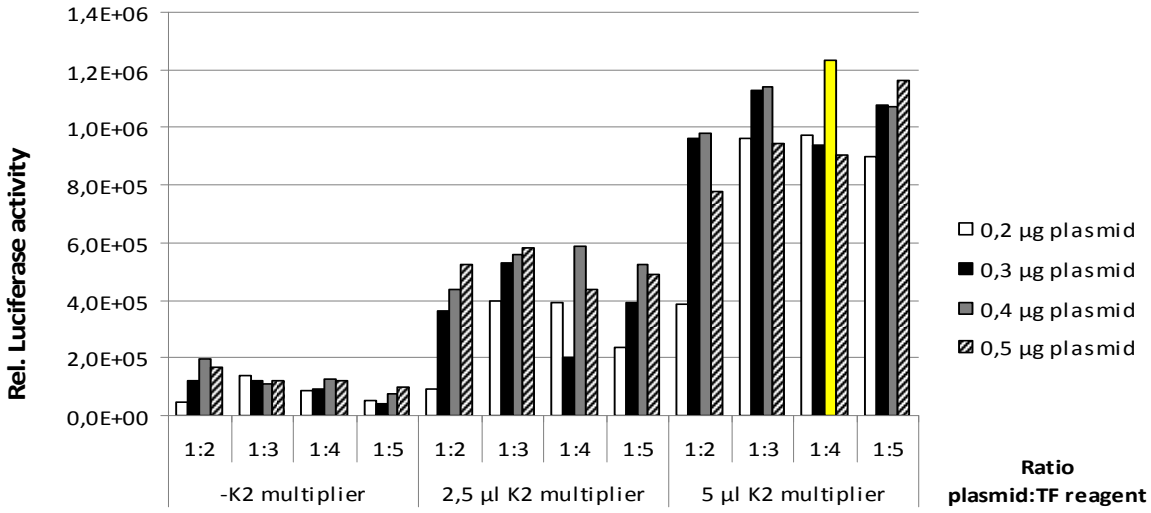

Figure 1: CT26 murine colon carcinoma cells were transiently transfected with the pGL3-Control luciferase expression vector (Promega) using the K2® Transfection System (Biontex) according to the optimization protocol of the manufacturer in a 48-well scale. At 48 h after transfection, cells were subjected to a Luciferase Assay (Promega) as suggested by the manufacturer. Luciferase activity was measured in an ELISA reader (TECAN, Männedorf, Switzerland). The yellow bar represent the conditions resulting in the highest Luciferase activity (5 µl K2® multiplier with 0,4 µg plasmid and 1:4 µg/µl plasmid:TF reagent ratio).

Expression of BLOCK-iT™ PolII miR RNAi GFP expression vectors

Efficient expression of BLOCK-iT™ PolII miR RNAi GFP vectors containing different miRNAs was monitored by fluorescence microscopy (Figure 2).

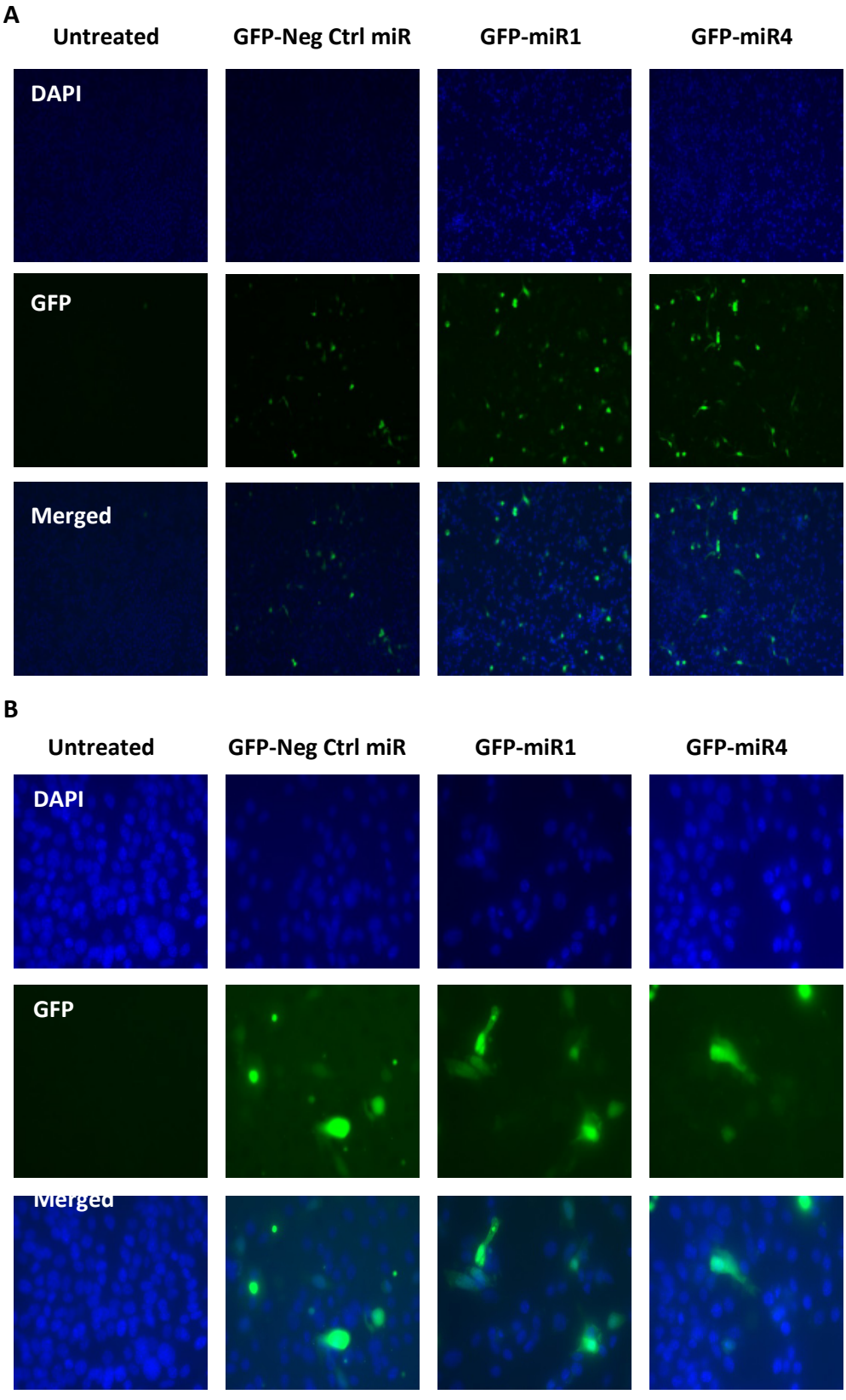
A
Untreated
GFP-Neg Ctrl miR
GFP-miR1
GFP-miR4
DAPI
GFP
Merged
B
Untreated
GFP-Neg Ctrl miR
GFP-miR1
GFP-miR4
DAPI
GFP
Merged

Figure 2: CT26 cells were transfected using 4 µg plasmid at a plasmid:transfection reagent ratio of 1:4 with K2® Multiplier treatment. Photographs were acquired with an Axiovert 40 CFL microscope (Carl Zeiss, Göttingen, Germany). A: 10x magnification, B: 40x oil magnification.

Summary

Transfection with the K2® Transfection System (Biontex) of the difficult-to-transfect CT26 colon carcinoma cells was highly efficient with transfection efficiencies up to 40%. Transfection efficiency and expression were higher than that of commonly used commercially available transfection reagents a, b, c, and d with transfection efficiencies of maximally 13% in CT26 cells (data not shown). Taken together, the Biontex K2® Transfection System is a useful tool for efficient transfection of CT26 murine colon carcinoma.