Plasmid DNA-transfection of LN319 human glioblastoma cells using the "Biontex K2 Transfection System".

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Material & Methods:

Vector

The transfection experiments were conducted using the pGFP-V-RS shRNA expression vector (OriGene) containing a scrambled shRNA coding sequence and a GFP coding sequence under a separate CMV promoter.

Cell culture

LN319 human glioblastoma cells were cultured in Dulbecco's modified Eagle medium (DMEM; Life Technologies) supplemented with 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin at 37°C under 5% CO_2 atmosphere. Prior to transfection, 500.000 cells were seeded in three wells of a 6-well plate (Greiner) and incubated for 48h. Transfection was conducted at approximately 80-90% confluence. Fifteen minutes before adding the K2 Multiplier, the DMEM medium on the cells was replaced by fresh medium (2 ml per well).

Transfection with K2 Transfection System (Biontex)

Cells were treated with 20 µl K2 Multiplier per well, 2h prior to transfection. For the transfection procedure the Plasmid-DNA and the K2 transfection reagent (K2TR) were diluted separately in a final volume of 100 µl Opti-MEM medium (Life Technologies) each. The diluted DNA was then added to the diluted K2TR, gently

mixed by pipetting and incubated for 20 min at room temperature. Three different K2TR [µl] to DNA [µg] ratios were tested: 3:1, 4:1, 8:2. After incubation the whole volume (200 µl) was added drop-wise to one well, respectively.

Results:

Analysis of transfection efficiency

After 24h the transfection efficiency was monitored by inverse fluorescence microscopy at an AxioVert-A1 microscope (Carl Zeiss). The fluorescence density was not quantified but rather estimated by eye.

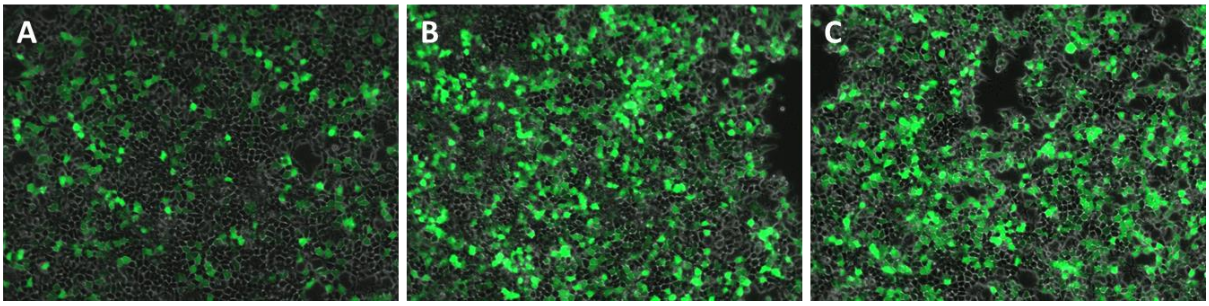

Figure 1: Transfection efficiency in LN319 cells. A) 3:1 ratio, B) 4:1 ratio, C) 8:2 ratio. 100x Magnification.

Stable transfection:

It is also possible to generate stable LN319 cell lines with the presented K2 transfection protocol, by simply replacing the medium 24h – 48h after transfection with medium containing an appropriate selection antibiotic (for the vector presented in this test setting; DMEM with 2 µg/ml Puromycin) and exchanging the medium every 48h with fresh selection medium. After approx. 7 – 10 days, the cells can be splitted and/or if necessary single clones can be selected by colony picking or limiting dilution.

Conclusion:

The K2 Transfection system is very efficient in transfecting LN319 cells, even with relatively low amounts of DNA and transfection reagent. As little as 4 µl K2TR and 1 µg DNA per well (2 µl K2TR per ml medium and 500 ng DNA per ml medium) was sufficient for ~70-80% transfection rate. An increase to double the amount of K2TR and plasmid DNA did not strongly increase the transfection rate in our test setting. We therefore recommend using 4 µl K2TR and 1 µg of Plasmid DNA in 200 µl OptiMEM per well when transfecting LN319 cells in a 6-well format. The K2 Transfection system is also very well tolerated by the LN319 cells, which show almost no signs of cytotoxicity after transfection. This also allows for simple selection of stable clones by addition of selection antibiotics.