K2 Transfection System Technical Note

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Materials:
Plasmid pcDNA3-EGFP
GFP-mRNA (Biontex)
Sterile 48-well tissue culture plates (Corning)
Poly-L-Lysine (Sigma)
Human brain vascular pericytes (HBVP; ScienCell)
Pericyte medium + supplements (ScienCell)
Transfection reagent: K2 transfection system + K2 Multiplier (Biontex)

Optimization of the transfection of primary human brain vascular pericytes:

Transfection using plasmid-DNA

For transfection using pcDNA-3-EGFP plasmid 60.000 HBVP (passage 4) per well were seeded in 250 µl of pericyte medium (including all supplements) on poly-L-Lysine coated 48-well plates and allowed to attach overnight. The cells were transfected as recommended by the provider using the transfection optimizing protocol for DNA transfection. 24h after transfection the medium was removed and standard culture growth medium was added. After further 24 h EGFP positive cells were detected using a fluorescence microscope (Zeiss Axiovision).

Transfection using mRNA

For transfection using GFP-mRNA 60.000 HBVP (passage 4) per well were seeded in 250 µl pericyte medium (including all supplements) on poly-L-Lysine coated 48-well plates and allowed to attach overnight. The cells were transfected as recommended by the provider using the transfection optimizing protocol for mRNA transfection with the exception that only 10 µg mRNA instead of 30 µg mRNA was used in total. 24h after transfection the medium was removed and standard culture growth medium was added. After further 24 h EGFP positive cells were detected using a fluorescence microscope (Zeiss Axiovision).

Conclusion:

Human primary cells such as HBVP are normally hard or nearly impossible to transfect using standard liposome based transfection technology. Therefore, lentiviral transduction of primary cells is mainly necessary to generate stable cell lines or to genetically modify these cells. Disadvantage of lentiviral transduction is the imperative need of a S2 biosavety laboratory. In the past we tried to transfect HBVP using a variety of liposom-based transfection reagents, but the determined transfection rates using these reagents and pcDNA3-EGFP as a sensor were zero or nearby. For this we analyzed whether HBVP at low passage could be transfected using the K2 transfection reagent.

We seeded low passage HBVP at subconfluency to avoid a reduced cell proliferation rate induced by massive cell-cell-contact and followed the standard optimizing protocols for either DNA or mRNA transfection as recommended.

For DNA transfection a broad spectrum of DNA/K2 ratio worked well, but 0.4 µg of plasmid DNA/well has to be used in a minimum. Pretreatment of the cells with 5 µl of the K2 multiplier enhanced the transfection rate approximately 2-fold if compared to transfected cells having seen no K2 multiplier or with cells pretreated with 2.5 µl K2 multiplier (for example see Fig. 1). For mRNA transfection an mRNA/K2 ratio of 1:1 gave best transfection rates, even if the transfection rate was generally lower than using plasmid DNA. Increasing concentrations of K2 multiplier did not show further enhancement of the transfection rate (for example see Fig. 2). Variability of EGFP expression in the transfected cells was high and both $EGFP^{high}$ and $EGFP^{low}$ expressing cells were detectable 48h after transfection. No toxic effects were detected at any of the conditions used during transfection, all observed cells were in good condition (observation time >72 h).

Thus, the surprisingly good transfection rate of approximately 10-15 % of HBVP is of great advantage for the establishment of stable cell lines from primary cells using SV 40 large T-Antigen or TERT-expressing plasmids. Beside this, transient transfection followed by FACS-based sorting of transfected low passage primary cells offers the option of generating genetically engineered primary cells in an adequate quantity.

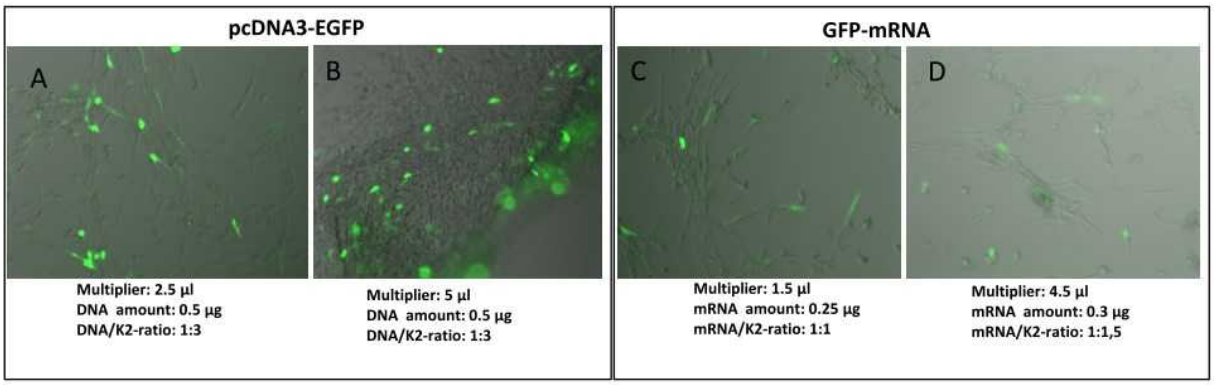

Figure legend: HBVP were transfected with a plasmid coding for EGFP (A, B) or with GFP-mRNA (C, D) using the K2 transfection system. Fluorescence image of the cells 48 h after transfection showed cells expressing EGFP (merged microphotographs showing both bright field and fluorescence). Legends show the amount of nucleic acid and K2 multiplier used for transfection as well as the ratio of nucleic acid / K2 transfection reagent.