Transfection of human melanoma cell line SKMEL-5 with the Biontex K2® Transfection System

Erdem Yilmaz, N. C. Tolga Emre

Boğaziçi University, Department of Molecular Biology and Genetics, Laboratory of Genome Regulation, Kuzey Park Binasi, 3. Kat, Bebek 34342, Istanbul, TURKEY

Materials and Methods

Cell Culture

Human melanoma cell line SKMEL-5 was cultured in 500 µl of Dulbecco's modified Eagle's Medium - high glucose (DMEM-Gibco) containing 10% fetal bovine serum albumin (FBS-Gibco) in a humidified atmosphere containing 5% CO_2 at 37°C. It was passaged in 24-well plates (TPP) 1 day before in order to reach ~70-80% of confluency at the time of transfection.

Transfection

The medium in each well was replaced with fresh DMEM (500 µl) containing 6 µl of K2 Multiplier and cells were incubated for 2 hours at 37°C. Adjusting the time to add the transfection mix at the end of 2 hours of incubation period, it was prepared as indicated below:

- Solution A was prepared by adding 0.5 µg of DNA (green fluorescent protein (GFP) expressing pEGFP plasmid - prepared with Macherey-Nagel endotoxin-free maxi prep kit) to 30 µl of serum-free DMEM in a polypropylene microcentrifuge tube (Eppendorf).

- Solution B was prepared by adding 3 µl of K2 Transfection Reagent to 30 µl of serum-free DMEM in a polypropylene microcentrifuge tube.

- Solution A was added onto Solution B and mixed gently by pipetting up and down.

- The mixture (60 µl) was incubated for 15 minutes at room temperature and then added to the corresponding wells of a 24-well plate in a drop-wise manner.

- Transfected cells were incubated at 37°C (5% CO_2) for 6 hours and then the transfection medium was replaced with fresh DMEM containing 10% FBS.

- After 48 hours of incubation at 37°C (5% CO_2), cells were analyzed for GFP signal by a fluorescent microscope and by flow cytometry (BD FACSCalibur) for transfection efficiency.

Results

In Figure A, SKMEL-5 cells transfected with pIRES-GFP are shown under fluorescent microscope (48h after transfection). In this cell line, we also observed dead cells which can also be detected as round shape-like structures. Using flow cytometry to detect the GFP signal

from the transfected GFP-expressing plasmid, transfection efficiency (after dead cells were washed away) was measured to be 42% (Figure C vs Figure B).

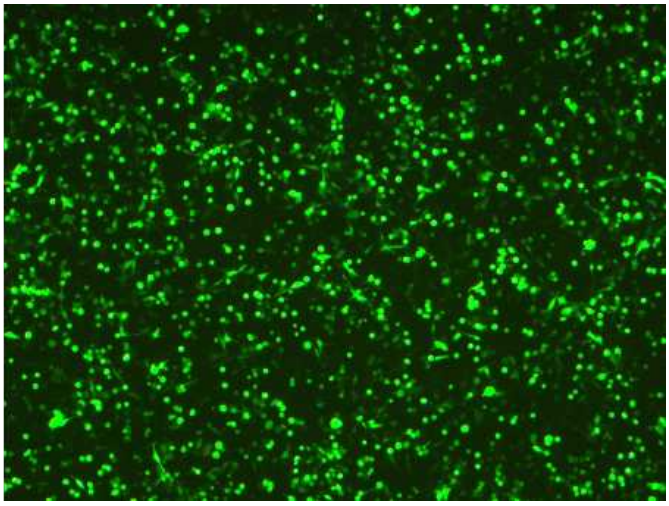
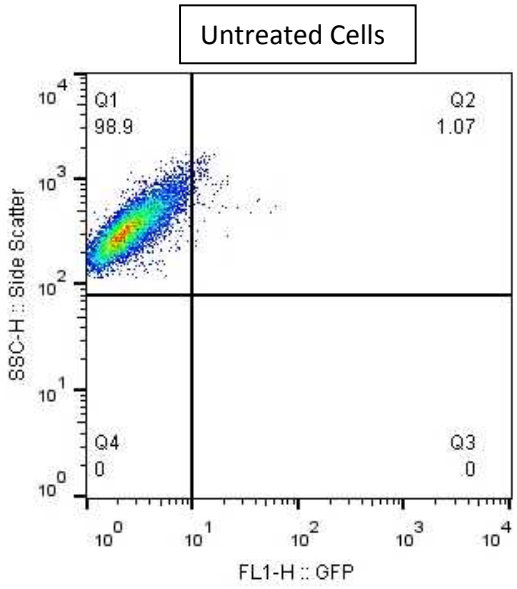


Figure A



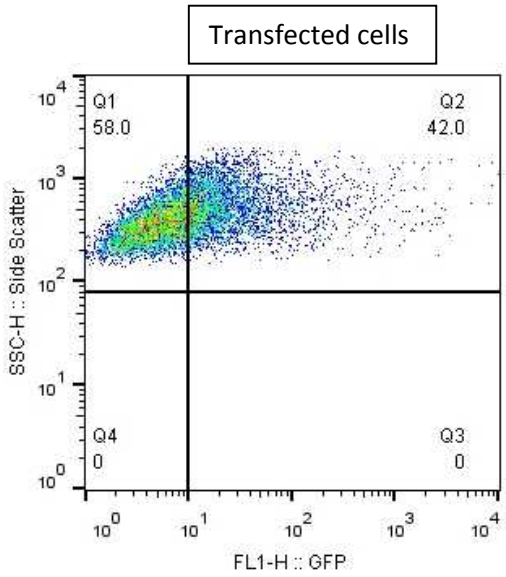


Figure B Figure C

Conclusion

In order to find a good transfection, we have tried different transfection conditions by changing the relative amounts of DNA, K2 Transfection Reagent, and K2 Multiplier, or transfection incubation times (data not shown). The protocol presented in this report represents the optimal that we have achieved for the SKMEL-5 melanoma cell line. In contrast, our previous transfection trials with other commercial reagents did not yield such satisfactory results in this cell line. In conclusion, we were able to achieve moderately high

transfection efficiencies in SKMEL-5 melanoma cell line using the Biontex K2® Transfection System.