DNA-transfection of human prostate cells (PNT2) and human prostate cancer cells (Pc3) using "Biontex K2 Transfection System"

Stephanie Burnell and Professor Shareen Doak Institute of Life Science 1, College of Medicine, Swansea University, Swansea, SA2 8PP, Wales, UK

Materials and Methods

Cell Culture

Human prostate cell line (PNT2) and human prostate cancer cell line (Pc3) were cultured in 6 well plates (Greiner Bio-one, Cell Star) in 2 ml of 1640 RPMI medium (Gibco, Life Technologies, UK) supplemented with 10% Fetal Bovine Serum, 1% Glutamine and 1% Penicillin/Streptomycin at 37°C and 5% CO_2. Cells were seeded at a density of $1.75x10^4$ cells per well 24 hours prior to transfection to ensure 90-100% confluency at time of transfection.

Cell Transfection

K2 Multiplier (45 µl) was applied in a drop-wise manner to the cells. The plate was then agitated gently to evenly distribute the multiplier and the plate was incubated at 37°C, 5% CO_2 for 2 hours. In 1.5 ml microcentrifuge tubes, solution A and solution B were prepared with Optimem serum free media (Gibco, Life Technologies, UK) (Table 1). The plasmid DNA was an empty vector containing only a GFP tag for detection.

	Solution A	Solution B
Optimem	135 µl	135 µl
Plasmid-DNA	2.4 µg	
Transfection Reagent		10.8 µl

Table 1: Contents of solutions A and B

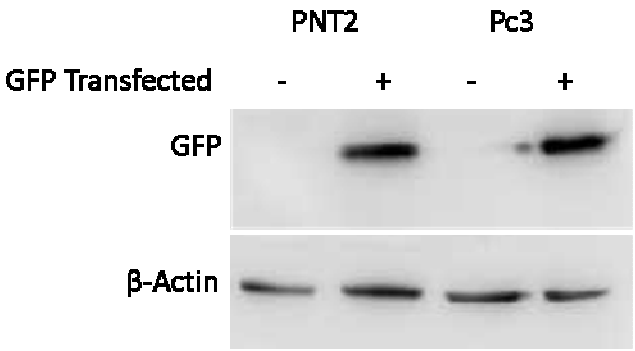
Each solution was prepared and gently mixed by pipetting. Solution A was then added to solution B, not the reverse order and mixed by gently inversion. The resultant solution was then incubated at room temperature for 20 minutes. The plasmid DNA-transfection reagent solution was then added in a drop-wise manner to the appropriate well of the 6 well plates and again, the plates were gently agitated to ensure an even spread of the complex. The transfections were incubated at 37°C, 5%CO_2 for 24 hours. Following this initial 24 hour incubation, the complex was removed from the cells and replaced with complete media, this was then incubated for a further 24 hours.

Cells were harvested using RIPA buffer, quantified using the Pierce Assay (Thermoscientific, USA) and stored at -20°C. Protein expression was observed via western blotting, using antibodies against GFP and β-actin (housekeeping control).

Results

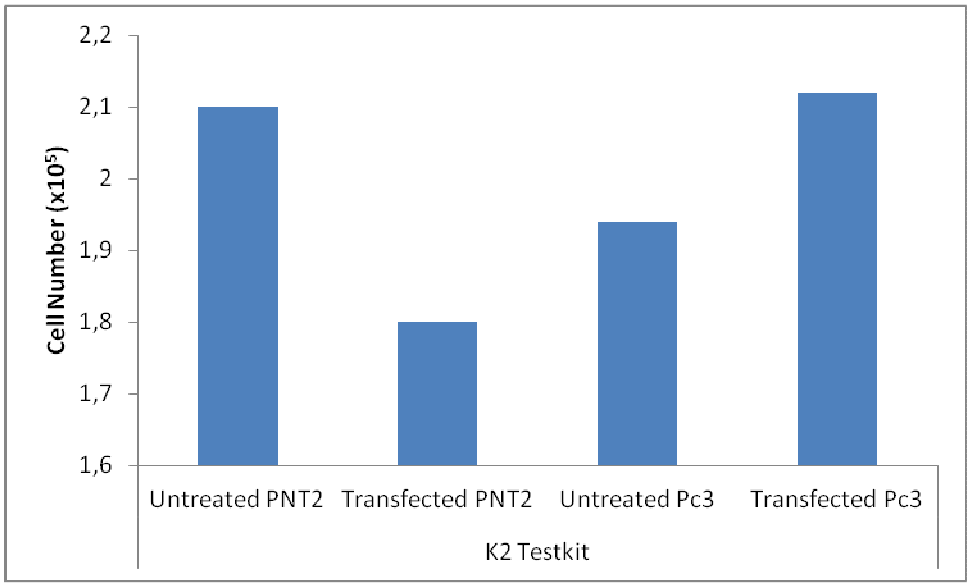
Western Blot

Blots were probed with Anti-GFP antibody to detect successful transfection



Cell Counts

The cells were counted upon harvesting to determine any cytotoxic damage



Conclusion

From the western blot image, it is clear that both cell lines have been successfully transfected with the GFP tag. There was a slight drop in cell number in the treated PNT2 cells when compared to their untreated counterparts, but this was not present in the Pc3 cell line.