Expression of Nucleolin (Nu) in osteosarcoma cell line (U2OS)

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Introduction:

The experiment was to determine which ration of DNA:Metafectene Pro is better for transfection and to analyze steady-state level of Nu-wt and mutant expression in U2OS cells.

Experimental procedures / transfection protocol:

U2OS cells were transfected after ~24h of seeding at 60-70% confluency with Metafectene Pro in 24-well plate.

GFPNu-wt and GFP-Nu-mutant were either transfected at 0.5ug/well with 1:1,1:2 or 1:3 DNA:Metafectene Pro ratio for 4hr. The cells were then washed once with DPBS and replaced cells in fresh medium. The complex formation was done with OPTIMEM and transfection was carried out with complete serum containing medium. Post ~36h cells were harvested for protein.

The protein was then analyzed by SDS-PAGE and Western blotting using anti-GFP antibodies.

Results and discussion:

Transfection efficiency was very good for both wt and mutant plasmids (~80-90%).

For wild-type there is a preferable 1:4 ratio for transfection. Mutant is probably getting degraded at the steady-state level although, even at lower ratio of 1:1 the protein is detected efficienctly.

Conclusion / summary:

Metafectene Pro is an efficient transfection reagent even at 1:1 DNA:Metafectene Pro ratio. The mutant under investigation appears to be lesser amount probably due to rapid degradation. Nonetheless, the reagent was efficiently transfecting the mutant plasmid and detectable by western blotting.

Appendix: Tables and/or figures: