Expression of the nuclear myc protein using a secretion vector and HeLa cells

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Introduction:

Previous attempts to purify native recombinant myc protein from mammalian cells failed because of the strong binding of the protein to nuclear structures. Therefore, we considered to clone the myc sequence in frame with a signal peptide for the secretion pathway, aiming at recovering the recombinant protein in the culture medium.

Experimental procedures / transfection protocol:

Cell transfection with Metafectene Easy was performed according to the instructions provided with the METAFECTENE EASY KIT. An aliquot of 10x EASY buffer was diluted in the presence of 9 aliquots of sterile water under sterile conditions to prepare 1x EASY buffer. Transfections with HeLa cells in suspension was performed in a single well of a 24-well-plate containing 500µl of cells suspended in complete culture medium (MEM+10%FBS+Glutamine+Pen/Strep+non essential aminoacids+sodium pyruvate) for a final concentration of $8*10^5$ cells/ml. Transfected cells were cultured on cover glasses. Transfection of HeLa adherent cells cultured in the same buffer was performed plating cells, at a concentration of $6*10^4$ cells/well, two days in advance on cover glasses in order to reach 70-80% of cell confluence at the moment of transfection. Lipoplexes was prepared pipetting 5µl of Metafectene Easy in 100µl 1x EASY buffer. 5µg of DNA (pSecTag2B-myc) were added, the suspension was mixed and incubated 15 min at RT before supplementing it to the cells. These were incubated at 37ºC in the presence of 6%CO_2. After 6h the medium containing the lipoplexes was removed and substituted with the complete culture medium. Cells were cultured 24 h before analyzing the protein accumulation and distribution by immunofluorescence.

Results and discussion:

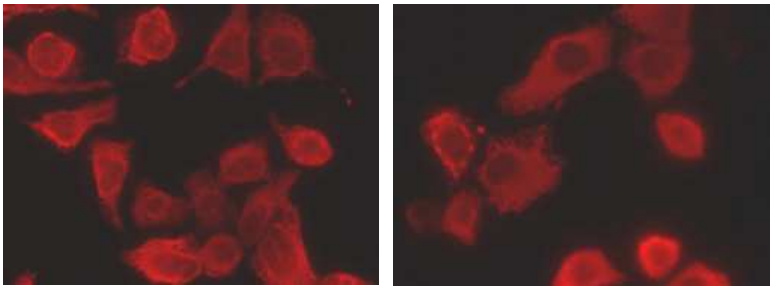
We have noticed three major points.

1) As expected, the transfection efficiency is higher in cells treated with metafectene than in those transfected using calcium phosphate. However, we did not perform statistical analysis and the conclusion is inferred by simple eye observations.
2) The transfection time is crucial for getting good results using metafectene. It had to be removed after 6 hours to avoid cell damage and death, observed when metafectene was washed out only the day after transfection (cells maintained 18 hours in the presence of metafectene). Metafectene toxicity should be indicated and tested with cell lines more sensitive than the HeLa used in our experiments.
3) Metafectene transfection showed the same efficiency when performed with adherent cells or cells in suspension (see picture). This result has clear technical advantages.

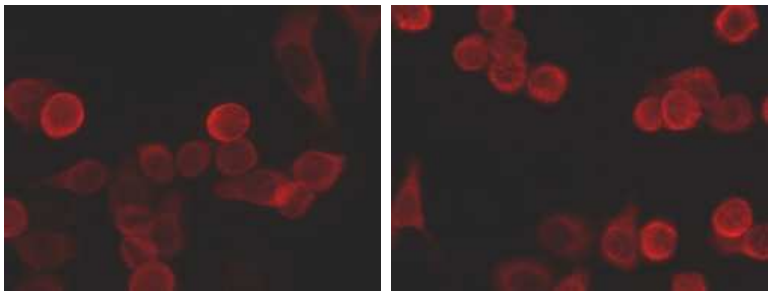
4) The cytoplasmic distribution of the protein inside the cell has been previously observed for constructs expressed fused to secretion signal peptides but having other sub-cellular localization signals inside their sequence.

Conclusion / summary:

Metafectene Easy appears to be a valuable medium for cell transfection and is applicable to both cells in suspension and adherent cells. However, caution must be paid because of the cell toxicity that has been observed when the reagent has been incubated for long period in the presence of the cells.



Transfection with cells cultured in adhesion



Transfection with cells cultured in suspension