P-4 Simple, Efficient and Reproducible Gene Transfection of Mouse Embryonic Stem Cells by Magnetofection

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Objectives: The aim of this work was to define whether magnetofection for genetic transfection was an efficient way to genetically manipulate stem cells without adversely affecting their proliferation or self-renewal capacity.

Methods: One day prior to transfection, NIH3T3 or D3 mES (1×10^4 cells/well) cells were seeded into 24-well plate. For transfection, 1 μg DNA was mixed with MagnetofectionTM or FuGENE 6 for 15 min, and each complex solution was added into the cell plate, and then incubated for 15 min or 24 hours at 37℃ in 5% CO_2, respectively. eGFP activity was assessed by measuring cell epi-fluorescence microscopy. To select antibiotic resistant eGFP-positive D3 mES (D3-eGFP) cell clone, transfected cells were treated using 250 μg/ml G418 for 2 weeks and then grown cloned cells were replated on gelatin coated plate in ES culture medium.

Results: Using enhanced green fluorescent protein (eGFP) as a reporter gene in D3 mouse ES cells, we found that magnetofection gave a significantly higher efficiency of gene delivery in the stem cells (45%) than did the FuGENE 6 method (15%), while both demonstrated efficient transfection in NIH3T3 cells (60%). Though the transfected D3 cells (D3-eGFP) had undergone a large number of passages (>50), they retained their undifferentiated characteristics and displayed markers such as Oct-4 and SSEA-1. They also retained the ability to form embryoid bodies, differentiated into neurons and glia. The eGFP expression was continuously sustained during stem cell proliferation and differentiation.

Conclusion: Based on our results, we conclude that magentofection system is a fast, simple, safety and efficient method for transfecting mouse ES cells. Thus, we suggest that magnetofection transfection systems using nanoparticle may be a useful method to achieve high level of transfection in the human ES cells.