

Protocol for siRNA transfection into primary human cells

Müge Ogrunc, Oliver Bischof and Anne Dejean
Institut Pasteur, Paris, France

INTRODUCTION

Efficiency of transfection into primary cells is a well known problem limiting experimental design, and available methods such as amaxa biosystems depend on usage of large quantities of cells. In addition, the procedure itself causes stress for cells making it hard to study certain phenotypes like cellular senescence.

We used WI38 normal human fibroblasts as our experimental system and performed experiments using scrambled siRNA smart pool in order to set up our controls for possible defects of manipulations. We optimized the conditions for using Dharmacon siRNA SMART pools in 6-well plates. Using this transfection reagent and fluorescent siRNA control, we visualized the delivery of siRNAs into primary cells at a 99% of efficiency.

MATERIALS

- siIMPORTER DNA transfection kit (Millepore)
- DMEM medium
- Fetal Bovine Serum
- Dharmacon SMART pool siRNA

METHODS

1. The primary human fibroblast cells were seeded 48 hours before the transfection at 3×10^4 cells/well in order to have 60-70% confluency at the day of transfection.
2. In a microcentrifuge tube, 20 μ M siRNA was mixed with 25 μ l of siRNA diluent and 10 μ l serum-free medium. In another tube, 5 μ l of transfection reagent was diluted with 25 μ l serum-free medium. The two tubes were mixed and incubated for 15 minutes at room temperature.
3. The mixture was added to a 6-well, which held cells in 930 μ l of serum-free medium.
4. After incubating the mixture for 3-4 hours, the medium was changed.
5. 72 hours after the application of the smart pools, the cells were harvested for qRT-PCR or western blotting to check the levels of knock-down.

Tips

While preparing master mixtures, it should be noted that the efficiency decreases as the number of samples increases because of the low complex formation. Hence, the number should not exceed four times the optimized conditions.