Comparison of Transfection of HeLa cells with GFP-connexin43 with Metafectene Pro and siPORT™ transfection reagent

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Introduction:

Connexin43 is the most common gap junction protein and is responsible for intercellular communication in a variety of cell types. In order to follow Cx43 trafficking in cells, we transfected HeLa cells, which are gap junction deficient, with GFP tagged Cx43 plasmid. By using the GFP tagged Cx43, we are able to follow Cx43 trafficking over time without fixing the cells.

Materials and methods:

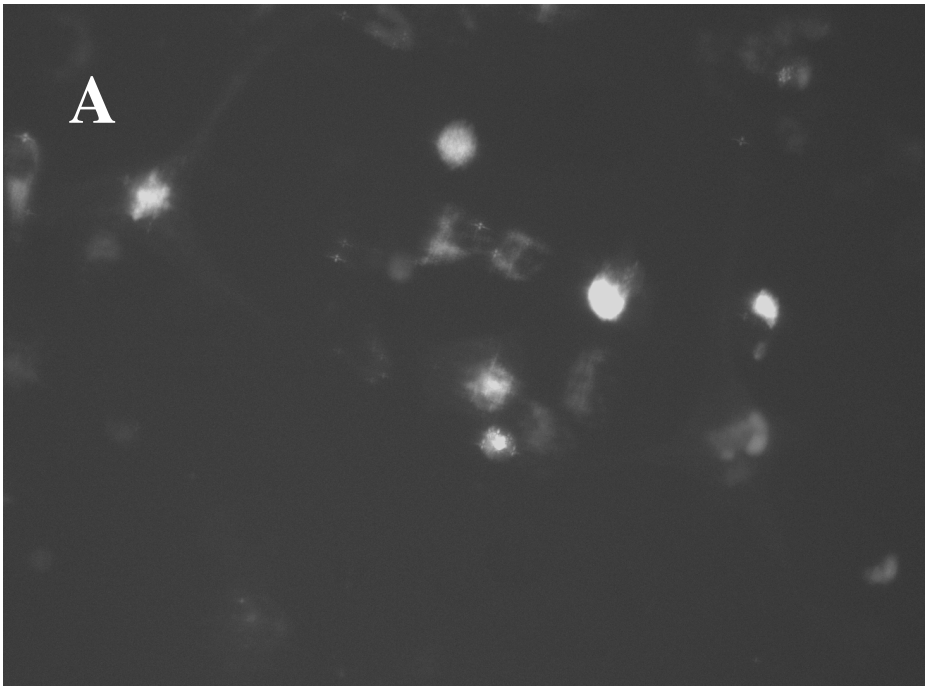
HeLa cells, kindly provided by Dr. Steven Scherer (University of Pennsylvania), were cultured in DMEM with 10% newborn calf serum, penicillin, and streptomycin.

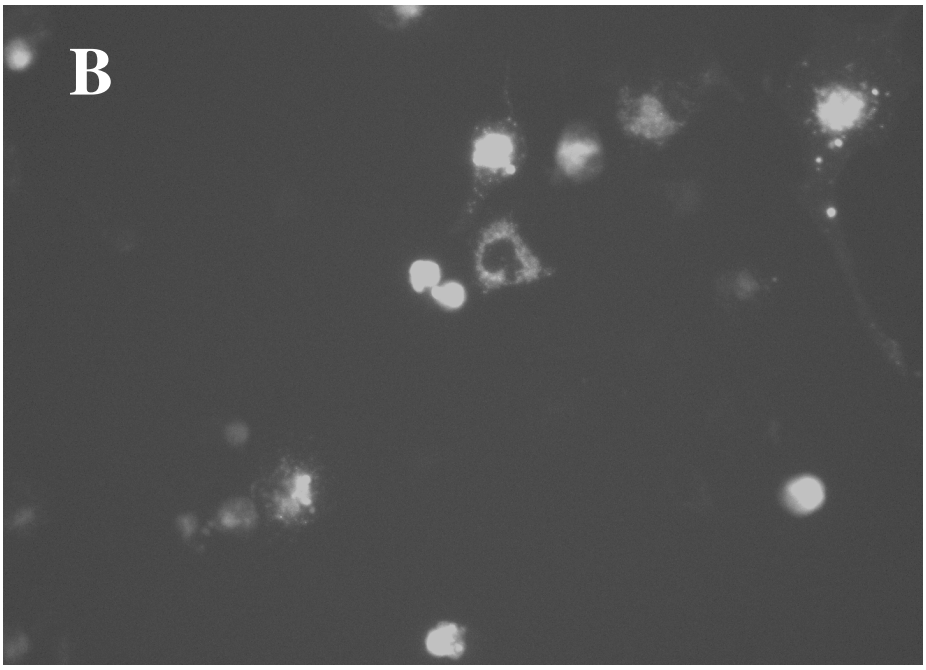
Experimental procedures / transfection protocol:

For transfection, HeLa ($2x10^5$ cells/well) were seeded in 1 ml of DMEM in a 12 well plate and, after 24 hours, used at 50% confluence. Metafectene Pro or SiPORT were complexed with GFP-Cx43 plasmid at reagent:DNA ratios of 1ul:1ug, 2ul:1ug, 4ul:1ug, 6ul:1ug using serum and antibiotic free DMEM. Approximately 50% expression of GFP-Cx43 was obtained at 2ul Metafectene PRO:1 ug DNA and 6 ul SiPORT:1 ug DNA.

Results and discussion:

Below are the results from the transfection experiment. The top image (A) is a fluorescence micrograph of GFP-Cx43 transfected HeLa cells using Metafectene PRO (2:1) and the bottom image (B) is a micrograph of GFP-Cx43 transfected HeLa using SiPORT (6:1).





Conclusion / summary:

Both reagents were effective in transfecting HeLa cells with GFP-Cx43 plasmid. The Metafectene reagent was used at lower volumes compared to the SiPORT reagent with similar results.