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# Testing of Metafectene Pro in human HeLa cells by immunoblotting

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## Introduction

*Salmonella enterica* serovar Typhimurium is a facultative intracellular pathogen responsible for self-limited gastroenteritis and systemic infections in humans and a variety of animalspecies. Our laboratory is interested in the study of the interactions between the pathogenic bacteria *Salmonella enterica* and their host cells. Human HeLa cells and murine RAW264.7 cells are widely used as models to study invasion or intracellular survival and proliferation of *Salmonella*, respectively. *Salmonella* has the ability to inject bacterial proteins inside the cytosol of the host cell through two type III secretion systems. These proteins, called effectors, are thought to interfere with signal transduction pathways in the host and, in this way, contribute to bacterial virulence. In order to study the effects of a secreted protein in the host it is sometimes useful to express this effector from a transfected plasmid. This is usually achieved in our laboratory by electroporation with good results. However, electroporation in HeLa cells results in a high per cent of dead cells, which, in some cases, can obscure the study of the effector overexpression. Here we test the use of Metafectene PRO as an alternative to electroporation in order to get high level of expression of the *Salmonella* effector SlrP in mammalian cells with low level of cell death.

## Materials and methods

### *Materials*

Metafectene PRO, a polycationic liposomal transfection reagent, was obtained from Biontex Laboratories GmbH (Munich, Germany). For transfection experiments, a 3xFlag tagged version of *slrP* was cloned into the eukaryotic expression plasmid pCS2+.

### *Eukaryotic cell lines*

HeLa human epithelial cells (ATCC) were grown in DMEM containing 10% foetal calf serum, 1mM glutamine, penicillin and streptomycin (PAA Laboratories GmbH, Austria).

### *Transfection protocol*

For transfection, HeLa ($10^5$cells/well) were seeded in 0.5ml of DMEM with serum and antibiotics in 24-well culture plates one day beforetransfection, and used at approximately 60-80% confluence. Metafectene PRO was complexed with the plasmid at reagent: DNA ratios of 0.5ml:0.25mg, 1ml:0.25mg, 2ml:0.25mg, 3ml:0.25mg, 1ml:0.5mg, 2ml:0.5mg, 4ml:0.5mg, 6ml:0.5mg, 2ml:1mg, 4ml:1mg, 6ml:1mg, or 8ml:1mg DNA with the pCS2+slrP-3xFlag plasmid. Complexes were prepared by mixing Metafectene PRO with 30ml of PBS, followed by the addition of plasmid DNA previously diluted in 30ml PBS. The mixture was incubated for 17min at room temperature and immediately added to the cells. Transfection efficacy was evaluated after 24 hours by western blot with anti-Flag monoclonal antibodies (Sigma).

### *Cell lysis, western blotting and antibodies*

Cells were detached, washed with PBS and incubated at 4ºC for 20min in 10ml/well lysis buffer (10mM Tris-HCl (pH 7.4), 150mM NaCl, 10% glycerol, 1% NP40, 1% aprotinin, 1mM PMSF, 1mg/ml pepstatin and 1mg/ml leupeptin). The extracts were centrifuged at 20.000g for 20min and the supernatants were boiled for 5min in sample buffer. Proteins were separated by SDS-polyacrilamide gel electrophoresis on 10% acrylamide gels. Gels were electrophoretically transferred to nitrocellulose filters. Monoclonal anti-Flag was from Sigma. Peroxidase-coupled donkey anti-mouse IgG was from Bio-Rad. Detection was performed with Supersignal west pico chemiluminescent substrate from Pierce.

## Results and discussion

Metafectene Pro was complexed with pCS2+slrP-3xFlag plasmid at ratios reagent/DNA indicated in Materials and Methods. Inspection under the microscope 24h

after transfection and the yield of protein recovered from each well indicated low toxicity under the different conditions assayed. Expression was measured by western blotting of protein extracts with anti-Flag antibodies (Fig.1).

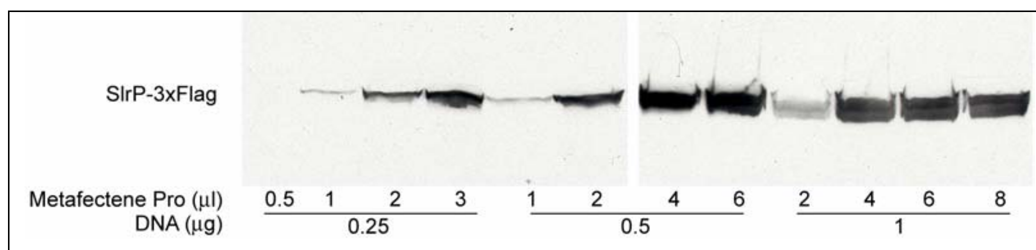


**Fig. 1** Optimization of transfection efficiency in HeLa cells. Extracts from HeLa cells transfected with the indicated amount of Metafectene Pro andthe pCS2+slrP-3xFlag plasmid were resolved by 10% SDS-PAGE. Immunoblotting was performed with a monoclonal anti-Flag antibody.

## Conclusion

Metafectene Pro was very effective in transfecting HeLa cells with low toxicity. The optimum ratio range of Metafectene Pro (ml) to DNA (mg) for these cells is between 4:1 and 8:1.