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Expression of recombinant SARS-CoV-2 nucleocapsid protein in mammalian cells

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The SARS-CoV-2 nucleocapsid (N) protein plays a significant role in the coronavirus life cycle and participates in a variety of critical events following viral invasion[1]. In infected patients, high titers of immunoglobulin G (IgG) targeting N protein were detected and correlated with the clinical course of the disease[2]. Therefore, N protein and anti-N protein IgGs were recognized as important diagnostic indicators of COVID-19 infection in serological and quick antigen tests[3]. In this study, we optimized the expression of the recombinant form of SARS-CoV-2 N protein in a mammalian cell line HEK293T by comparing the transfection efficiency between Polyethylenimine (PEI) and Calcium Phosphate (CaP) DNA-complexing agents. Transfection potency was tested at different cell confluence and passage number, in several cell culture media, pre-transfection and post-transfection media change and in conditions of reduced serum. Chloroquine and glycerol treatments were included to enhance transfection efficiency as they might inhibit DNA degradation in lysosomes or increase membrane permeability. Protein expression was monitored in cell supernatants up to 7 days post-transfection in dot-bot and Western blot using anti-N protein antibodies. Both transfection methods have shown moderate to relatively high transfection efficiency dependent on the applied conditions, making them affordable and easy to use techniques for recombinant N protein production on a small-scale in adherent mammalian systems. PEI acts as a good delivery system regardless of the presence of the fetal bovine serum (FBS), while CaP transfection is more dependent on the presence of FBS which in turn favors N protein degradation. However, we have optimized both methods to achieve optimal expression of unfragmented N-protein in serum-free conditions. Apart from setting up a cost-effective platform for expression of N protein in mammalian cells, we plan on investigating the mechanisms behind the PEI and CaP non-viral gene delivery systems as there are still some uncertainties in the scientific community.

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