Abstract

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Title of Master thesis: Optimization of Transfection of Eukaryotic Cells

An optimization of a transfection is a process of searching for the best conditions for the transfection. Expression systems corresponding to concrete requirements are chosen first of all. A speed, an economy and a functionality of prepared recombinant protein that can be influenced by posttranslational modifications must be considered. Expression vectors are used to insert genes of the interest into expression systems.

The goal of this diploma thesis was to optimize conditions for transfection of eukaryotic cell lines CHO PF a HEK293 using of three commercially available kits. It was followed by a comparison of these transfection efficiency with standard procedures that are used in the company Generi Botech s.r.o. A plasmid pMaxGFP was used for the optimization. A cell counting in Neubaurer's chamber and a quantification of GFP in cell lysate were used to evaluate the efficiency of the transfection. Commercially available kits Electroporation Solution, TansIT-293 Reagent and TransIT_PRO were used for the optimization. Later the transfection efficiencies of these kits were compared with formerly established methods of electroporation in Ex-Cell ACF CHO and with the transfection by Effectene.

The best transfection efficiency was reached in Electroporation Solution that was 4 times higher 48 hours later then in the case of the electroporation in Ex-Cell ACF CHO and 12 times higher compare to Effectene. The efficiency was 3 times higher using of Trans-293 Reagent compare to the electroporation in Ex-Cell ACF CHO and 9 times higher compare to Effectene. The transfection by TransIT_PRO kit was 0.3 times lower than the electroporation in Ex-Cell ACF CHO and 0.8 higher compare to Effectene.