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Ligation Independent Cloning of Polycistronic, Genetically Modified, HuMAb4D5-8 F (ab`)2, in Bacterial Plasmid

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Abstract

Background: Over the recent years, the recombinant monoclonal antibodies and their derivatives are considered among the key elements of targeted therapy. Monoclonal antibodies are regularly expressed in mammalian cells and the costs of their commercial production are too high. Accordingly, the final price of drugs thus produced is high – what further adds to the financial pressures on consumers. Therefore, finding an easy and cheap mechanism such as bacterial hosts to reduce the costs for producing similar products is a main objective in the field of studying the expression systems of recombinant monoclonal antibodies.

Methods: In the present research, a polycistronic construct of F(ab')2 Fragment of anti HER-2 antibody which can be expressed in a bacterial system has been designed. Also, changes have been applied in the antibody hinge region to increase the efficiency of its expression as well as its bending so as to prevent the development of inactive and heterogenic constructs. Eventually, the designed construct was cloned in pET-32 Ek/LIC vector without using the regular cloning methods.

Results: Polycistronic construct of anti HER-2 F(ab')2 fragment was designed successfully. All components for transcription, translation, proper folding, identification and purification were considered. Hinge region modifications were performed successfully. F(ab')2 region amplified by specific primers. Expression cassette was cloned into the framework of the E.coli plasmid pET-32 Ek-LIC vector at the LIC site, successfully.

Conclusion: The results of this study showed that modified F(ab')2 fragment was inserted in E. coli using the Ligation Independent Cloning technology simply and successfully. Although the rate of studies regarding F (ab') 2 fragment design and production in bacterial hosts are very limited, the results of these pieces of research suggest that these hosts are alternative routes and potentially more economical expression systems for the production of antibody fragments.

Keywords: Recombinant antibody, cloning, Polycistronic construct, Bacterial Host, Her2 Receptor.

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