Abstract

Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Biochemical Sciences

Candidate: Iva Vomočilová
Supervisor: Prof. Ing. Vladimír Wsól, Ph.D.
Title of diploma thesis: Cloning, expression and purification of human AKR1C1

This work is focused on synthesis of human AKR1C1. AKR1C1 coding sequence (cDNA), incorporated into plasmid pOTB7, was purchased and delivered in *Escherichia coli* cells. These cells with modified plasmid were multiplied in LB medium. After the multiplication plasmid was isolated and purified by the alkaline lysis process. Coding sequence for AKR1C1 was amplified by PCR method. The primers were designed in advance and contained restriction sites for XhoI and NdeI endonucleases. The results of PCR were validated by gel electrophoresis. Then the PCR product was purified on ultra-pure agarose gel.

In the next step plasmid pET-28b(+) was used to insert prepared coding sequence. Plasmid was multiplied in competent cells *E. coli* HB101 and purified by the alkaline lysis process. Purified PCR fragment and plasmid were double digested by a pair of restriction endonucleases mentioned above. These digested fragments were purified and PCR fragment was put into the open vector pET-28b(+) by T4 DNA ligase enzyme. This modified plasmid was transferred into the competent cells HB101. Cells were multiplied and plasmid was purified. The results of ligation were validated by gel electrophoresis method, later by back restriction with different mixtures of restriction endonucleases. Finally, the results of ligation were proved by sequencing analysis.

After confirmation the product of ligation was transferred to the competent cells *E. coli* BL21. The expression of protein was induced by IPTG. The synthesized protein was purified by application of the affinity chromatographic method. Finally, the concentration of protein was measured by spectrophotometric method (Breadford reagent was used).