Abstract

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Title of diploma thesis: Cloning, expression and purification of human AKR1C2

The coding sequence of AKR1C2 inserted in vector pOTB7 was multiplied by PCR and purified by alkali hydrolysis. Then it was ligated together with TOPO 2.1 vector. Prepared sequence inserted in vector TOPO 2.1 was transformed into the competent E. coli cells and multiplied. To verify these steps we did incubation of cell culture with ampicilin and incubation of coding sequence with restriction endonucleases.The samples inserted in vector TOPO 2.1 were sent to do sequencing.

The next step was the digestion of the coding sequence inserted in vector TOPO 2.1 and the opening of purchased expressed vector pET-15b. Then a several attempts to ligation were made. The control of these steps was done by transformation of vector pET-15b with coding sequence into Hb101 cells, by incubation of transformed cells with antibiotics and by restriction sequence.

Finally, the vector with the coding sequence was transformed into competent BL-21 cells and the expression was done. The result of expression we could observe after sonication and digestion of the cells by lysosym using SDS PAGE.