Research in Pharmaceutical Sciences, 2012;7(5)
School of Pharmacy and Pharmaceutical Sciences
Isfahan University of Medical Sciences

Optimization of the production and purification of the mutant N666E Taq polymerase

H. Mirmohammad Sadeghi, M. Rabbani, S. Assarzadeh*, F. Moazen

Department of Biotechnology, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran

Background and Aims: The aim of this project was to isolate and purify the highly active recombinant Taq DNA polymerase from the strain of Escherchia coli BL21. This enzyme is weighted about 9.4× 104 Daltons molecular weight that is widely used in PCR. In PCR reaction, Taq's activity and fidelity can significantly influence the results. Taq polymerase active site is suggested to bear the O-helix region of the enzyme. In previous work an expression vector containing mutated Asn 666 Glu taq Taq polymerase gene was designed, in order to investigate the effect of this mutation on the enzyme's function, first it was necessary to purify this enzyme.

In this study after transformation of competent cells and induction of expression by IPTG, Desai and method, modified Desai protocol with DNase and RNase, Ni-NTA His.Bind Resins, TCA and refolding protocols was used for purification of this enzyme and comparison these protocols together to find the better method. In comparison the results, using of Desai protocol causes a sharp band in expected region (94 kDa) and several other bands were also visible, but in modified Desai protocol an expected band just observed. In TCA and Ni-NTA His.Bind Resins protocol, the expected bands were so weak. Refolding protocol causes a band in unexpected region (66kDa).

From the different purification techniques that were used in this study, the modified method of Desai and Pfaffle containing RNase and DNase worked best. Addition of TCA for precipitation of proteins that were not affected by heat and using Ni-NTA His.Bind Resins resulted elimination unwanted bands. However, the amount of Taq polymerase enzyme also decreased. About unexpected band was caused because of using refolding protocol it is probable that during the isolation of bacterial inclusion body other enzymes such as proteases are also isolated which could break down the Taq polymerase enzyme.

Keywords: Taq polymerase; Fidelity; Desai; Purification