Recombinant Expression and Purification of Heat-stable Taq DNA Polymerase Gene in E.coli

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Background & Objectives: Heat-stable DNA polymerase enzyme is much attention because of its application on the PCR and molecular biology research and consequently is important the production of enzymes with better functional properties. This enzyme is known as one of the first enzymes that can tolerant high temperature during the PCR denaturation. The purpose of this study was to investigate the recombinant production of a cold sensitive Heat-stable DNA polymerase in bacterial host and to applied a speed and in expensive methods for enzyme purification.

Methods: After synthesis of the desired gene artificially, was cloned into the pET28 vector and then was transform into an *E.coli*. Then the recombinant pET28 was transformed to a BL21 strain. As IPTG induction was used in the study of gene expression. Initial purification of the enzyme was performed by heat treatment at 72°C and precipitation of other insoluble proteins by centrifugation. The DNA synthesis activity of extracts was compared with commercial enzymes. The recombinant enzyme purify and activity were compared to an available industrial enzyme.

Results: Recombinant cold- sensitive Taq DNA polymerase showed similar characteristics such as activity, heat resistance to commercial enzyme.

Conclusion: Since the widespread application of Heat-stable DNA polymerase and simple and cost effective extraction used in this study, it is recommended to use in the production of local enzyme.

Keywords: Taq DNA Polymerase; *E.coli*; Heat-stable; Cold Sensitive