

Production of *thermus aquaticus* DNA polymerase in *Escherichia coli*

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Background and Aims: Taq DNA polymerase is widely used in laboratories for the PCR amplification of target DNA. In the present study, experimental conditions for producing Taq DNA polymerase in E.coli using pET-15b expression vector were optimized.

Methods: Correct framing of the gene in the expression vector pET-15b and its orientation were analyzed by digestion and sequencing. Production of Taq DNA polymerase in E.coli BL21 (DE3) cells was induced by incubation with different concentrations of IPTG. Optimum production occurred with the addition of 1mM IPTG for 2 hours. The activity of the obtained enzyme was measured by comparing the intensities of the produced DNA bands in PCR reactions.

Results: Recombinant plasmid containing Taq polymerase gene was confirmed by restriction digestion and DNA sequencing. Purified protein was identified by Western blotting. Optimum condition for the production of the enzyme was induction with 1mM IPTG for 2-3 hours. Addition of NP-40 increased enzyme stability.

Conclusions: We expressed the recombinant Taq DNA polymerase in E.coli using a T7 based promoter system and obtained an active and stable enzyme.

Keywords: Taq DNA polymerase; Cloning, Expression; Optimization; pET-15b