Thesis title Molecular Cloning and Expression in *E. coli* of the DNA Polymerase Gene from Thermostable *Thermus aquaticus*

Name Plearnpis Luxananil

Degree Master of Science (Biochemistry)

Thesis Supervisory Committee

Sakol Panyim, Ph.D.

Wipa Chungjatupornchai, Ph.D.

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ABSTRACT

The thermostable DNA polymerase of *Thermus aquaticus* (Taq Pol I) greatly simplifies the PCR procedure by enabling the amplification reaction to be performed at higher temperatures. This thesis aims to clone *Taq Pol I* gene in *E. coli* and to partially purified the enzyme product suitable for the PCR reaction.

The total DNA of *Thermus aquaticus* ATCC 25104 was isolated. The *Taq Pol I* gene were amplified from the total DNA using the primer pairs. The 2.5 kb *Taq Pol I* gene was cloned into *E. coli* BL21 (DE3) with bluescribe plus (BS+) plasmid. 6 out of 60 transformants were characterized as the recombinant clones harbouring the *Taq Pol I* gene and designated as BS-Taq 3, 5, 8, 13, 20 and 57. The Taq Pol I product of BS-Taq 57 had the enzyme activity of 1980 U/L of cell cultures. In order to improve the gene expression, the *Taq Pol I* gene was subcloned into pMEX8 plasmid and transformed into *E. coli* JM

107. 5 out of 63 transformants were characterized as recombinant clones which were designated as MEX-Taq 3, 18, 25, 30 and 48. The Taq Pol I product of clone MEX-Taq 30 provided the highest enzyme activity which was 16960 U/L of cell cultures. Analysis of the total intracellular proteins of *E. coli* (MEX-Taq 30) in SDS-PAGE showed an intense protein band of 94 kDa. This 94 kDa Taq Pol I protein represents about 3.4% of the total solubilized protein content in the *E. coli* cells.

Taq Pol I was purified from *E. coli* using a simple purification scheme in two steps. First, since Taq Pol I protein was thermostable, any other *E. coli* proteins was inactivated and denatured by heating at 75°C for 20 min and then was removed by centrifugation. Second, the *E. coli* nucleic acids were removed using ion-exchange chromatography, DEAE-sephacel. The Taq Pol I protein was eluted from DEAE-sephacel with 450 mM KCl TMK buffer. This partial purified Taq Pol I had polymerase activity of 16700 U/L of cell cultures.

From the characterization of Taq Pol I, the enzyme exhibited the optimum activity at the temperature in the range of 70-75°C, pH in the range of 8.5-9, concentration of monovalent cations either KCl or NaCl at 50 mM while at 160 mM showed the inhibitory effects. The absolute requirement for Mg++ as the divalent cation cofactor at the optimum concentration was 2 mM whereas Mn++ displayed the inhibitory effect or to a lesser extent at 1 mM. The Taq Pol I enzyme lacks of exonuclease or endonuclease activity. More than 10% glycerol in the

total PCR reaction decreased the activity of Taq Pol I. Without adding any stabilizers, Taq Pol I could be stored at room temperature (25-30^{o}C) for a week with the loss of approximately a half of activity.