UNIVERSITI TEKNOLOGI MARA

CLONING OF THERMOSTABLE RECOMBINANT DNA POLYMERASE IN *ESCHERICHIA COLI*

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ABSTRACT

Background: The DNA polymerase from *Pyrococcus furiosus (Pfu)* has gained considerable attention in the field of DNA amplification as this enzyme have elevated temperature optima and have thermal stabilities that roughly correspond to the thermal extremes of the environment from which they were isolated. Due to thermostability of this enzyme, the structure and function relationships and the potential industrial applications of many thermostable enzymes such as DNA polymerases are of considerable interest to researchers.
Aim: The purpose of this study is to clone the Recombinant DNA polymerase from *Pyrococcus furiosus* in *Escherichia coli.*
Method: The PCR was conducted based on standard PCR protocol and was optimized under different annealing temperatures using Eppendorf Mastercycler Gradient to determine the optimal annealing temperature for this method. And also for further optimization, new primers batch was developed to eliminate contaminate primers.
Results: The PCR based method was failed to give the result. It shows that there was no DNA amplification but only DNA ladder band can be seen.
Conclusion: This study failed to amplify the *Pfu* DNA polymerase gene. More time needed to optimize the PCR condition.

CHAPTER 1

INTRODUCTION

A number of thermophilic DNA polymerases have been isolated and characterized from both mesophilic eubacteria and archaeae sources. These enzymes have elevated temperature optima and have thermal stabilities that roughly correspond to the thermal extremes of the environment from which were they isolated. More than 50 DNA polymerase genes have been cloned and sequenced from various organisms, including thermophiles and archaea. Amino acid sequences deduced from their nucleotide sequences can be classified into four major groups which are *Escherichia coli* DNA polymerase I (family A), DNA polymerase II (family B, α- like DNA polymerase), DNA polymerase III(family C), and others(family X). The DNA polymerase from *Pyrococcus furiosus (Pfu)* has gained considerable attention in the field of DNA amplification. The *Pfu* DNA was initially characterized for the preparation isolated directly from *P.furiosus*, but this thermophilic, anaerobic bacterium is difficult to grow so as to obtain large amounts of protein. A major advance was expression of recombinant *Pfu* DNA polymerase in a baculovirus-mediated system. This system makes possible production of commercial amounts, but is not as cheap and convenient as an *E.coli* bacterial expression system. The *Pfu* DNA polymerase was also produced