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A Simplified Protocol for Producing *Taq* DNA Polymerase in Biology Laboratory

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Abstract

Background: *Taq* DNA polymerase is a very important enzyme for molecular biological studies such as DNA amplification and DNA sequencing by the PCR. It is a standard enzyme that is used in 90% of molecular biology labs today. The aim of this study was to produce *Taq* DNA polymerase enzyme in *E. coli* by a reliable, practical, simple and low cost method.

Materials and Methods: In this study, the *Taq* gene was amplified from the genomic DNA of *Thermus aquaticus* and cloned into pTrc99A vector. Recombinant plasmid is expressed in *E. coli* strain TOP10. Product protein is extracted and purified. Expression of gene was analyzed by SDS-PAGE and gene amplification.

Results: In SDS-PAGE, bands were observed in the range of 94 KDa. The density of protein bands in agarose gel electrophoresis indicated that the purified enzyme is more active than the nonpurified one.

Conclusion: The protocols described in this paper lead to the production of pure and active enzyme that can be applied in both teaching and research laboratories.

Keywords: *Taq* polymerase; Expression; Purification

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Introduction

The polymerase chain reaction (PCR) and DNA sequencing are usually used techniques of molecular biology which utilize DNA polymerases. The high temperatures required for DNA amplification in PCR entail a thermo stable enzyme, and *Taq* DNA polymerase obtained from a heat stable bacterium called *Thermus aquaticus* has solved this problem. The high optimal polymerization temperature of this enzyme also makes it useful tools for sequencing of DNA (1).

Taq DNA Polymerase has a molecular weight of about 66-94 kilo Daltons (kDa). The full length 94 kDa of *Taq* polymerase has the highest activity and half-life of 10 minutes at 97 °C (2). *Taq* polymerase catalyzes the incorporation of dNTPs into DNA with the rate of about a thousand base pairs per minute. It requires a DNA template, a primer and the divalent cation magnesium. *Taq* polymerase contains a 5'-3' exonuclease activity. It does not have a 3'-5' exonuclease activity and thus, no proof reading function (3). *Taq* DNA polymerase has a terminal transferase activity.

It adds an adenine at 3' end found to be useful to produce TA cloning plasmids which possess a 3' thymine overhangs. This allows ligation using DNA ligase be quickly be accomplished with the adenine overhangs of the PCR product (4). However, the *Taq* DNA polymerase isolated from *Thermus aquaticus* was the first characterized thermo stable enzyme but until now, the more than fifteen DNA polymerase genes have been cloned and sequenced from various organisms by PCR cloning technique. The recombinant

Taq DNA polymerase expressed in *E. coli* represents the identical characteristics to native *Taq* from *Thermus aquaticus* with preservation of activity, specificity and thermo stability in PCR.

However, the lac promoter and its derivatives like ptac are frequently applied in order to control gene expression, and almost in most cases, Isopropyl β-D-1-thiogalactopyranoside (IPTG) is used as inducer for foreign gene expression (4). In this study, we describe a reliable, practical, simple and low cost method to produce the enzyme of *Taq* DNA polymerase in the *E. coli* for use in PCR, in any molecular laboratory.

Materials and Methods

PCR and Cloning

The Genomic DNA of Thermus aquaticus strain YT-1 (ATCC-25104) was isolated using high pure PCR template preparation kit (Roche, Germany). A pair of primers was designed based on the 5' and 3' ends of the DNA sequence of *Taq* DNA polymerase gene (Accession No. DD137468) and were used for PCR amplification. A 2.5 Kb fragment was amplified by primers of forward, 5´-CGGAATTCTGAGGAGG-TAACATGAGGG-3´ and reverse, 5´-CGTCGACT-AGATCACTCCTTGGCGGAGAG -3´ which created unique EcoRI and SalI restriction sites (underlined), respectively, at each end of the amplified DNA fragment. PCR amplification was performed using the following reagents: PCR reaction buffer (50 mM KCl, 20 mM Tris- HCl (pH=8.3), 1 µM each of primer, 1.5 mM MgCl2, 250 µM dNTPs, 30 ng template DNA, 2 Unit Pfu DNA polymeras (Fermentas, Germany). The final reaction volume was 30 µl. Thermocycling conditions were 95 °C initial denaturing for 4 minutes followed by 30 cycles of 94 °C denaturing for 30 seconds, annealing at 55 °C for 2 minutes, and extension at 68 °C for 2 minutes; and the last final extension at 68 °C for 10 minutes. Amplified products were analyzed by electrophoresis using 1% agarose gels. DNA fragments from agarose gels were extracted by an Agarose Gel DNA Extraction Kit (Roche, Germany). The fragment was ligated into the expression vector of pTrc99A (Addgene) that had been digested before with EcoRI and SalI (Fermentas, Germany) using Rapid DNA Ligation Kit (Roche, Germany). Ligate was transformed into competent *E. coli* strain, TOP 10 (Invitrogene) by CaCl2 (Sigma, Germany) using heat shock method at 42 °C for 45 seconds. These mixtures were then plated on Lauria Bertaini (LB) agar media (Merck, Germany) containing 100 µg/ml ampicillin and incubated at 37 °C overnight. The obtained colonies were grown in 2 ml LB broth media (Merck, Germany) containing 100 µg/ml ampicillin at 37 °C with shaking at 200 rpm. The recombinant plasmids prepared by a Qiagen mini-prep kit and the presence of the amplicon had been confirmed by sequencing.

Expression and Extraction

The recombinant *E. coli* was cultured in 200 ml of LB broth overnight at 37 °C containing 100µg/ml ampicillin. The expression of recombinant protein was induced by 0.125 mg/ml IPTG (Sigma, Germany) until the bacteria reach OD600 of approximately 0.7. The culture was continued overnight. No addition of inducer was used as negative control. The bacteria were harvested by centrifuging in 6000 rpm for 15 minutes at 4 °C and washed in 100 ml of sterilized A Buffer [50 mM Tris-HCl pH=7.8, 50 mM Dextrose, 1 mM EDTA (Merck, Germany)]. The cells were spin down and resuspend in 2 ml of A Buffer and 4 mg/ml lysozyme (Sigma, Germany) for 15 minutes at room temperature (RT). The bacteria were then lysed by adding 2 ml of sterilized lysis buffer [50 mM Tris-HCI pH=7.8, 50 mM KCI, 1 mM EDTA pH=7.8, 1 % Triton x-100 (Merck, Germany)] and incubated in 15 ml falcon tube at 70 °C for 1 hour. The lysis mixture was then centrifuged at 14000 rpm for 10 minutes at 4 °C, and the clarified supernatant was transferred to a sterile 15 ml falcon tube.

The enzyme was recovered from the clarified supernatant by adding 1 gram of Ammonium sulphate (Merck, Germany) while stirring rapidly for 10 minutes at RT. The solution was then centrifuged at 14000 rpm for 10 minutes and protein precipitate was collected (pellets and surface). The pellet was resuspended in 3 ml of A buffer and dialyzed against 25 ml sterilized storage buffer [20 mM Tris-HCI pH=7.8, 100 mM NaCI, 0.1 mM EDTA pH=7.8, 1% Triton x-100, 50% glycerol (Merck, Germany)] overnight at 4 °C. The resulting protein was diluted 1:1 with storage buffer.

Enzyme assay

The expression of *Taq* DNA polymerase was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (1). The activity of the enzyme was determined using a PCR amplification reaction with titration against a commercial *Taq* DNA polymerase (Fermentas, Germany).

Human genomic DNA was used as template for amplifying a 470 base paired fragment. To obtain the optimum pH in which the enzyme is stable for a longer time, the storage buffer was prepared in a gradient pH range 7.2 to 8.2. After two months storage in -20 °C, the activity of enzyme was assayed by PCR.

Results

In SDS-PAGE analysis, protein segments were observed in the range of 94 KDa which confirmed that Taq gene was expressed. In this assay, the

expression levels of Taq protein were measured in three steps; No inducing *E. coli* with IPTG, induced *E. coli* before dialysis and induced *E. coli* after dialysis.

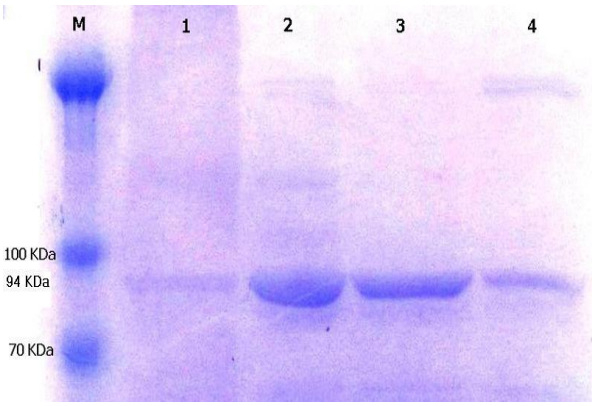

Figure 1. SDS-PAGE analysis of *Taq* DNA polymerase. 7 µl of commercial *Taq* equal to 5 units (line1). 7 µl IPTG induced protein after dialysis (line 2). 7 µl IPTG induced before dialysis (line 3). 7 µl non-induced protein after dialysis (line 4).

To estimate the enzyme activity, the recombinant enzyme was titrated in five dilutions in both dialyzed and non-dialyzed against a commercial *Taq*. Bands in agarose gel electrophoresis indicated that the purified enzyme is active (Figure 2). As shown in figure 2, the activity of the non-dialyzed enzyme is less than dialyzed. Assessment of the enzyme activity at different pH of storage buffer showed that *Taq* DNA polymerase enzyme has remained active longer at pH close to 7.8 after a couple of months.

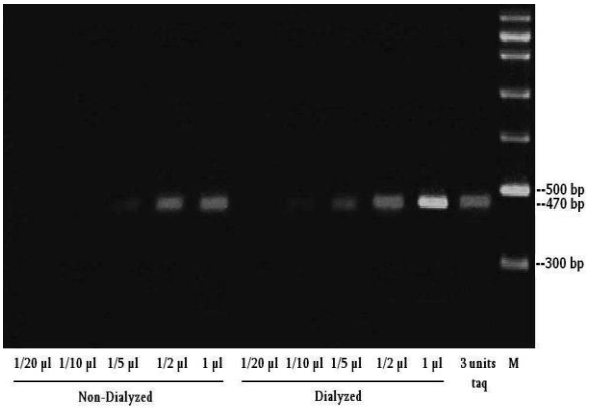

Figure 2. Enzyme activity assay.Comparison of PCR band intensity in dialyzed and non-dialyzed *Taq* protein at different dilutions.

Discussion

Taq DNA polymerase is an important enzyme for DNA amplification and sequencing by the PCR in molecular biological studies (5). It is a standard enzyme used in most of molecular biology laboratories today. Despite all the problems associated with culture of *T. aquaticus*, gene isolation and cloning and also purification of *Taq* DNA polymerase, maintain the stability of the enzyme for long-term storage is also huge challenge that must be considered. The SDS-PAGE results show that the intensity of expressed *Taq* protein is close to the *Taq* protein expressed by some group of molecular scientists (3, 6, 7). Comparison of the induced and non- induced pattern of protein concentrations in SDS-PAGE shows that trc promoter in ptrc99a plasmid is a powerful promoter in gene expression in *E. coli*.

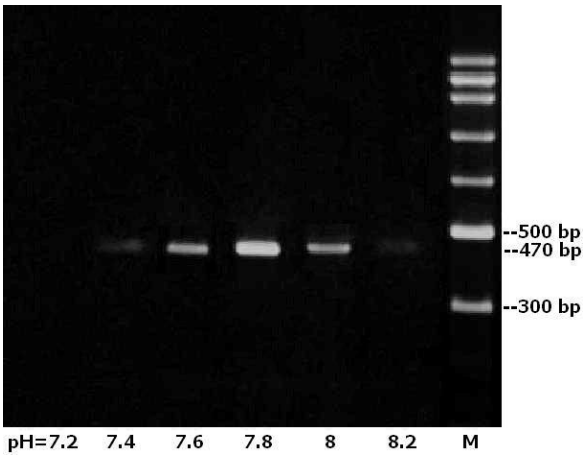

Figure 3. Enzyme stability assay.Comparison of PCR band intensity at different pH of storage buffer two months after purification.

Nowadays, more patents of *Taq* DNA polymerase production have recently expired which provide an opportunity for many molecular biology researchers to produce and use their own *Taq* DNA polymerase. The protocols described in this paper lead to the production of pure and active enzyme that can be applied in both teaching and research laboratories. *Taq* DNA polymerase was remained active for at least one year.

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