High-Efficiency Separation and Purification of Taq DNA Polymerase

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1 Introduction

The thermostable Taq DNA polymerase was firstly isolated by Ms. Qian Jiayun from the YT-1 strain of the aquatic thermophilic bacteria (Thermus aquatics) [1–3]. Its high specificity, yield and sensitivity make it widely used in PCR [4, 5] and other related techniques for many years. Taq polymerase has a half-life of 9 min at 97.5 °C and its optimum temperature is 75–80 °C. It can replicate a DNA fragment of 1000 bp within 10 s at 72 °C. Its higher enzyme activity is temperature dependent. At low temperature, the enzyme activity of replicating DNA was significantly lower comparing to that at higher temperature. However, its activity on DNA synthesis is also quickly reduced when temperature is higher than 90 °C.

At present, the main task in optimizing Taq DNA polymerase is to improve its yield by genetic engineering as well as simplifying the purification and shortening the production process [6, 7]. Large-scale production of efficient and cost-effective Taq DNA polymerase relys on new engineering method, and therefore the PCR technology still has much room to improve [8].

Engelke et al. constructed recombinant plasmid pTTQ18 and expressed the recombinant Taq DNA polymerase in Escherichia coli. It was purified by denaturing heat- hybrid protein and PEI. Then used BioRex 70 Ion Exchange Chromatography to obtain recombinant Taq DNA polymerase. However, the yield, purity and enzyme activity of this method are not very high. Qinchuan et al. used ammonium sulfate precipitation and freezing and thawing to extract Taq DNA polymerase. It showed that ammonium sulfate precipitation is relatively simple and low in cost. The activity of

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prepared Tap DNA polymerase can be effectively amplified DNA fragments. Freeze-thawing method did not sufficiently purify protein. The activity of Taq DNA polymeraseis low. PCR amplification effect of this method is not ideal.

In this study, we established a highly efficient and rapid method for the expression and purification of Taq DNA polymerase in an engineering strain of Taq DNA polymerase. This method optimized various conditions to elevate the production and purity of the enzyme, providing high quality Taq DNA polymerase for routine molecular biology experiments. His6 refers to a fusion tag consisting of six histidine residues that can be inserted at the C-terminus or N-terminus of the protein. It can constitute an epitope that facilitates purification and detection, it also can format the unique structural features (binding ligands) to facilitate purification. So the constructed plasmid of Recombinant Escherichia coli strain uesd His-tag. The Taq DNA polymerase Recombinant Escherichia coli strain has the advantage of producing large quantities of this enzyme in E. Coli using IPTG as an inducer. The technique does not require tedious work, and purified enzyme is effective and can be used with relatively low amount. Therefore it reduces the cost of each experiment to a certain extent [9]. Taq DNA polymerase is an important biotechnology tool, and is widely used in diagnosis and treatment of infectious diseases, drug mechanism and other medical fields. Therefore, this study not only has clear potential in applications, but also has some theoretical significance for the research of polymerase expression, separation and purification.

2 Materials and Methods

2.1 Materials

Taq DNA polymerase Recombinant Escherichia coli strain preserved in our laboratory. Chemicals and culture medium: Luria-Bertani (LB, 1% Tryptone, 0.5% Yeast extract, 1% NaCl), 50× TAE Buffer (Tris, acetate, EDTA, pH 8.5), DNA extracts (100 mmol/L Tris-Cl pH 8.0, 50 mmol/L EDTA pH 8.0, 500 mmol/L NaCl, 1% SDS, 10 mmol/L βHere) [10]. Buffer A (500 ml): 0.6057 g Tris (pH 7.9), 0.2033 g $MgCl_2$, 0.1982 g $(NH_4)_2SO_4$, 0.99 g Glucose, 1.86 g KCl.

2.2 Definition of Taq DNA Polymerase

The enzyme activity of one unit of Taq polymerase was defined by the amount of enzyme required to incorporate 10 nmol deoxynucleotides into acid-insoluble matter at 74 °C for 30 min using activated salmon sperm DNA as template/primer [11].

2.3 Selection and Activation of Culture

The *E. coli* strain of this experiment contains an exogenously expressed Taq DNA polymerase gene. To purity Taq DNA polymerase, the strains were streaked on LB plates (Amp 100 μg/mL), cultured for 16–20 h at 37 °C. Single colony is picked and inoculated into 6 ml LB medium containing ampicillin (100 μg/mL), and cultured overnight at 37 °C.

When the bacteria reached their semi-growth phase(OD660 = 0.4), induction of Taq DNA polymerase was performed using different concentrations of IPTG and samples were shook for 12 h at 37 °C.

2.4 Separation, Purification and Concentration of Taq DNA Polymerase

Using lysozyme, freezing-thawing and heparin-Sepharose affinity chromatograph to separate and purify Taq DNA polymerase. Lysozyme, also known as the cell wall enzyme or N-acetyl cell wall polysaccharides hydrolase, is an alkaline enzyme that can effectively hydrolyze the peptidoglycan of bacterial cell wall [12]. Freezing and thawing method cooled brakes cells to −15 to −80 °C, and then melt quickly at 30–40 °C, so repeated freezing and thawing many times, the formation of ice particles in the cell to make the remaining fluid solution of salt Concentration increased caused by cell swelling and fragmentation. Repeated freezing and thawing method is more gentle, avoiding the destruction of high temperature on the active substance.

The procedures of lysing bacteria and denaturing by high temperature to remove some miscellaneous proteins are (1) Adding 5 μL Buffer A to the centrifuge tube. After the cells were completely dissolved in buffer A, added 200 μL lysozyme, placed at 70 °C for 1 h. (2) Lysed cells were placed at −80 °C for 10 min, remove the cells and place in water at 80 °C for 5 min. After repeated freezing and thawing for three times, transfer the bacteria into water with a temperature of about 75–80 °C for 30 min, mix upsiding down the bacteria culture every 5 min. (3) Centrifuging at 8000 rpm for 30 min at 4 °C, transferring the supernatant to a clean tube, and placed on ice. (4) Filtering with 0.45 nm filter, taked 1 mL as a crude enzyme.

In this study, the heparin affinity chromatography was used to further purify. Finally the high concentration of Taq DNA polymerase was obtained. Heparin affinity chromatography medium (Heparin QZT 6FF) was prepared by conjugating heparin to an agarose gel using a self-made cross-linked agarose as the matrix. In this paper, Taq DNA polymerase was concentrated by dialysis.

2.5 Detection of Taq DNA Polymerase

Taq DNA polymerase were separated by SDS-PAGE [13–15]. A 12% polyacrylamide gel was used for electrophoresis and Coomassie blue R-250 (0.1%) was used for gel staining. The Odyssey Infrared Imaging System, and the corresponding application software version 3.0 from Li-Cor Biosciences [16].

2.6 DNA Extraction and PCR Analysis

Total DNA was extracted by using the Kit (Solarbio) according to the manufacturer's instructions. Using extracted cell genome as PCR template through culturing cell. Three kinds of primers were designed and verified by PCR [17]. The length of the target fragment was 2200, 750 and 600 bp respectively. It can verify the amplify efficiency of PCR.

3 Results

3.1 Optimal Screening of Taq DNA Polymerase Strain

Drawing three section line [18] with a small amount of Taq DNA polymerase solution. The treated plate was then incubated overnight at 37 °C and the growing rounded single colony was picked as the candidate Taq DNA polymerase strain.

3.2 Induction of Taq DNA Polymerase

Recombinant bacteria were inoculated into LB medium. After incubating at 37 °C for 12 h, 5 mL bacteria solution was inoculated into 100 mL culture medium (Containing 100 μg/mL ampicillin). Adding IPTG in the latter part of the growth curve [19]. The final concentration of IPTG is 0.5 mmol/L. After inducting for 12 h, the bacteria were collected by centrifugating at 5000 rpm for 10 min. Using SDS polyacrylamide gel electrophoresis to examine the induced effect of protein of the crude Taq DNA polymerase.

It can be seen from Fig. 1 that the level of protein expression of Taq DNA polymerase increased significantly after IPTG induction for 12 h compared to the strain that was not induced by IPTG. The production of Taq DNA polymerase increased 50% by the gray level analysis through Quantity One.

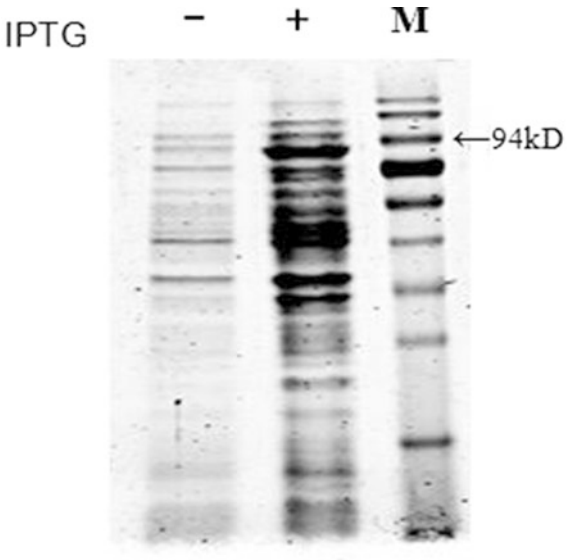

Fig. 1 Induction of Taq DNA polymerase

3.3 Purification of Taq DNA Polymerase

The crude Taq DNA polymerase was obtained by repeated freezing and thawing cycle as previously described [20] with constantly switching high and low temperature. The protein was purified by affinity chromatography. The purified Taq DNA polymerase that was obtained after the elution and detected the purification effect of protein by SDS polyacrylamide gel electrophoresis [21].

As shown in Fig. 2, compared with the purchased Taq DNA polymerase (control), the crude enzyme solution contains a small amount of hybrid protein, but the purity of Taq DNA polymerase has been greatly improved after affinity chromatography. The purity of Taq DNA polymerase is up to about 90% by the gray level analysis through Quantity One.

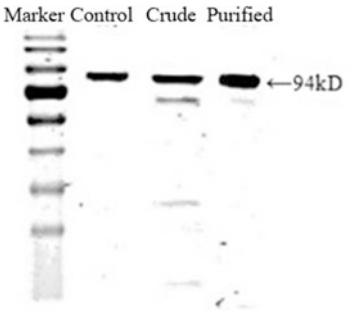

Fig. 2 Affinity purification of Taq DNA polymerase

3.4 Detection of Residual Nucleic Acids

The obtained crude Taq DNA polymerase and purified enzyme were checked to see whether there are residual amount of nucleic acid by using agarose gel electrophoresis. As shown in Fig. 3, there was no ribonucleic acid bands in the obtained Taq DNA polymerase extracts, thus it could be used for PCR experiment.

3.5 Detection of the Protein Concentration

200 μL Coomassie Brilliant Blue G-250 Formulation Solution and 5 μL Taq DNA Polymerase Purified Enzyme were added to each well of a cleaning 96-well plate, reading the OD at 595 nm. The protein concentration of Taq DNA polymerase was calculated according to the standard curve [22]. As shown in Fig. 4, after purification, protein concentration of Taq DNA polymerase had increased.

3.6 Detection Heat Tolerance

Toleration of the enzyme to high temperature is critical for PCR, therefore we determined the thermal stability of the enzyme. As shown in Fig. 5, the Taq DNA polymerase was heated at 95 °C for 5, 10, 15 and 20 min, respectively. PCR and agarose gel electrophoresis were used to detect the heat tolerance of Taq DNA polymerase.

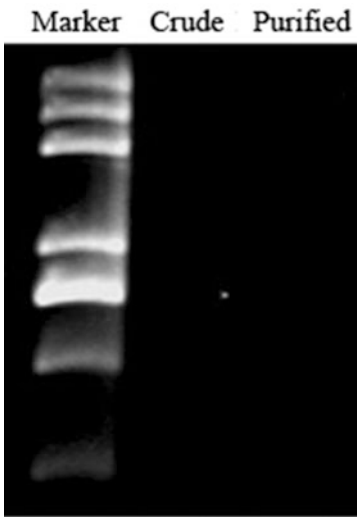

Fig. 3 Detection of residual nucleic acid

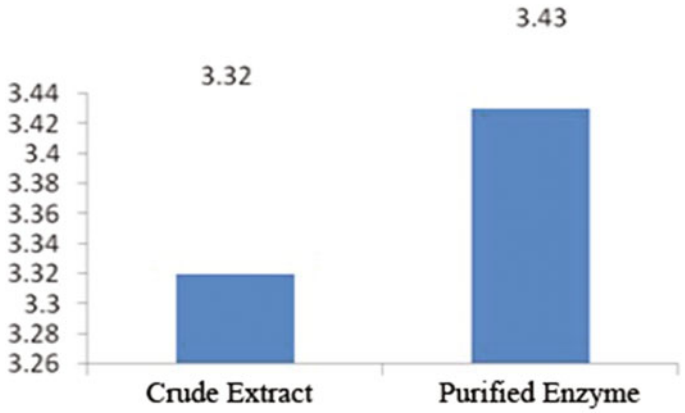

Fig. 4 Protein concentration of Taq DNA polymerase (μg/mL)

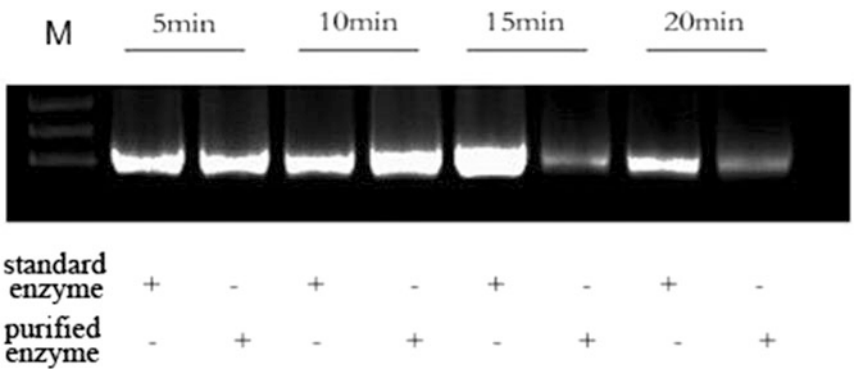

Fig. 5 Detection of Taq DNA polymerase heat resistance

It can be found that the purified enzyme of Taq DNA polymerase was heated to 15 min. PCR target band did not differ from the standard enzyme, which can meet the high temperature conditions during PCR. Thus it could prove that the extracted Taq DNA polymerase has good heat resistance.

3.7 The Amplification Efficiency of Taq DNA Polymerase

Using PCR to analyze the efficiency of the obtained Taq DNA polymerase. The length of the target fragment was 2200, 750 and 600 bp respectively. The results indicated that our purified Taq DNA polymerase not only showed the high efficiency on PCR as that of the standard enzyme, but even more powerful in amplifying short length DNA template as it revealed higher specificity. Our purified

Taq DNA polymerase was not do the transformed, so fidelity will not have any increase or decrease. Fidelity was not significantly different from the purchased standard (Fig. 6).

4 Discussion

This study characterized and optimized the procedure of produce Taq DNA polymerase using a strain developed in our lab. Here we optimized the induction time of IPTG for Taq DNA polymerase, and the protein content after induction was significantly increased. Taq DNA polymerase was isolated by the method of preliminary protein extraction combined with freeze-thawing and thermal denaturation, followed by further purified using heparin affinity chromatography. The production of Taq DNA polymerase was in high yield that reached ~3.43 mg/mL. Taq DNA polymerase was not do the transformed, so enzymatic activity will not have any increase or decrease. The yield of purified enzyme was 5.15 mg and the final yield was 2.6 mg/mL. The study also verified the quality and efficiency of the extracted

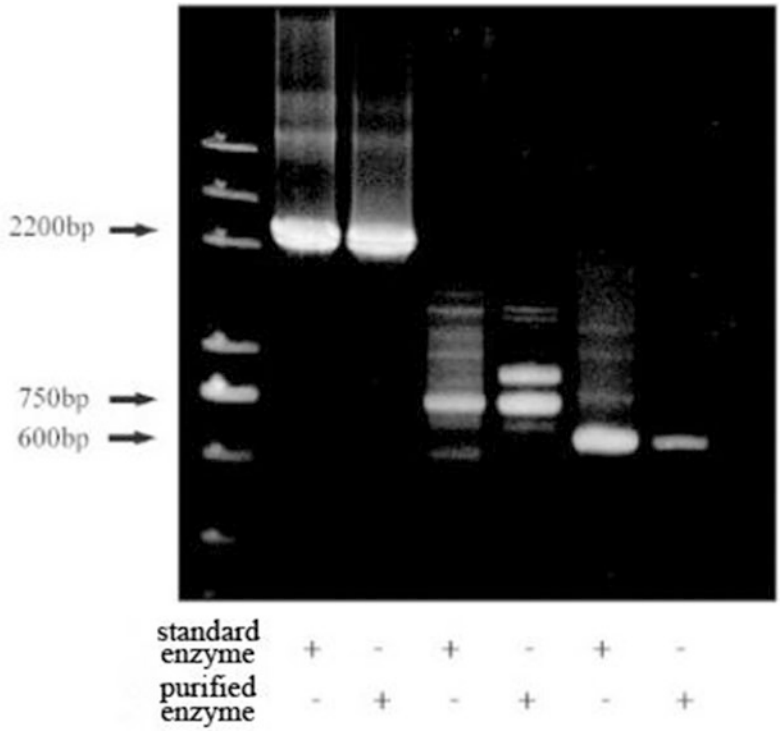

Fig. 6 Effect of Taq DNA polymerase amplification

Taq DNA polymerase, and our results showed that Taq DNA polymerase could amplify the DNA fragment of different length with even higher specificity than that of the standard commercial enzyme.

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