Cloning, Expression, Production and Activity Test of Taq Polymerase

Arife Kaçıran (ORCID: https://orcid.org/0000-0002-6881-8568) [1,3*], Süleyman Abdulhekim (ORCID: https://orcid.org/0000-0002-7080-4755) [2,3], Ayşe Nur Akmehmet (ORCID: https://orcid.org/0000-0001-9599-8050) [1,3], Sabriye Çanakçı (ORCID: https://orcid.org/0000-0003-0132-7198) [1,2], Ali Osman Beldü z (ORCID: https://orcid.org/0000-0003-2240-7568) [1,2]

[1] Karadeniz Technical University, Faculty of sciences, Department of Biotechnology, Trabzon, Türkiye
[2] Karadeniz Technical University, Faculty of sciences, Department of Biology, Trabzon, Türkiye
[3] Eryiğit Medical Devices Inc., R&D Department, Ankara, Türkiye
E-mail; 407958@ogr.ktu.edu.tr

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Abstract

Polymerase chain reaction (PCR) is a basic and primary technique used in clinical, forensic and food laboratories. This technique is a key tool for forensic DNA analysis, food safety, clinical diagnosis, and safety decision-making in bioterrorism. In addition, it is used in the diagnosis of infectious diseases such as tuberculosis, AIDS, COVID-19, to characterize the infection and to treat it appropriately. DNA polymerase is the enzymeused in PCR. DNA polymerase makes it possible to detect and analyze the target DNA sequence by producing copies of specific DNA and amplifying traces of target DNA sequence billions of times this way. Although various DNA polymerases are available for various PCR techniques, Taq polymerase obtained from *Thermus aquaticus* bacteria is widely used and, it has a high resistance to denaturing conditions such as high temperature required during PCR. The importance of Taq polymerase has been further realizedduring the COVID-19 pandemic and ithas become a great need for COVID-19 detection kits produced across the country. In this product-oriented study, it was aimed to produce Taq DNA polymerase in our laboratory and to make it more stable and effective by optimizing it with different buffers. To achieve this, the Taq DNA polymerase gene, which is a GC-rich region, was amplified by PCR using specific primers and was first cloned into a cloning vector and, then into pET28a(+) expression vector. Cloned sequence was confirmed by next generation sequencing. Taq polymerase was expressed in E. coli BL21(DE3) cells and partially purified. Partially purified enzyme was tested in PCR and it was observed that our enzyme works as efficient as commercial Taq polymerases. In the continuation of the study, this enzyme will be purified to high purity level and optimized with different buffers. Thus, more efficient, domestic and long-lasting enzyme production will be achieved.

Keywords: Taq polymerase, Cloning, Expression, Purification, PCR Activity Test, Domestic Product

Introduction

Polymerase chain reaction (PCR) is a basic technique used in various research laboratories such as clinical laboratories, forensic and food laboratories. Since its initial development in the early 1980s, the basic polymerase chain reaction (PCR) has been adapted to a wide variety of tasks in molecular cloning, including DNA sequencing, in vitro mutagenesis, mutation detection, cloning of cDNA and genomic DNA, and allelotyping. In addition, it is used in the diagnosis of infectious diseases such as tuberculosis, AIDS, hepatitis, COVID, to determine the amount of infection and to treat it appropriately. Therefore, it constitutes the basic need of all health and biology research laboratories (1). The most basic requirement for the realization of the polymerase chain reaction process is the polymerase enzymes. Polymerases are such enzymes that make DNA from nucleotides by catalyzing the synthesis of long chains or polymers of nucleic acids(2). There are several forms of DNA polymerase with a role in DNA replication and usually, work in pairs to copy one molecule of double-stranded DNA into two new double-stranded DNA molecules (figüre 1).

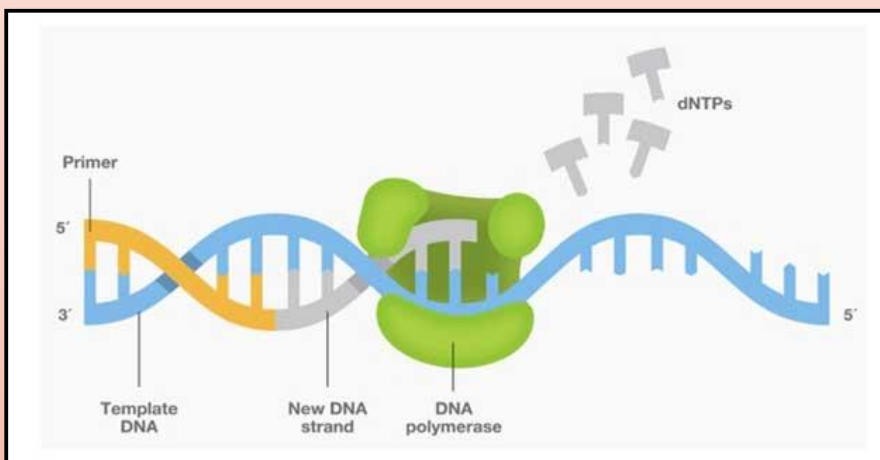

Figure1. Activity of Taq DNA polymerase at 72 C (4)

Although various DNA polymerases are available for various PCR techniques, Taq polymerase obtained from *T. aquaticus* bacteria is widely used, which has a high resistance to denaturing conditions such as high temperature required during PCR.

Among the DNA polymerases, some are highly thermostable like *Taq* DNA polymerase and can work at elevated temperatures in comparison with other polymerase enzymes. Thus, *Taq* DNA polymerase is widely used in all molecular studies nowadays. It was first isolated in 1976 from *T. aquaticus* in a hot spring in Yellowstone National Park. It uses single-stranded DNA as a template for the synthesis of the complementary DNA strand. The best temperature for *Taq*'s activity is 75–80 °C. However, it can also stand at 97.5 °C and can replicate 1-kb strand of DNA in less than 10 s at 72 °C. These thermostable properties of *Taq* DNA polymerase from *T. aquaticus* have contributed greatly to the specificity and yield of the polymerase chain reaction, which has been recently emerged as a valuable technique for gene amplification and identification in clinical diagnosis and sequencing. The purification of the *Taq* polymerase enzyme from the native host results in low yield (3). So, the gene encoding this enzyme has been cloned and engineered in different ways for obtaining high-level expression in *E. Coli*.

In this study in progress Taq DNA polymerase obtained from *T. aquaticus* bacteria is cloned into pET28 a vector to get higher amounts. In the continuation of this study, the aim is to extend the half-life of Taq polymerase by treating it with different buffers.

Materials & Methods

Amplification and cloning of *Taq* DNA polymerase gene

Taq DNA polymerase gene from "*T. aquaticus*" was obtained in a construct. Routine PCR was conducted for the amplification of Taq DNA polymerase gene. Gene fragment with an approximate size of 2500 bp was amplified using specific gene primers with designed linkers and, optimized PCR conditions were used. The resulting PCR fragment was first cloned into the pJET1.2 vector. In the cloning studies into the pJet1.2 vector, E.coli dh10b host cell was used for transformation host and, the ligation and transformation methods recommended for the vector were used. Colonies obtained as a result of the cloning process were confirmed by setting up the reaction for the colony PCR method. Subsequently, plasmid isolation was performed by alkaline lysis method and the obtained plasmids were treated with restriction enzymes to obtain the Taq polymerase gene region. The resulting Taq polymerase gene region was ligated into the pET28a expression vector and transformed into E.coli DH10B host cell. Colonies obtained as a result of transformation were confirmed by the colony PCR method. By NGS analysis of the obtained recombinant vector, Taq polymerase gene region was found to be present and then transformed into E.coli BL21(D3) host cell.

Expression and purification of Taq Polymerase

Different expression conditions were tested for the expression of Taq polymerase. As a result of the optimization studies carried out, the best expression was achieved with 0.5 mM IPTG in LB medium, with an incubation at 37 °C for 4 hours. Different methods were tested to obtain purified Taq polymerase and the method with the best results was selected. After the expression process, the product was centrifuged and the pelleted part was taken. The resulting pellet was treated with buffer A (50 mM Tris-Cl pH 8.0, 1 mM EDTA, 50 mM glucose). Afterwards, the cells were blasted with the help of sonication. Finally, the lysate was exposed to heat shock at 75°C. The product obtained as a result of these processes was centrifuged at 14000 rpm for 15 minutes and Taq polymerase was removed from cell residues and some proteins. Complete purification of Taq polymerase was achieved through the dialysis membrane.

Optimization of taq polymerase with different buffers

Taq polymerase was expressed in E. coli BL21(DE3) cells and partially purified. Partially purified enzyme was tested in PCR and it was observed that our enzyme works as efficient as commercial Taq polymerases. In the continuation of the study, this enzyme will be purified to high purity level and optimized with different buffers. Thus, more efficient, domestic and long-lasting enzyme production will be achieved.

Results

The gene length of Taq polymerase enzyme is 2470 bp and it has a high GC content sequence. For this reason, optimization was performed with different polymerase enzymes in the PCR reaction. A proofreading Taq Polymerase (Q5 High-Fidelity DNA Polymerase, New England Biolabs) working efficiently at high GC rate was utilized. The obtained PCR product was visualized in gel electrophoresis and the results were examined (Figure 2). According to the results of gel electrophoresis, a band of desired size was obtained.

Fgure 2. The PCR amplification result of Taq DNA polymerase gene: 1) Taq DNA polymerase gene (~ 2470 bp).

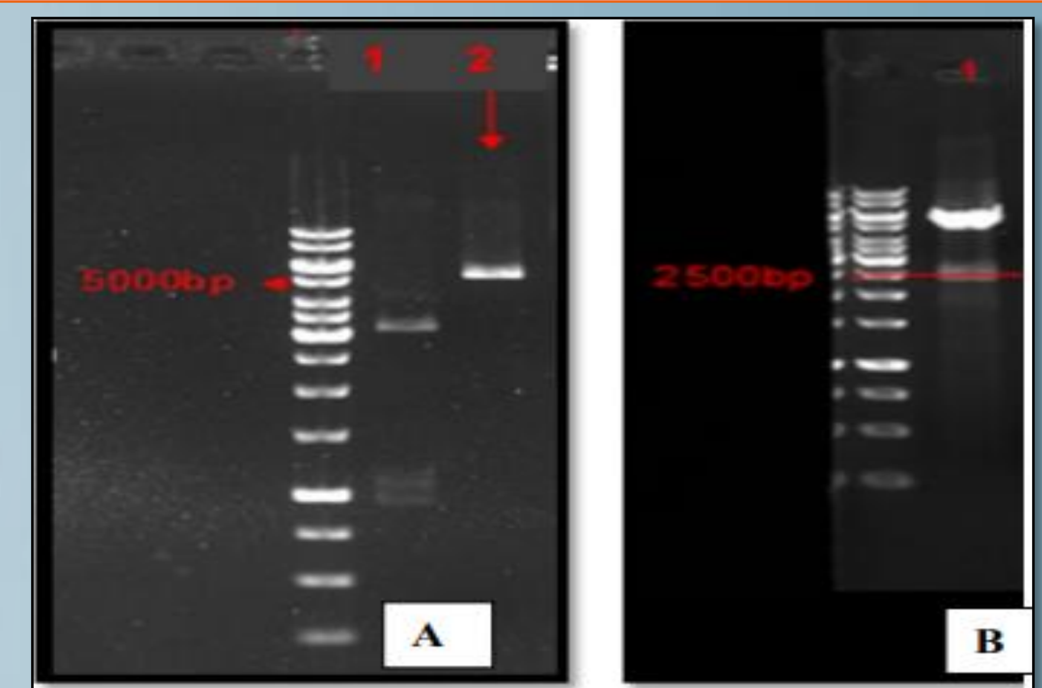

Figure 3. Gel electrophoresis image of the digestion result of Taqpol-pJET1.2 recombinant vector with restriction enzymes; A) 1: Digestion result of Taqpol-pJET1.2 vector with XhoI 2: Digestion result of Taqpol-pJET vector with EcoRI B) 1: Digestion result of Taqpol-pJET1.2 vector with EcoRI and NcoI

Plasmid isolation was performed to confirm the cloning. Analysis was performed by colony PCR and PCR bands were observed at 2500 bp. At the same time, the reaction was carried out with restriction enzymes. The result of the digestion reaction with the restriction enzymes was visualized by gel electrophoresis and confirmed that cloning had taken place (Figs. 3A, 3B). First, the Taqpol-pJET vector was treated separately with the enzymes XhoI and EcoRI (Fig. 3A). There are three distinct XhoI cut sites in the Taq polymerase gene and one XhoI cut site in the pJET 1.2 cloning vector. The locations of these regions were determined in the SnapGene program. When the vector was cut with XhoI, three different bands were observed in the gel electrophoresis (~900, ~1100 and ~3100). (Figure 3A-1). Digestion was made with EcoRI only and the Taqpol-pJET vector was linearized, observing the expected result since there is only one cut site (Fig. 3A-2). In the next step, NcoI and EcoRI restriction enzymes, which were predetermined and added in the primer design, were used, and a band of Taq Polymerase gene with a size of 2500 bp was seen as a result of the cut (Figure 3B-1).

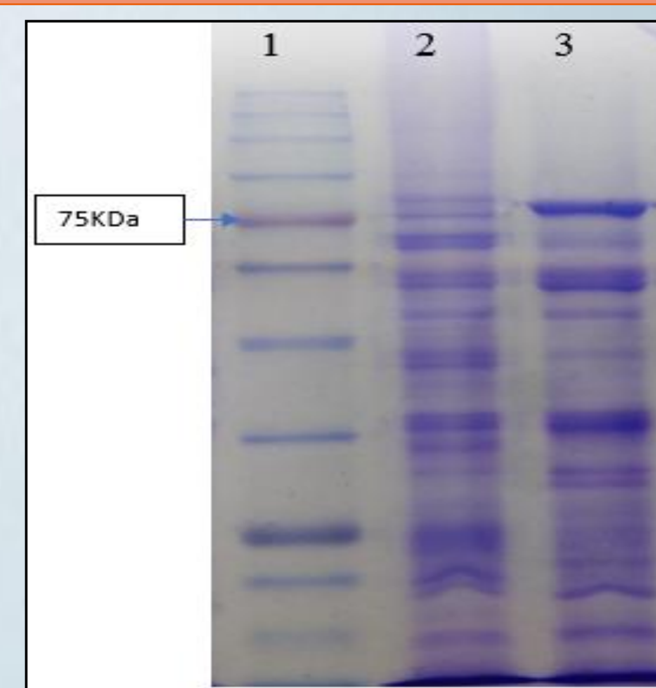

Figure 4. Expression of Taq Polymerase shown by SDS-PAGE. 1) Marker, 2) Expression with empty pET28a (+) vector; 3) Expression of Taq DNA polymerase in pET28a (+) vector

Figure 5. PCR activity results of Taq Polymerase produced and purified in our laboratory**.** Beta-Actin (1 and 2) and alfa-glucosidase (3 and 4) genes were amplified. **2 and 4)** PCR result with commercial Taq polymerase (using genomic DNA).**1 and 3)** PCR result (genomic DNA was used) performed with Taq polymerase produced in the laboratory.

Cloning of Taq polymerase into the pET28a (+) expression vector was verified by colony PCR and restriction enzyme digestion as described above, then confirmed by NGS analysis. As a result of NGS analysis, it was seen that Taq polymerase was successfully cloned into pET28a. The protein expression was verified by SDS-PAGE (Figure 4). Taq Polymerase was subjected to purification and the activity of purified Taq pol. was tested in PCR reaction in parallel with the commercial enzyme. It was observed that Taq Polymerase produced and purified in our laboratory can demonstrate activity in PCR (Figure 5).

Discussion and Conclusion

As a result of the studies, the gene region of Taq DNA polymerase obtained from *T. aquaticus* was successfully cloned into the pET28a (+) expression vector and expressed. Expressed Taq DNA polymerase enzyme was purified and tested in PCR reactions established using different buffers. Both genomic DNA and plasmid were used as templates in the PCR reactions. As a result of the studies, it was demonstrated that the Taq DNA polymerase activity produced in the laboratory is good and can be used in laboratory studies and kit use.

References

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