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Original Article

Amplification and cloning of Taq DNA polymerase gene from Thermus Aquaticus strain YT-1

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Abstract

DNA amplification using Taq DNA polymerase is one of the most widely used techniques in molecular biology and biotechnology. The aim of this study was to amplify the gene of this enzyme from a thermophilic bacteria called *Thermus aqauticus* and clone it into a vector for future use. Using specific primers the cDNA of Taq DNA polymerase was amplified and ligated into the cloning vector pTZ57R using TA cloning technique. The recombinant plasmids were identified using restriction enzyme digestion. The presence of the Taq DNA polymerase gene was confirmed by DNA sequencing. In conclusion, Taq DNA polymerase gene has been cloned in our laboratory and can be used for the production of large quantities of this enzyme.

Key words: Cloning, Taq DNA polymerase, *Thermus aquaticus*

INTRODUCTION

Taq DNA Polymerase is an enzyme obtained from a heat stable bacteria called *Thermus aquaticus* having a molecular weight of about 66,000-94,000 daltons (1). This enzyme is used for the amplification of selective DNA segments using polymerase chain reaction (PCR; 2). The Taq DNA polymerase isolated from *thermus aquaticus* was the first characterized thermostable enzyme and is one of the most widely used enzymes of this category. This thermostable enzyme enables the amplification reaction to be performed at higher temperatures and makes the automation of PCR possible (3). The full length 94 kDa Taq polymerase has maximal activity and half life of 9 min at 97.5 °C (4).

More than 50 DNA polymerase genes have been cloned and sequenced from various organisms including thermophiles and archaea (5). Although some laboratories have reported the cloning of Taq DNA polymerase (6), no study has been conducted in Iran. Because of the economic value of this enzyme, obtaining this clone from external sources for laboratory production of Taq DNA polymerase is extremely difficult. Therefore, in order to produce Taq DNA polymerase, reduce the cost of research in our laboratory, and having the gene of this enzyme for future modifications, we decided to amplify and clone Taq DNA polymerase gene. For this purpose, TA cloning technique was used, which has not been utilized by any previous study reporting cloning of this gene

MATERIALS AND METHODS

Bacterial strains and plasmids

Thermus aquaticus strain YT-1 (ATCC-25104) a gift from Dr. Kutellu Ulgen

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(Turky, Bogazici University), was used as a source to isolate the thermostable Taq DNA polymerase gene. *E. coli* XL1-Blue strain was used as a host for recombinant plasmids. The plasmid pTZ57R obtained from Fermentas company was utilized as a cloning vector.

Growth conditions

E. coli strains were grown at 37° C in Luria Bertani (LB) broth or plated on LB agar containing 80 μg/ml ampicillin as described by Sambrook et al. (7).

Genomic DNA preparation

The Genomic DNA from *Thermus aquaticus* strain YT-1 was isolated using high pure PCR template preparation kit, which was purchased from Roche Co. (Germany). Electrophoresis in 0.7% agarose gel was used to confirm the size of the isolated DNA (7).

Amplification protocol

A pair of primers were designed based on the 5' and 3' ends of this gene and were utilized for PCR amplification. The sequence of these primers were as follows:
Forward: 5′-CACGAATTCGGGGATGCT GCCCCT CTTTGAGCCCAAG-3′
Reverse: 5′-GTGAGATCTATCACTCCT TGGCGGAGAGCCAGTC-3′
PCR amplification was performed using the following reagents: 1 X PCR reaction buffer (50 mM KCl, 20 mM Tris- HCl (pH 8.4), primers (each 2.5 μM), 3 mM $MgCl_2$, 0.5 mM dNTPs, 0.4 μg template DNA, 1X Q solution, 5 Unit Taq polymeras (QIAGEN, Germany). The final reaction volume was 50 μl. PCR cycles were as follows: one cycle of 5 min at 94 °C, 35 cycles of: l min at 94 °C, 2 min at 55 °C, 3 min 72 °C, and one cycle of 20 min at 72 °C (8).

Confirmation of PCR product

The amplified PCR product was analyzed by electrophoresis in 0.7% agarose gel. In addition to checking its sized, using *Hin*dIII restriction enzyme the amplifi-cation of Taq DNA polymerase gene was confirmed. Finally, after cloning of this PCR fragment, its sequencing was carried out using T7 primer in Fazapajooh Co.

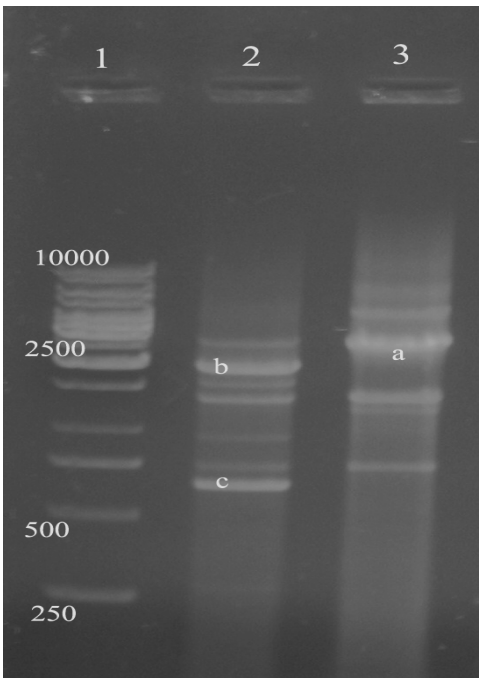

Figure 1. Amplification of the Taq polymerase gene.

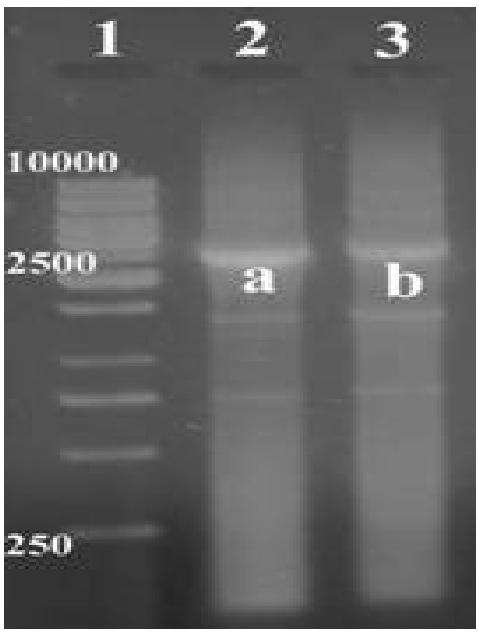

Figure 2. Restriction digestion of the PCR product.

Ligation

The PCR product was extracted by QIA quick gel extraction kit obtained from Germany. The concentration of the insert was determined using λ DNA (*Hin*dIII digested). This insert was then ligated into pTZ57R vector using InsT/A clone PCR product cloning kit (Fermentas, Germany). Ligation was performed in 10 µl volumes under the following conditions: The molar ratio of 3/1 for insert to vector, 1X ligase buffer, 1X PEG 4000, BSA (0.44 ng), 5 Units T4 DNA ligase (Fermentas), and dH_2O. The reaction mixtures were incubated over night at 16 ºC.

Transformation and plasmid preparation

The ligated mixture were transformed to XL1-Blue competent cells ($CaCl_2$ method) using heat shock method (42 ^oC, 45 sec). These mixtures were then plated on LB agar containing 100 µg/ml ampicillin and incubated at 37 ^oC overnight. The obtained colonies were used for plasmid preparation (7). Restriction enzymes, *Eco*RI, *Bam*HI, *Kpn*I and *Hin*dIII were used for the digestion of these plasmids

RESULTS

The isolated DNA from from *Thermus aquaticus* colonies used as a template for PCR amplification of Taq DNA polymerase gene. The electrophoresis of this product is shown in Figure 1 matching the expected size of the gene which is 2500 bp. Digestion of this DNA with *Hin*dIII restriction enzyme gave the expected two bands of 1900 bp and 600 bp as is shown in Figure 2 (Lanes 2 and 3).The amplified product corresponding to Taq DNA polymerase gene was ligated into pTZ57R vector. The presence of the insert within the plasmid was confirmed by restriction enzyme digestion. *Hin*dIII enzyme produced two bands (1974 bp and 3400 bp), *Kpn*I enzyme also produced two bands (617 and 4800 bp), and double digestion with *Bam*HI and *Eco*RI enzymes

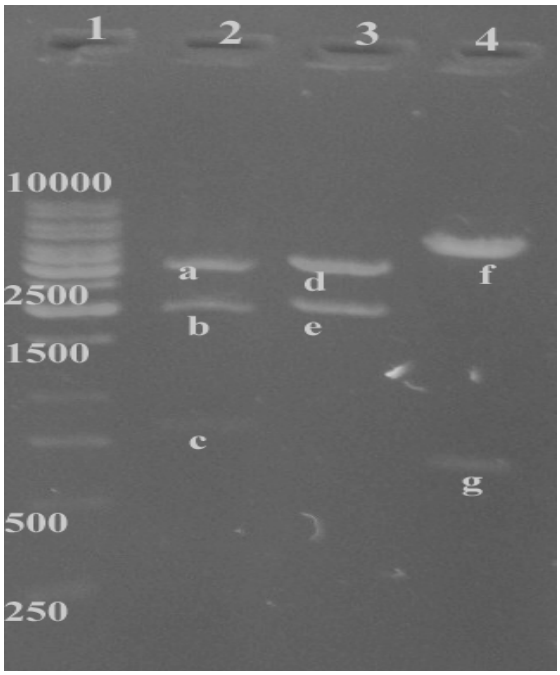

Figure 3. Restriction analysis of colonies for the presence of the recombinant plasmids.

TTAGCTTGAGTGAGGGCCCGGGATCCGATTACG
AATTCGGGGATGCTGCCCCTCTTTGAGCCCAAGG
GCCGGGTCCTCCTGGTGGACGGCCACCACCTGG
CCTACCGCACCTTCCACGCCCTGAAGGGCCTCAC
CACCAGCCGGGGGGAGCCGGTGCAGGCGGTCTA
CGGCTTCGCCAAGAGCCTCCTCAAGGCCCTCAA
GGAGGACGGGGACGCGGTGATCGTGGTCTTTGA
CGCCAAGGCCCCCTCCTTCCGCCACGAGGCCTAC
GGGGGGTACAAGGCGGGCCGGGCCCCCACGCCG
GAGGACTTTCCCCGGCAACTCGCCCTCATCAAGG
AGCTGGTGGACCTCCTGGGGCTGGCGCGCCTCG
AGGTCCCGGGCTACGAGGCGGACGACGTCCTGG
CCAGCCTGGCCAAGAAGGCGGAAAAGGAGGGCT
ACGAGGTCCGCATCCTCACCGCCGACAAAGACC
TTTACCAGCTCCTTTCCGACCGCATCCACGTCCT
CCACCCCGAGGGGTACCTCATCACCCCGGCCTG
GCTTTGGGAAAAGTACGGCCTGAGGCCCGACCA
GTGGGCCGACTACCGGGCCCTGACCGGGGACGA
GTCCGACAACCTTCCCGGGGTCAAGGGCATCGG
GGAGAAGACGGCGAGGAAGCTTCTGGAGGAGTG
GGGGAGCCTGGAAGCCCTCCTCAAGAACCTGGA
CCGGCTGAAGCCCGCCATCCGGGAGAAGATCCT
GGCCCACATGGACGATCTGAAGCTCTCCTGGGA
CCTGGCCAAGGTGCGCACCGACCTGCCCCTGGA
GGTGGACTTCGCCAAAAGGCGGGAGCCCGACCG
GGAGAGGCTTANGGCCTTTCTGGAGAGGCTTGA
GTTTGGCAGCCTCCTCCACGAGTTCGGCCTTCTG
GAAAGCCCCAAGGNCCTGGANGAAGCCCCCTGG
CCCCCGCCGGAAAGGGCCTTCGTG

Figure 4. Nucleotide sequence of a segment of the recombinant plasmids

resulted in three bands (719, 1700 and 2900 bp) as shown in Figure 3 (Lane 2, 3 and 4). A segment of the recombinant plamid was also sequenced (Figure 4).

DISCUSSION

Because of the widespread use of Taq DNA polymerase, we decided to amplify and clone its gene. Several attempts varying the experimental conditions, such as PCR cycles and $MgCl_2$ concentrations were made for its amplification without any success. Surprisingly, by changing the brand of Taq DNA polymerase, the desired product was obtained. Considering that Taq DNA polymerase should act the same when purchased from any company, our results indicate that some of the local companies sell faulty products and one has to be selective in ordering reagents from these sources.

Desai and Pfaffle have reported the cloning of Taq DNA polymerase into pUC18 plasmid (8). Other reports are also available regarding the cloning of this gene (6, 9). However, our study is the first to clone the Taq DNA polymerase gene using TA cloning method. This is a more convenient and much faster procedure as compared to those used in other studies. The sequencing of the obtained clone in our laboratory indicated that for the first time in Iran, Taq DNA polymerase gene has been successfully cloned. This would allow us to perform many studies including expression of this gene, mass production of the enzyme and introducing mutations for enhancing its performance.

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