A fusion of *Taq* DNA polymerase with the CL7 protein from *Escherichia coli* remarkably improves DNA amplification

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Abstract

Background

DNA polymerases are important enzymes that synthesize DNA molecules and therefore are critical to various scientific fields as essential components of in vitro DNA synthesis reactions, including PCR. Modern diagnostics, molecular biology, and genetic engineering require DNA polymerases with improved performance. This study aimed to obtain and characterize a new CL7-Taq fusion DNA polymerase, in which the DNA coding sequence of *Taq* DNA polymerase was fused with that of CL7, a double-stranded DNA binding-like protein from *Escherichia coli*.

Results

The resulting novel recombinant gene was cloned and expressed in *E. coli*. The recombinant CL7-*Taq* protein exhibited excellent thermostability, extension rate, sensitivity, and resistance to PCR inhibitors. Our results showed that the sensitivity of CL7-*Taq* DNA polymerase was 100-fold higher than that of wild-type *Taq*, which required a template concentration of at least 1.8×10^5 aM. Moreover, the extension rate of CL7-*Taq* was 4 kb/min, which remarkably exceeded the rate of *Taq* DNA polymerase (2 kb/min). Furthermore, the CL7 fusion protein showed increased resistance to inhibitors of DNA amplification, including lactoferrin, heparin, and blood. Single-cope human genomic targets were readily available from whole blood, and pretreatment to purify the template DNA was not required.

Conclusions

Thus, this is a novel enzyme that improved the properties of *Taq* DNA polymerase, and thus may have wide application in molecular biology and diagnostics.

Background

Discovered in the 1980s, the polymerase chain reaction (PCR) has become one of the most significant technological advancements in the fields of biology and clinical medicine. Technology that detects a broad range of bacteria or viruses based on PCR is widely applied for the screening of clinical, environmental, and manufacturing samples to diagnose infectious diseases or to detect bacterial contamination. Therefore, PCR technology plays a vital role in molecular biology, genetic engineering, and diagnostics [1]. The *Taq* DNA polymerase that is now widely used in PCR was first isolated from the extreme thermophilic bacteria *Thermus aquaticus* YT-1 [2].

Currently, *Taq* DNA polymerase is crucial for almost all PCR-based techniques, including RT-PCR and digital PCR. However, *Taq* DNA polymerase has the drawback of a relatively higher error frequency than other enzymes [3]. Usually, product yields will decrease when the amplification fragment becomes longer

than 1 kb due to the relatively low processivity and thermostability of the wild-type enzyme. Generally, the efficiency of amplification by *Taq* polymerase for targets shorter than 1 kb is approximately 80% [4]. However, *Taq* DNA polymerases become completely inhibited when the PCR mixture contains 0.2% blood. It seems that hemoglobin and lactoferrin have important roles in inhibiting the amplification process [5]. Therefore, these drawbacks limit the applications of *Taq* DNA polymerase.

Recently, a novel strategy was employed to overcome these limitations [6]. Generating fusion proteins with *Taq* DNA polymerases and a thermostable DNA-binding protein such as the Sso7d DNA-binding protein from *Sulfolobus solfataricus* has been shown to enhance the processivity [7], thermostability, and overall stability compared with wild type *Taq* polymerase. The purified S-*Taq* protein has shown acceptable limits of host genomic DNA levels without the use of DNases or other DNA precipitating agents, which highlights its potential for use in PCR-based diagnostics, *in-situ* PCR, and forensic science [7].

A previous study also found that adding 0.6% bovine serum albumin (BSA) to reaction mixtures containing *Taq* DNA polymerase reduced the inhibitory effect of blood and allowed DNA amplification in the presence of 2% instead of 0.2% blood [8]. Furthermore, BSA was found to be the most efficient amplification facilitator. As rapid and simple diagnostics methods are urgently required for blood analyses, modifications to *Taq* DNA polymerase that enhance its tolerance to inhibition by blood are significant to medical diagnostics. Mutations to *Taq* DNA polymerase that render it resistant to inhibition by blood components are now commercially available [9]. Additionally, there are *Taq* polymerase mutants that are used in PCR-based tests of blood and soil samples that are widely used for diagnostics and forensic analyses and do not require pretreatment to purify the template DNA and others that allow increased dye concentration overcomes fluorescence background and quenching in real-time PCR analyses of blood. Because of deficiencies in the 3′-5′ exonuclease domain, *Taq* DNA polymerase is widely applied to ARMS-PCR to detect single nucleotide polymorphisms (SNPs).

In recently reported studies, *Taq* polymerase was fused to DNA binding proteins to improve other properties [10]. To further improve the properties of *Taq* polymerase, we fused the DNA binding protein of CL7, which is a mutant of the CE7 protein derived from *E. coli*, to the *Taq* DNA polymerase with a 7-amino acid linker [11]. We demonstrated and functionally characterized the fusion enzymes and demonstrated that they showed improved processivity, sensitivity, amplification rates, and eliminated pre-PCR treatment steps. Finally, we demonstrated the practical benefits for PCR applications of enhancing the sensitivity of the polymerase.

Results

Expression and purification of Taq and CL7-Taq

To obtain the purified enzymes, we designed the experiment as follows. The gene encoding a fragment of *Taq* DNA polymerase was cloned into the pET-30 vector to generate a pET30/Taq plasmid. This led to the expression of a *Taq* DNA polymerase fusion protein with a C-terminal 6 × histidine tag. To achieve fusion

with the CL7 gene, the two PCR products were mixed together with the pET-30 vector to generate the pET30/CL7-Taq plasmid, which encoded the fusion enzyme with a C-terminal 6 × histidine tag. Then the pET30/Taq and pET30/CL7-Taq plasmids were transformed into *E. coli* BL21 (DE3) to express the C-terminally His-tagged *Taq* and CL7-*Taq* proteins, respectively. Following expression, the cells were harvested and sonicated. The recombinant DNA polymerases were then purified by passing the heat-denatured supernatant through a His-Bind Ni^{2+} affinity column. After each purification step, the purity of the DNA polymerase was monitored by SDS-PAGE (Fig. 1), which separated the following major protein bands: 93 and 108 kDa for *Taq* and CL7-*Taq*, respectively; this result was in agreement with the molecular masses of 92.7 and 107.7 kDa that were calculated based on the amino acid sequences. The *E.coli* overexpression system used in this study enabled the production of 720 mg of *Taq* polymerase and 620 mg of CL7-*Taq* fusion protein per 1 L of induced culture. After being measured by the EvaEZ Fluorometric Polymerase Activity Assay Kit, the specific activities of the purified *Taq* and CL7-*Taq* polymerases were found to be 1426.4 and 1572.6 U/mg, respectively. These results indicated that the CL7 fusion had a positive effect on the relative activity of *Taq* DNA polymerase. The production efficiency of *Taq* and CL7-*Taq* DNA polymerase in this study were satisfactory.

Characterization of Taq **and CL7-**Taq

In all experiments, the activity of 1 μL of enzyme was determined for *Taq* and CL7-*Taq* DNA polymerases and compared with commercial *Taq* polymerase with an activity of 1 U/μL using the EvaEZ Fluorometric Polymerase Activity Assay Kit (Biotium) in an isothermal reaction at 60 °C on a real-time PCR apparatus (Bio-Rad). For characterization purposes, the activity of *Taq* and CL7-*Taq* DNA polymerases were measured by PCR using various buffer compositions and concentrations of $MgCl_2$, KCl, and $(NH_4)_2SO_4$ and various pHs (Fig. 2).

The effect of pH on the activity of *Taq* and CL7-*Taq* DNA polymerases was evaluated using Tris-HCl buffers of pH ranging from 7.0 to 9.0. Both polymerases had the highest enzyme activity at pH 8.0 (Fig. 2c). The activity of *Taq* and CL7-*Taq* DNA polymerases were closely related to the concentration of $MgCl_2$, which was optimal at between 1 to 8 mM and 2 to 8 mM, respectively (Fig. 2a). DNA polymerase activity was completely inhibited when KCl concentrations surpassed 70 mM for *Taq* and 80 mM for CL7-*Taq* (Fig. 2b). The activity of *Taq* and CL7-*Taq* DNA polymerase were also strongly affected by $(NH_4)_2SO_4$ and were completely inhibited at concentrations over 30 and 40 mM, respectively (Fig. 2d). The fusion of CL7 to *Taq* DNA polymerase resulted in higher tolerance of the enzyme to salt inhibition.

Based on these results, we found that the optimal PCR buffer for CL7-*Taq* DNA polymerase consisted of 20 mM Tris-HCl (pH 8.0), 4 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, and 20 mM KCl.

PCR amplification rate and processivity

To measure the PCR amplification rate, we designed tests similar to those previously published [15]. The results showed that the fusion CL7-*Taq* DNA polymerase replicated template strands at a faster rate than

the *Taq* DNA polymerase (Fig. 3). The data showed thatCL7-*Taq* extended a 4,000-bp product during 60 s, while *Taq* DNA polymerase required 1 min for a 2,000-bp product. This showed that the fusion of CL7 protein with *Taq* DNA polymerase was twofold more efficient than *Taq* DNA polymerase without CL7, meaning DNA amplification required less time.

Sensitivity

To monitor enzyme sensitivity, we used the protocol published before [15]. Sensitivity was measured by PCR with CL7-*Taq* and *Taq* polymerases and 10-fold serial dilutions of template; the product size was 3,000 bp. The results showed that the fusion polymerase was more sensitive than the wide-type polymerase. In the case of CL7-*Taq* polymerase, it was sufficient to use 1.8×10^3 aM of plasmid DNA, while *Taq* polymerase required at least 1.8×10^5 aM of plasmid. These data showed that the sensitivity of CL7-*Taq* DNA polymerase was improved 100-fold compared with *Taq* polymerase (Fig. 4).

Thermostability of the DNA polymerases

To determine the thermostability of *Taq* and CL7-*Taq* DNA polymerases, the enzymes were monitored for decreased activity after preincubation at 95 °C and 99 °C. These experiments revealed that the CL7-*Taq* DNA polymerase had remarkably higher thermostability. Our data showed that *Taq* and CL7-*Taq* DNA polymerase were functional after 30 and 50 min at 99 °C, respectively (Fig. 5a). Additionally, *Taq* and CL7-*Taq* DNA polymerases remained active after 2 and 3 h at 95 °C, respectively (Fig. 5b).

Tolerance of the DNA polymerases to PCR inhibitors

To determine the limiting concentration of PCR inhibitors, the fusion CL7-*Taq* and the *Taq* DNA polymerases were PCR-tested for their resistance to serial dilutions of blood, lactoferrin, and heparin, which have been reported to be PCR inhibitors in many publications (Kermekchiev et al. 2008). The results showed that the CL7-*Taq* and *Taq* polymerase remained active in the presence of 3.5 μg and 2 μg lactoferrin, respectively (Fig. 6b), and 14 μg and 6 μg of heparin, respectively (Fig. 6a). CL7-*Taq* and *Taq* were resistant to 2% and 0% blood, respectively (Fig. 6c). Together, these findings revealed that the fusion CL7-*Taq* DNA polymerase was significantly more resistant to blood, lactoferrin, and heparin compared with the *Taq* enzyme.

Discussion

PCR technology has been widely applied in the fields of molecular biology, genetic engineering, and diagnostics [8]. PCR amplification efficiency is strongly dependent on the properties of the DNA polymerase and reaction conditions. Wild-type DNA polymerase has some drawbacks; therefore, modern diagnostic methods and genetic engineering techniques require modified DNA polymerases with better properties, such as those that possess higher sensitivity and/or amplification rates. For this reason, we

engineered fusions of DNA polymerases with single and double stranded DNA binding proteins to enhance the DNA binding ability.

In this study, the N-terminus of *Taq* DNA polymerase was fused with the thermoduric CL7 protein using a 7-amino acid linker (Gly-Asn-Let-Tyr-Phe-Gln-Cys). It has been reported that the template is picked up more selectively from the mixture in the presence of a binding domain and that PCR is more efficient even when inhibitors are present [4] Some studies have shown that covalently linking a DNA binding protein to a DNA polymerase can strongly enhance polymerase activity [3, 6]. Similar results are shown in this study, in which the CL7 protein doubled the elongation rate and increased sensitivity for the DNA template by 100-fold.

The most common challenges during amplification of environmental and blood samples are inhibitors present in the tested material [17]. Our observations of successful amplification of the *β-actin* gene directly from human blood using CL7-*Taq* is very promising and opens up a new avenue for this polymerase as a valuable tool for medical diagnostics and forensic sciences, where sample availability is minimal. We found that the enhanced salt tolerance of CL7-*Taq* is responsible for its successful use in direct PCR of the human genome without preprocessing. Previous studies have shown that PCR inhibitors alter DNA or block enzymes though these pathways, such as inhibiting the active site or blocking access to the active site for cofactors such as Mg^{2+} ions [8, 18]. Therefore inhibitors either weaken the efficiency of PCR amplification or block it completely. However, commercially available native enzymes are not always able to deal with these PCR issues. Our data showed that the fusion CL7-*Taq* DNA polymerase exhibited a higher tolerance to PCR inhibitors (blood, lactoferrin, and heparin) compared with *Taq* DNA polymerase. Therefore *Taq* DNA polymerase containing CL7 had better performance in the amplification of complicated templates.

Our data showed that the fusion of CL7 to *Taq* DNA polymerase improved enzymatic properties such as thermostability, amplification rate, and template sensitivity. In thermostable tests, we found that the thermostability of CL7-*Taq* DNA polymerase was remarkably higher than *Taq* DNA polymerase. The thermostability of CL7-*Taq* was over 1 h longer than that of *Taq* DNA polymerase at 95 °C. Mg^{2+} and other salts were critical components of PCR reactions; thus, optimizing their concentrations was essential for native DNA polymerase. Unlike *Taq* DNA polymerase, CL7-*Taq* DNA polymerase exhibited sufficient amplification efficiency within a wide range of Mg^{2+} concentrations. Furthermore, CL7-*Taq* DNA polymerase had a satisfactory amplification efficiency at various concentrations of KCl and $(NH_4)_2SO_4$. Thus, the salt tolerance of CL7-*Taq* was remarkably enhanced.

Our observation that CL7-*Taq* had higher sensitivity than *Taq* makes it an attractive enzyme for PCR-based diagnostics. Therefore, it is tempting to speculate that one could explore using CL7-*Taq* for ARMS-PCR to detect SNPs or common DNA viruses such as HPV, Herpesvirus, and Parvoviruses.

Conclusions

In this study, we aimed to obtain and characterize a new CL7-Taq fusion DNA polymerase, in which the DNA coding sequence of Taq DNA polymerase was fused with that of CL7, a double-stranded DNA binding-like protein from *Escherichia coli*. Our results showed that the sensitivity of CL7-Taq DNA polymerase was 100-fold higher than that of wild-type Taq, which required a template concentration of at least 1.8 × 105 aM. Moreover, the extension rate of CL7-Taq was 4 kb/min, which remarkably exceeded the rate of Taq DNA polymerase (2 kb/min). The recombinant CL7-Taq protein exhibited excellent thermostability, extension rate, sensitivity, and resistance to PCR inhibitors. Thus, this is a novel enzyme that improved the properties of Taq DNA polymerase, and thus may have wide application in molecular biology and diagnostics.

Methods

Construction of recombinant plasmids

First, DNA coding sequences of the DNA polymerase from *Thermus aquaticus* (GenBank: P19821.1) and of CL7, which is a mutant of CE7 from *E. coli* (GenBank: CP018986.1) were obtained [11]. The two fragments were synthesized by GeneCreate (Wuhan, China). The designed primers for amplification are listed in Table 1. The gene synthesis procedure was performed as described previously. PCR amplification was then used to obtain two products: the *Taq* DNA polymerase gene (2493 bp) and the CL7 gene (390 bp). The pET-30 vector was digested with the restriction endonuclease *Xba I* (Takara, Shiga, Japan), and the product was purified using the DNA Gel Extraction Kit (Promega, Madison, WI, USA). Then the PCR products were mixed together with the digested pET-30 vector. Finally, T5 cloning (New England Biolabs, Ipswich, MA, USA) was performed, in which CL7 was fused to the N-terminus of *Taq* DNA polymerase with the 7-amino acid linker (ENLYFQG) and a 6 × His tag to the C-terminus. This was necessary for the purification of recombinant protein by metal affinity chromatography. Nucleotide sequences of the resulting recombinant plasmids, pET30/Taq and pET30/CL7-Taq, were confirmed by DNA sequencing (Sangon, Shanghai, China).

Table 1
Primers used in this study for PCR.

Primer	Sequence(5'-3')
Cl7-F	aagcaatgaaccgggtaaagcaaccggtg
Cl7-Linker	accctgaaaatacaggttttcgcc
Taq-Linker	aacctgtattttcagggtcgtggtatgctgccgct
Taq-his	gtgatggtgatggtgatgttcttttgcagacagccagt

Protein expression and purification

Plasmids encoding Taq and CL7-Taq were used to transform the *E. coli* BL21 strain (DE3). Luria-Bertani (LB) medium was prepared. *E. coli* containing recombinant plasmid were grown to an A600 of 0.6 in LB

containing 50 μg/mL kanamycin at 37 °C. Then isopropyl-β-D-1-thiogalactopyranoside (IPTG) was added at a final concentration of 1 mg/ml to induce *Taq* or CL7-*Taq* expression from the T7 promoter with shaking at 18 °C. After 12 h incubation, the cells were centrifuged at 7,000 × *g* for 5 min, and the pellets were resuspended in 20 mL of lysis buffer (20 mM Tris-HCl [pH 9.0], 0.5 M NaCl, and 10 mM imidazole). Protein complexes were extracted by ultrasonic decomposition and the insoluble debris was removed by centrifugation at 12,000 × *g* and 4 °C for 20 min.

For heat treatment, the cleared lysate was immersed in a 75 °C orbital water shaker for 30 min, cooled on ice for 20 min, and then the denatured host proteins were removed by centrifugation at 12,000 × *g* and 4 °C for 20 min. Following heat treatment, the exogenous proteins were purified in a one-step process using the Ni^{2+}-affinity chromatographic technique. A His-bind resin and His-bind buffer kit (GE Life Sciences, Chicago, IL, USA) were used to purify the His-tagged proteins according to the manufacturer's instructions. The supernatant and produced enzyme were put into a column containing Ni-nitrilotriacetic acid (Ni-NTA) agarose (GE Life Sciences), which was previously prepared and equilibrated with lysis buffer. After binding to the Ni-NTA agarose column, the recombinant proteins were washed twice with washing buffer (20 mM Tris-HCl [pH 9.0], 0.5 M NaCl, and 50 mM imidazole), and then eluted with elution buffer (20 mM Tris-HCl [pH 9.0], 0.5 M NaCl, and 200 mM imidazole). An equal volume of each buffer (20 μL) was loaded onto a 12% (w/v) polyacrylamide gel for SDS-PAGE [12], followed by staining with Coomassie Brilliant Blue G-250. Finally, the eluted fractions were dialyzed three times with storage buffer (20 mM Tris-HCl [pH 8.0], 100 mM KCl, and 0.2 mM EDTA). Protein concentrations were measured using the Bradford method.

DNA polymerase activity assay

The DNA polymerase activity of purified proteins was assayed using the EvaEZ Fluorometric Polymerase Activity Assay Kit (Biotium, Hayward, CA, USA). All assays were conducted in an isothermal reaction at 60 °C using a CFX Real-Time PCR instrument (Bio-Rad, Hercules, CA, USA) in accordance with the definition of one unit of enzyme activity ("One unit of DNA polymerase activity is usually defined as the amount of enzyme that will produce 10 nmol of nucleotides during a 30-min incubation"). Enzymatic activity was determined relative to a commercial *Taq* DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA) with an activity of 1 U/μL. When the DNA polymerase was active, the primer was extended to form a double-stranded product that bound the EvaGreen dye, resulting in increased fluorescence. The rate of increase is positively correlated with polymerase activity [13].

Optimization of PCR amplification

To optimize the amplification process, polymerase activity was measured using various concentrations of $MgCl_2$, KCl, and $(NH_4)_2SO_4$ in the buffer as well as various pHs. All PCR reactions were performed using 1 mM of each dNTP, 0.4 mM of each primer and of the pET30a-GFP plasmid DNA as template, containing a known target sequence (PCR product of 445 bp). PCR assays were performed using 1 U of purified CL7-*Taq* or *Taq* DNA polymerase in a 20 μL reaction mixture containing 1 ng of DNA template. The PCR conditions were as follows: 3 min at 95 °C, and then 25 cycles of 15 s at 95 °C, 15 s at 60 °C,

and 30 s at 72 °C. To determine the optimum $MgCl_2$ concentration, PCR was performed at various pHs (7.0–9.0) with the use of the Tris–HCl buffer. Then, we used the Tris-HCl buffer (pH 8.0) containing increasing concentrations of $MgCl_2$ (0–9 mM) to detect the enzyme activity. Furthermore, PCR was performed with various concentrations of KCl (10–90 mM) and $(NH_4)_2SO_4$ (10–90 mM).

Temperature stability was also assayed [14]. One unit of purified CL7-*Taq* and *Taq* DNA polymerases were heated at 99 °C for 10, 20, 30, 40, 50, and 60 min, and at 95 °C for 1, 2, 3, 4, and 5 h. Then, the same amount of enzyme was used to amplify a 445-bp target fragment under the optimal reaction buffer (in the same PCR conditions determined in the optimization process): 20 mM Tris-HCl (pH 8.0), 3 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, and 20 mM KCl.

Measuring the PCR amplification rate

PCR amplification rates were measured using the protocol described by [15] CL7-*Taq* and *Taq* DNA polymerases were used to amplify PCR products of 1, 2, 3, and 4 kb under the conditions determined in the optimization process and using pET23a/dcas9 plasmid DNA as a template, which was recombined in our laboratory. PCR amplification started with an initial denaturation at 95 °C for 3 min and included 25 cycles of 30 s at 95 °C, 30 s at 60 °C, and 60 s at 72 °C.

Sensitivity

Following sufficient modifications, DNA polymerase sensitivity (affinity for template) was measured using the protocol by [16]. PCR was conducted under conditions optimized for the fusion polymerases CL7-*Taq* and *Taq*. We used pET23a-GFP Plasmid as a template along with the primers 5′-TGGTCTTCAATGCTTTGCGAGATAA-3′ (forward) and 5′-CTTTTCGTTGGGATCTTTCG-3′ (reverse). The product of the reaction was 445 bp. The reaction was conducted at decreasing template concentrations (serial 10-fold dilutions of the template) and included an initial denaturation at 95 °C for 3 min followed by 35 cycles of 30 s at 95 °C, 30 s at 60 °C, and 90 s at 72 °C. The amplified fragments were analyzed in a 1.5% agarose gel stained with ethidium bromide.

Resistance to inhibitors

The effect of PCR inhibitors such as heparin (Sigma-Aldrich, St. Louis, MO, USA) at a range from 16 to 1 μg, lactoferrin (Sigma-Aldrich) at a range from 4 to 0.5 μg, and blood at a range from 8–1%, on the catalytic activity of the CL7-*Taq* and *Taq* DNA polymerases was assessed by a PCR reaction using human genomic DNA as a template and the specific *β-actin* primers 5′-AGAGATGGCCACGGCTGCTT-3′ (forward) and 5′-ATTTGCGGTGGACGATGGAG-3′ (reverse) [16]. The amplified fragments were analyzed in a 1.5% agarose gel stained with ethidium bromide.

Abbreviations

PCR
polymerase chain reaction;

Declarations

Authors' contributions

WF, MLX and WYP conceived and designed the experiments. WF and RB performed the experiments. NJ, WYP analyzed the data. WF wrote the manuscript. All authors reviewed the manuscript.

Ethics statement and consent to participateand

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included with in the article and its additional files. All strain materials were obtained from Hubei University, Wuhan, China.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

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Not applicable.

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Figures

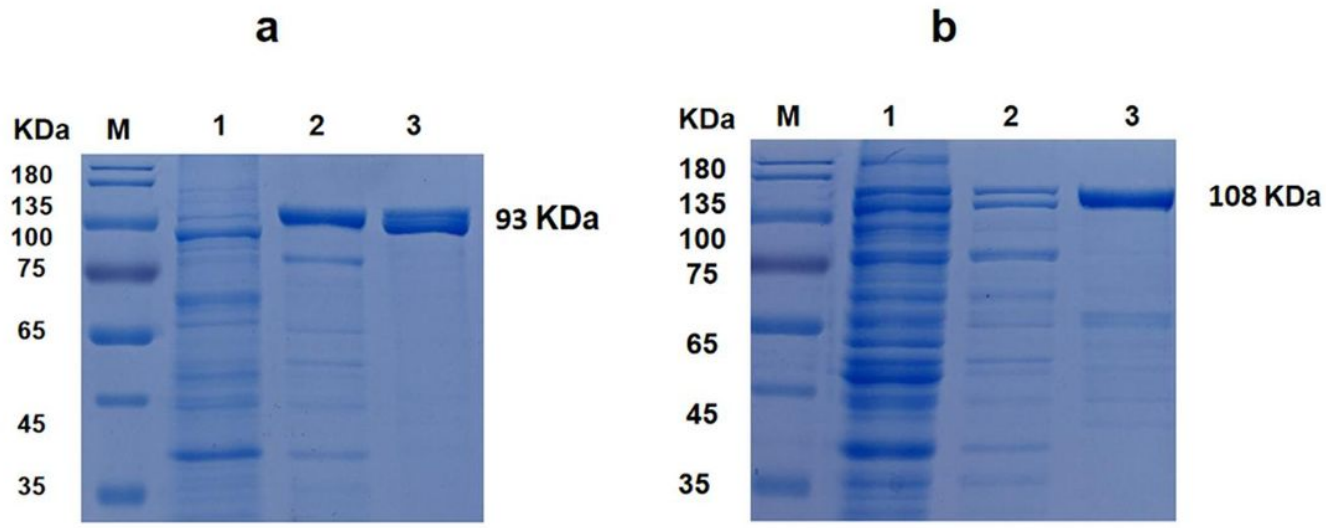

Figure 1

Expression and purification of Taq (a) and CL7-Taq (b) polymerases. The proteins were analyzed on a 12% polyacrylamide gel (SDS-PAGE). Lane M, PageRuler (Thermo Fisher Scientific), with the molecular masses of the reference proteins marked. Lane 1, sonicated extract of induced cells; lane 2, heat treatment; lane 3, purified protein after elution with storage buffer.

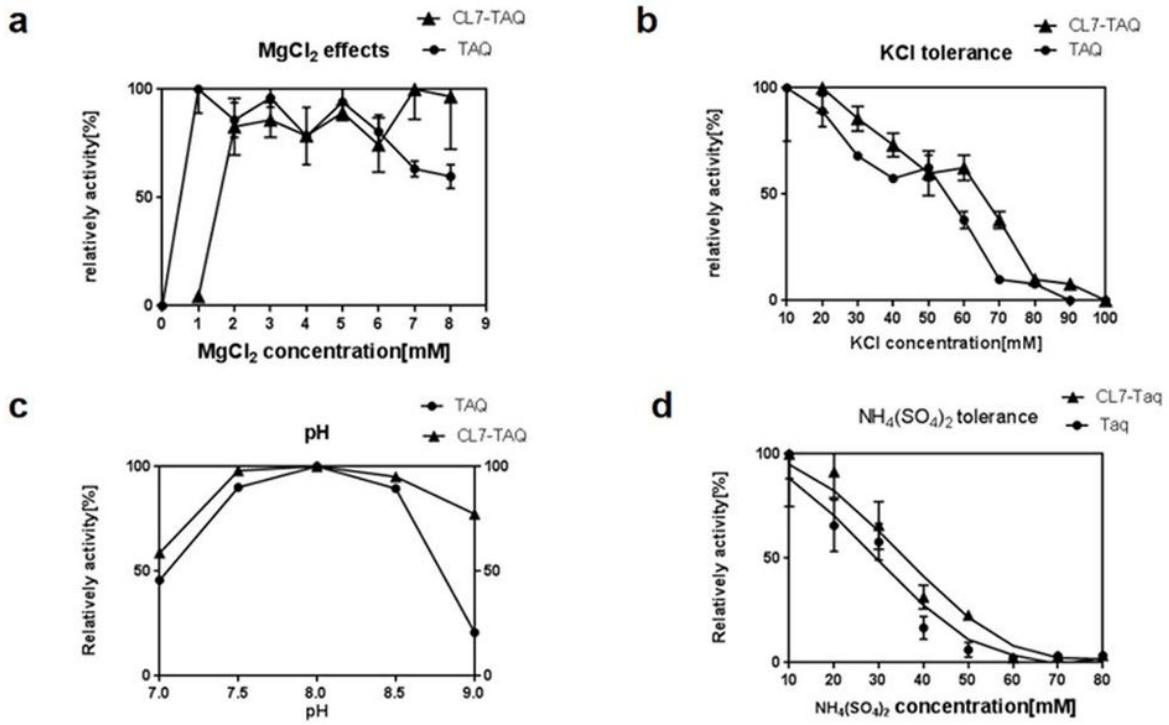

Figure 2

Characterization of the fusion Cl7-Taq DNA polymerase in comparison with Taq DNA polymerase. The effects of MgCl2 (a), KCl (b), pH (c), and (NH4)2SO4 (d). Error bars for Cl7-Taq DNA polymerase have an end bar, while those for Taq DNA polymerase do not have an end bar.

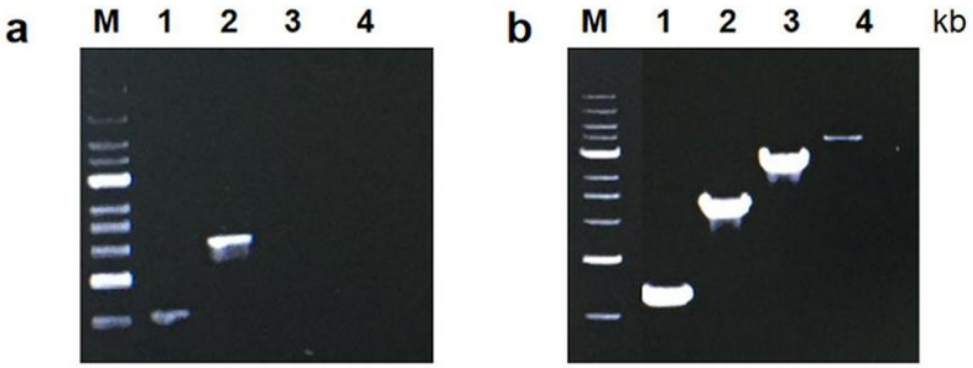

Figure 3

Comparison of the PCR amplification rates of the fusion Cl7-Taq DNA polymerase (b) and Taq DNA polymerase (a). The elongation times used for PCR amplification are shown at the top. LaneM is the GeneRuler 1 kb DNA ladder marker (250–1,0000 bp) (Thermo Fisher Scientific). The amplified products were analyzed in a 0.8% agarose gel stained with ethidium bromide.

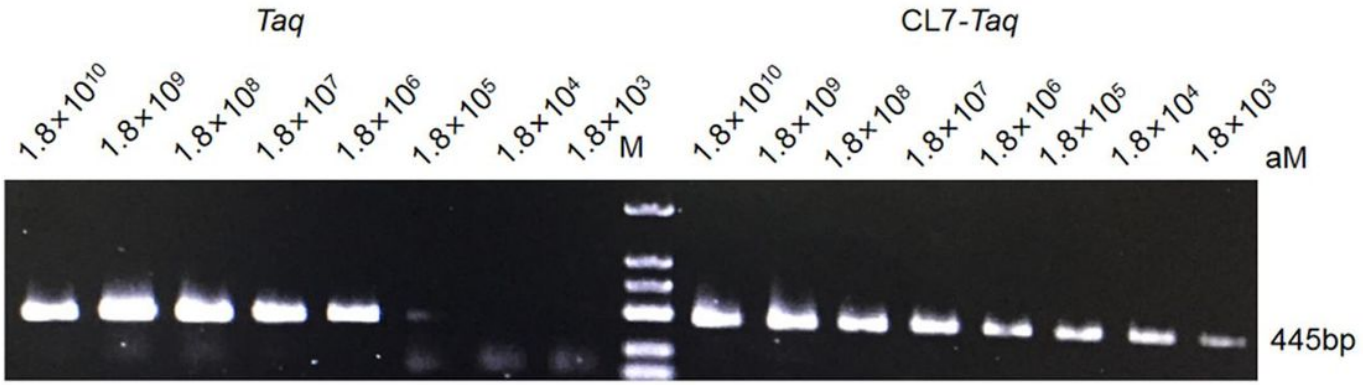

Figure 4

Electrophoretic separation showing the products of plasmid DNA amplification as a function of template concentration for the fusion CL7-Taq polymerase and Taq polymerase. Lane M, DL2000 (100–2000 bp) (TsingKe, China). The amplified products were analyzed in a 1% agarose gel stained with ethidium bromide.

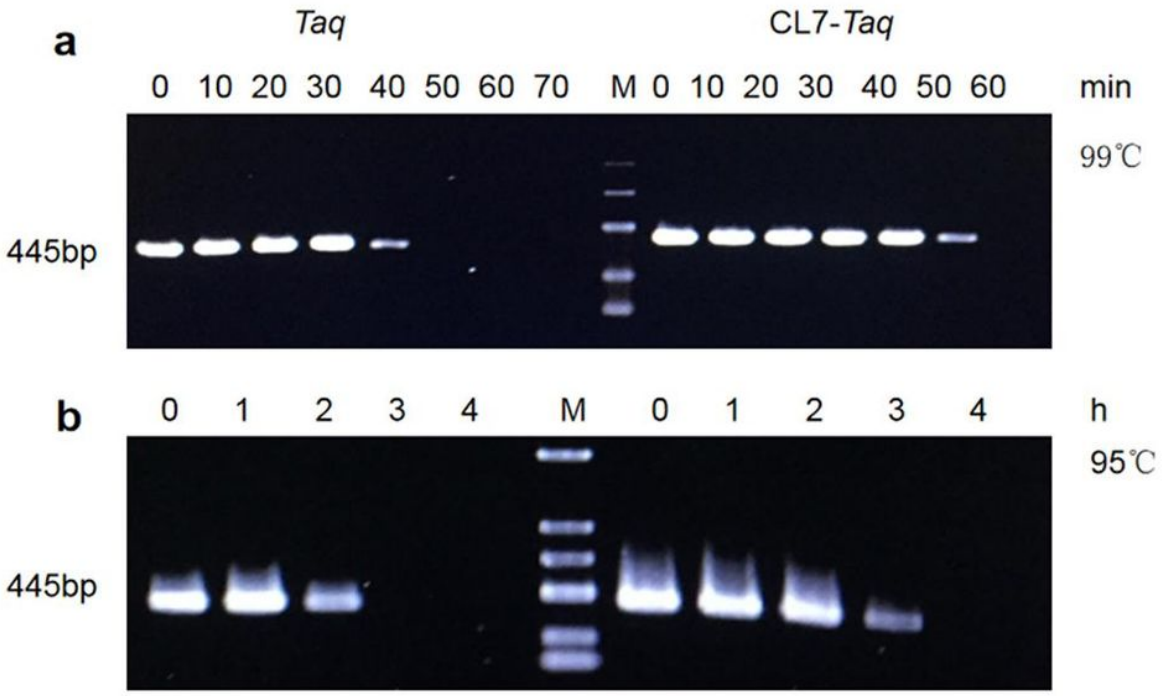

Figure 5

Residual activity was assayed by PCR after incubation at 99°C (a) or 95°C (b). Differences in the amplification efficiency of the fusion Cl7-Taq DNA polymerase and Taq DNA polymerase after incubation at 99°C for 0–70 min and after incubation at 95°C for 0–4 h. Lane M: the DNA molecular size marker DL2000 (TsingKe).

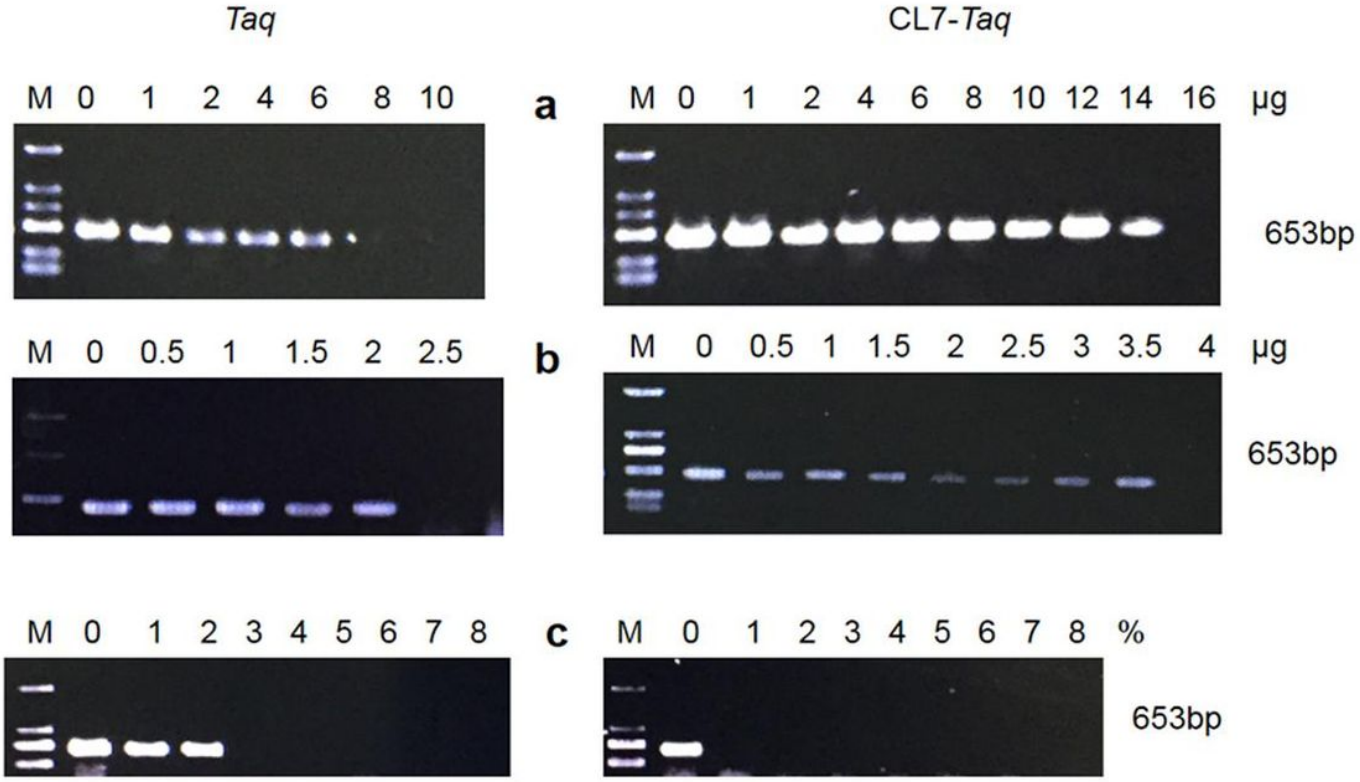

Figure 6

The effect of heparin (a), lactoferrin (b), and blood (c) on DNA amplification using human genomic DNA as a template and primers for β-actin. Control reactions were performed without any inhibitors. Lane M, DL2000 (100–2000 bp) (TsingKe, China). The amplified products were analyzed in a 1 % agarose gel stained with ethidium bromide.

Supplementary Files

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- Fig1Original.tif
- SupplementaryInfoFile.pdf