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# Characterization and application of a Family B DNA polymerase from the hyperthermophilic and radioresistant euryarchaeon *Thermococcus gammatolerans*

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## Abstract

*Thermococcus gammatolerans* is anaerobic euryarchaeon which grows optimally at 88°C and its genome encodes a Family B DNA polymerase (Tga PolB). Herein, we cloned the gene of Tga PolB, expressed and purified the gene product, and characterized the enzyme biochemically. The recombinant Tga PolB can efficiently synthesize DNA at high temperature, and retain 93% activity after heated at 95°C for 1.0 hr, suggesting that the enzyme is thermostable. Furthermore, the optimal pH for the enzyme activity was measured to be 7.0-9.0. Tga PolB activity is dependent on a divalent cation, among which magnesium ion is optimal. NaCl at low concentration stimulates the enzyme activity but at high concentration inhibits enzyme activity. Interestingly, Tga PolB is able to efficiently bypass uracil in DNA, which is distinct from other archaeal Family B DNA pols. By contrast, Tga PolB is halted by an AP site in DNA, as observed in other archaeal Family B DNA polymerases. Furthermore, Tga PolB extends the mismatched ends with reduced efficiencies. The enzyme possesses 3′-5′exonuclease activity and this activity is inhibited by dNTPs. The DNA binding assays showed that Tga PolB can efficiently bind to ssDNA and primed DNA, and have a marked preference for primed DNA. Last, Tga PolB can be used in routine PCR.



**Keywords**: Archaea; DNA polymerase; DNA replication; Routine PCR

## Introduction

DNA polymerase (pol) is a ubiquitous enzyme that replicates DNA in all living cells. Since the first DNA pol was discovered and characterized from *Escherichia coli* in the 1950s by Arthur Kornberg, a large number of DNA pols have been biochemically and structurally characterized from diverse organisms. Currently, DNA pols have been grouped into six families: A, B, C, D, X, and Y, based on amino acid sequence similarities [1]. In addition, reverse transcriptase has been proposed as the seventh family since it can synthesize DNA [2]. Family B DNA pols have been found in bacteria, archaea, eukarya, and viruses/phages. A typical characteristic of most of Family B DNA pols is that they possess 3′-5′ exonuclease activity in addition to polymerase activity [3; 4], which can remove a misincorporated nucleotide opposite a template strand, thus enhancing fidelity approximately 100-fold.

As the third life, Archaea are a simplified version of eukarya with respect to DNA metabolism [5; 6; 7]. Currently, DNA pols of Family B, D, and Y have been found in Archaea [8]. Family B DNA pols are ubiquitous in both *Euryarchaeota* and *Crenarchaeota*, which are two major branches of Archaea. In addition to possessing five conserved motifs, archaeal Family B DNA pols have a unique uracil binding motif [9; 10], which can recognize uracil and hypoxanthine bases derived from deamination in template DNA. Replication of uracil and hypoxanthine before repair leads to GC to AT mutation and AT to GC mutation, respectively. However, replication by archaeal Family B DNA pols would be stalled if deaminated base is encountered with these pols [11; 12]. Such an error avoidance mechanism would be expected to be evolutionary favorable for hyperthermophilic Archaea thriving in high-

temperature environments where the rates of deamination are increased. Hence, the recognition of these pro-mutagenic lesions and the following replication stop observed in archaeal Family B DNA pols might be a specific adaptation of hyperthermophilic Archaea to their hostile environments.

As an important branch of euryarchaea, *Thermococcus* thrives in high-temperature environments, including deep-sea hydrothermal vents and hot springs. Increasing numbers of *Thermococcus* species have been isolated and identified [13]. Since the first archaeal Family B DNA pol was reported from *Thermococcus litoralis* [14], a dozen of archaeal Family B DNA pols have been biochemically characterized from *Thermococcus* species: *Thermococcus* 9°N-7 [15], *Thermococcuscelericrescens* [16], *Thermococcus fumicolans* [17], *Thermococcus* sp. NA1 [18], *Thermococcus peptonophilus* [19], *Thermococcus zilligii* [20], *Thermococcus aggregans* [21], *Thermococcus celer* [22], *Thermococcus marinus* [23], *Thermococcus pacificus* [24], *Thermococcus thioreducens* [25], *Thermococcuswaiotapuensis* [26], and *Thermococcus barophilus* Ch5 [27]. All these DNA pols have been proven to be used efficiently in routine PCR.

The euryarchaeon *Thermococcus gammatolerans* was isolated from a hydrothermal vent located in the Gulf of California [28]. This euryarchaeon is a hyperthermophilic archaeon with an optimal growth temperature of 88°C which can fully withstand a 5.0 kGy dose of gamma irradiation without loss of viability [29], and thus is the most radioresistant archaeon to date. In reason of its extreme tolerance of extreme temperatures and irradiation, it was very interesting to test whether the DNA

polymerases of *T. gammatolerans* has any specific features in comparison to other DNA pols from this family. The genome of *T. gammatolerans* encodes two DNA pols, a Family B DNA pol (Tga PolB) and Family D DNA pol [29]. In this work, we cloned the Tga PolB gene, expressed its product and characterized its biochemical characteristics. The recombinant Tga PolB was found capable of bypassing uracil in a template DNA, which is distinct from other Family B archaeal DNA pols. Furthermore, biochemical characterization of Tga PolB is discussed.

## Materials and methods

### 2.1. Materials

Materials were purchased from the following companies: pET-30a (+), Novagen (Merck, Darmstadt, Germany); Plasmid Extraction Kit, PCR Cycle Pure Kit, and Gel Extraction Kit, Omega (Guangzhou, China); *Escherichia coli*DH5α cells, Transgene (Beijing, China); dNTPs, T4 DNA ligase, *Nde*I, *Xho*I, and Pfu DNA pol, Thermo Fisher Scientific (Waltham, MA); Chemicals, Amresco (WA, USA).

### 2.2. DNA substrates

All the oligonucleotides used were synthesized by Sangon Company (Shanghai, China) and their sequences are listed in Table 1. Primed DNA substrates were prepared by annealing the Cy3-labeled oligonucleotide with its complementary oligonucleotides in an annealing buffer containing 20 mM Tris-Cl pH 8.0 and 100 mM NaCl. The annealing reactions were performed at 100$^{o}$C for 5 min and cooled slowly to room temperature at least 4 hours.

### 2.3. Cloning of the Tga PolB encoding gene

The TGAM_RS07365 gene encoding a Family BDNA pol of *T. gammatolerans* was amplified by Pfu DNA pol using the genomic DNA as the template in the presence of the forward primer (5′-GGG AAT TC*C ATA TG*C ATTCTCGATACCGACTACATC-3′, the italic nucleotides represent *Nde*I restriction site) and the reverse primer (5′- CCG *CTC GAG* TCA CTT CTT CCC TTT CAC CTT CAG-3′, the italic nucleotides represent *Xho*I restriction site).The PCR product was extracted and cleaved by *Nde*I and *Xho*I, and cloned into a pET-30a (+) vector. The sequence of the recombinant plasmid was verified by sequencing, and the confirmed plasmid was transformed into *E. coli* BL21 (DE3) RIL cells for expressing Tga PolB protein with a 6 x His-tag in its C-terminal.

**2.4. Overexpression and purification of Tga PolB**

For Tga PolB protein expression, the transformant was cultured at37$^{o}$C in LB medium containing 10 μg/mL kanamycin and 17 μg/mL chloramphenicol until the $OD_{600}$ reached 0.6.Then, isopropyl thiogalactoside (IPTG) was added at a final concentration of 0.8 mM for 12 hours at room temperature to induce the expression from the recombinant plasmid.

The induced cells were harvested by centrifugation and resuspended in a Ni column buffer A containing 20 mM Tris-HCl pH 8.0, 1 mM dithiothreitol (DTT), 500 mM NaCl, 50 mM imidazole and 10% glycerol. The cells were immediately disrupted by ultrasonication at 4$^{o}$C. After removing cell debris by centrifugation (16,000 g) at 4$^{o}$C, the supernatant was heated at 70$^{o}$C for 20 min. The supernatant was then collected after centrifugation (16,000 g) at 4$^{o}$C and was loaded onto a HisTrap FF

column (GE Healthcare, Uppsala, Sweden). The column was eluted with NCG™ Chromatography System (Bio-Rad, Hercules, CA, USA) using a linear gradient of 50–500 mM imidazole with a Ni column buffer B containing 20 mM Tris-HCl (pH 8.0), 1 mM DTT, 500 mM NaCl, 500 mM imidazole and 10% glycerol. Fractions containing the His-tagged Tga PolB protein were collected and analyzed by migration on a 10% SDS-PAGE. The purified Tga PolB protein fractions were dialyzed against a storage buffer containing 50 mM Tris-HCl (pH 8.0), 1 mM DTT, 50% glycerol and 50 mM NaCl, and stored at -80°C. The Tga PolB protein concentration was quantitated by measuring the absorbance at 280 nm. The theoretical molar extinction coefficient of the enzyme protein is predicted to be 122,300$M^{-1}$ $cm^{-1}$.

**2.5. Polymerase assays**

The standard reaction mixture (10μL) contained 50 nM Tga PolB, 200 nM Cy3-labeled primed DNA substrate (p22/t59), 20 mM Tris–HCl pH8.0, 2 mM DTT, 100 μg/mL BSA,2 mM $MgCl_2$, and 200 μM dNTPs. The polymerization reactions were performed for 15 min at 65°C. The polymerized product was analyzed by electrophoresis in a 15% polyacrylamide gel containing 8 M urea. The gel was visualized with a Molecular Image analyzer (Bio-Rad). ImageQuant software was used for quantitative analysis. All polymerase experiments were repeated three times.

**2.6. 3′–5′exonuclease assays**

The standard reaction mixture (10 μL) contained an indicated amount of Tga PolB, 200 nM Cy3-labeledssDNA, 20 mM Tris–HCl pH 8.0, 2 mM DTT, 100 μg/mL BSA, and 2 mM $MgCl_2$.Reactions were carried out for 15 min at 65°C. The cleaved

products were subjected to electrophoresis in a polyacrylamide urea gel as described above. The gel was visualized with a Molecular Image analyzer (Bio-Rad). ImageQuant software was used for quantitative analysis. All exonuclease experiments were repeated three times.

### 2.7. Electrophoretic mobility shift assays (EMSA)

200 nM Cy3-labeled ssDNA or primed DNA were incubated with Tga PolB with varied concentrations in 20 mM Tris-HCl pH 8.0, 1 mM DTT and 8% glycerol at room temperature for 10 min. The retarded product was analyzed by electrophoresis in a 4% native polyacrylamide gel in 0.1 x TBE buffer and visualized with a Molecular Image analyzer (Bio-Rad). ImageQuant software was used for quantitative analysis. All DNA-binding experiments were repeated three times.

### 2.8. PCR amplification assays

To determine whether Tga PolB can be used in routine PCR, we performed PCR reactions containing 50 mM Tris-Cl pH8.0, 0.1 mg/ml BSA, 5 mM $Mg^{2+}$, 50 mM NaCl and 130 nM Tga PolB to amplify the gene TBCH5v1_0629 (744 bp) using the genomic DNA of *Thermococcus barophilus* Ch5 as a template and PCR primers described in our previous publication [30]. The cycling of PCR reactions was performed as follows: one initial denaturation at 98$^{o}$C for 5 min followed by 30cycles of 95$^{o}$C for 30s, 50 $^{o}$C 30s and 72 $^{o}$C1min, and the final step was 72 $^{o}$C 10 min. PCR products were resolved by electrophoresisin a 0.8% standard agarose gel.

## Results

### 3.1. Cloning, expression and purification of Tga PolB protein

The genome of *T. gammatolerans* encodes a Family B DNA polymerase. To characterize Tga PolB biochemically, its gene was cloned into a pET30a (+) expression vector, and overexpressed in *E.coli*. The recombinant Tga PolB protein was purified to near homogeneity after Ni-column affinity purification (Fig. 1A). The purified protein displayed an approximate MW of 90 kDa (Fig. 1A), which correlates with the deduced amino acid sequences.

Using the primed DNA (p22/t59), we determined the polymerase activity of the purified Tga PolB protein using concentrations ranging from 1 to 500 nM. As shown in Fig.1B, the full length product was gradually created with increasing Tga PolB concentrations at 65$^{o}$C, and maximal activity (>90 polymerization percentage) was obtained at ≥20 nM, suggesting that the enzyme is able to efficiently synthesize DNA at high temperature.

The genome of *E.coli* encodes five DNA pols that can synthesize DNA, which might interfere with our above observations [31]. To rule out this possibility, we prepared the *E.coli* cell extract with the empty pET-30a (+) plasmid as described for preparation of the recombinant pET30a (+) plasmid with the Tga PolB gene. We used the cell extract to perform above DNA synthesis reactions, and found that no synthesized product was observed (data not shown). Thus, the possibility of contamination of *E.coli* DNA pols was removed, thereby indirectly confirming that Tga PolB can synthesize DNA at high temperature.

### 3.2. Biochemical characterization of Tga PolB

The biochemical characteristics of DNA synthesis by Tga PolB, which includes

thermostability, the optimal pH, divalent cation availability, salt adaptation and dNTPs availability, were investigated using the primed DNA described above as the substrate. Considering the instability of primed duplex used in this work at >65°C, we performed DNA synthesis reaction of Tga PolB at <65°C. As shown in Fig. S1, the enzyme is capable of synthesizing DNA at increasing temperatures ranging from 25°C to 65°C with varied efficiencies High synthesis efficiency (99%) was observed from 45°C to 65°C, further confirming that Tga PolB can replicate DNA at high temperature.

To investigate its thermo-tolerance, 500 nM Tga PolB was heated at varying temperatures for different times, and the heated enzyme (50 nM) was used to perform DNA synthesis reactions at 65°C. Our results showed that the enzyme is able to fully withstand 95°C for at least 1.0 hr, retaining a 93 ± 2.6% activity (Fig. 2A). After 2 hours at 95°C, the enzyme retained ca. 20 ± 2.1% activity. Thus, these observations confirm that, as expected from DNA pols from other *Thermococcus*, Tga PolB is a thermostable DNA pol.

To investigate the effect of pH on Tga PolB activity, we reduced enzyme concentrations to be 15 nM in DNA synthesis. As shown in Fig. 2B, the enzyme could synthesize DNA in a broad pH range from 5.0 to 10.0, at which the activity begins to be altered. No product is synthesized at pH=11. The maximal synthesis activity was observed between pH 7.0 and 9.0, but our test did not allow having more precision on the effective optimum.

It is well-known that DNA pols utilize a divalent cation to synthesize DNA. To

investigate the effect of a divalent cation on Tga PolB activity, we reduced enzyme concentrations to be 15 nM in DNA synthesis. As expected, no product was observed in DNA synthesis by Tga PolB without a divalent cation or with EDTA (Fig. 2C). Only two divalent ions, $Mg^{2+}$ and $Mn^{2+}$, could restore DNA pol activity to the enzyme. The polymerization percentage of Tga PolB was estimated to be 72 ± 5.7% and 33 ± 7.1% in the presence of $Mg^{2+}$ and $Mn^{2+}$, respectively, demonstrating that similarly to other *Thermococcus* DNA PolB, DNA synthesis activity of Tga PolB is dependent on a divalent cation. Thus, $Mg^{2+}$ is the preferred ion for polymerization activity of Tga PolB.

We investigated the salt tolerance of the enzyme from 0 to 600 mM by using 15 nM enzyme in DNA synthesis reactions. In absence of NaCl, Tga PolB displayed 67 ± 2.2% polymerization activity (Fig. 2D). From 50 to 200 mM NaCl, the activity was higher than 90%, but it was significant reduced at NaCl concentrations above 400mM. Thus, NaCl is required for optimal Tga PolB activity. But, enzyme activity was inhibited by 400 mM NaCl, and no synthesis was observed at 600 mM NaCl.

### 3.3. Effect of dNTPs on the balance of the polymerase and exonuclease activity of Tga PolB

Most of Family B DNA pols, including the members from Archaea, possess 3′-5′ exonuclease activity in addition to 5′-3′ polymerase activity. To examine whether Tga PolB has exonuclease activity, we used ssDNA as a substrate. As expected, we found that Tga PolB started to degrade DNA when adding 5 nM enzyme to the exonuclease reaction, and exonuclease activity increased with increasing enzyme concentration

(Fig. 3A). Maximal exonuclease efficiency (>90%) was obtained when for concentrations of 15 nM Tga PolB and higher. Furthermore, DNA products with various sizes were created in the presences of varied concentrations of Tga PolB.

Next, we investigated the effect of dNTPs on the balance of the polymerase activity and exonuclease activity of Tga PolB. We designated Exo/Pol as the effect of dNTPs concentration on the balance of exonuclease activity and polymerase activity. As the the dNTPs concentrations increased, the values of Exo/Pol decreased, suggesting that dNTPs concentrations are favor in polymerase activity. At 4.0 μM dNTPs, the values of Exo/Pol reached 0.07 (Fig. 3B), indicating that Tga PolB exhibits extremely weak exonuclease activity in the presence of 4.0 μM dNTPs. Thus, dNTPs stimulates Tga PolB to synthesize DNA rather than degrade DNA.

**3.4. Mismatch extension of Tga PolB**

We determined mismatch the extension efficiency of Tga PolB by using primed DNA with a match end (C/G) and primed DNA with a mismatch end (C/A, A/G and T/G) as substrates. As shown in Fig. 4, no matter what concentration of Tga PolB was used in polymerase reactions, >95% polymerization percentage was observed during DNA synthesis by Tga PolB when using the primed DNA with a match as the substrate. However, Tga PolB displayed only about 18 ± 1.5%, 15 ± 2.8% and 14 ± 4.3% activity for extending A/G, G/G and T/G mismatch at 20 nM, respectively. Furthermore, at 50 nM, the efficiencies of the enzyme were still lower than for matched DNA, with 83 ± 5.7%, 72 ± 7.8% and 77 ± 0.8% for extending A/G, G/G and T/G mismatch at 50 nM, respectively. Only at 200 nM the enzyme displayed full

efficiency for extending mismatched DNA. Overall, these results suggested that Tga PolB has a low efficiency to extend mismatched DNA, although capable to extend it at high DNA/protein ratios.

### 3.5. Bypass activity of uracil and AP in DNA by Tga PolB

Archaeal Family B DNA pols can recognize uracil in DNA, which results in polymerization arrest. Using uracil-containing primed DNA as the substrate, we tested this ability in Tga PolB. Note that the full length template DNA is 59 mer and uracil in the template DNA is located at 42 mer described in Table 1. As shown in Fig. 5, the full length product was observed at 200 nM Tga PolB in the control reaction using the normal primed DNA as the substrate. Compared with the control reaction, two products corresponding to 42 mer (partial length) and 59 mer (full length) were synthesized by 200 nM Tga PolB using the uracil-containing primed DNA as the substrate. Furthermore, more synthesized full length product than partial length product was created by Tga PolB, suggesting that this pol can bypass the uracil of the DNA template, which is a distinct feature from typical archaeal Family B DNA pols.

Since Tga PolB could bypass U bases in its DNA template, we tested whether it could also bypass AP sites in DNA. Like uracil-containing DNA, the AP site in the full length template DNA (59mer) is also located in 42 mer position. By using AP-containing primed DNA as a substrate, we conducted DNA synthesis by Tga PolB. As shown in Fig. 5, only one band corresponding to 42 mer product was observed by Tga PolB, which is sharply inconsistent with the observation of the replication of uracil containing DNA by this pol. In contrast, no full length product was synthesized by

Tga PolB, suggesting that this pol cannot bypass AP sites, as observed in other archaeal Family B DNA Pols.

### 3.6. DNA Binding by Tga PolB

Since Tga PolB can degrade ssDNA and extend primed DNA with both matched and mismatched ends, we employed the EMSA to determine whether Tga PolB is able to bind DNA. We incubated ssDNA, primed DNA and primed DNA with a mismatch (T/G) with Tga PolB at varying concentrations, respectively. As shown in Fig. 6A, the binding of free ssDNA substrate increased gradually with increasing Tga PolB concentrations. No ssDNA binding was observed at 100 nM and maximal binding was observed at 500 nM.

dsDNA binding also increased as Tga PolB concentration increased, from 49 ± 5.0%, 80 ± 4.8%, and 86 ± 3.6% at 100 nM, 200 nM and 300 nM enzyme (Fig. 6B). At higher concentrations (>400 nM), the binding percentages reached >90% and reached its maximum (~97%) at 500 nM. Thus, Tga PolB has a slightly higher affinity for primed DNA binding than for ssDNA binding. Tga PolB binding to primed DNA is not affected by the presence of mismatches (Fig. 6C).

### 3.7. PCR amplification of Tga PolB

Last, we investigated whether or not Tga PolB can be used for routine PCR to amplify DNA. Using the genomic DNA of *T. barophilus* Ch5 as a template, we performed the PCR reaction to amplify the UDG247 gene with Tga PolB. As shown in Fig. 7, the PCR product was clearly created by Tga PolB, corresponding to 744 bp, thus suggesting that this Pol can be employed in routine PCR.

## Discussion

In this work, we characterized biochemically the Family B DNA pol from the hyperthermophilic and radioresistant euryarchaeon *T. gammatolerans*, and revealed that Tga PolB can replicate DNA at high temperature. Consistent with other *Thermococcus* DNA pol homologues, Tga PolB displays similar biochemical characteristics for DNA synthesis. As expected, Tga PolB can be used to amplify DNA by routine PCR reaction, suggesting that this Pol also resembles other *Thermococcus* DNA pols. Interestingly, Tga PolB has extraordinary properties that are distinct from other archaeal Family B DNA pols.

Crystal structures of archaeal Family B DNA pols demonstrate that they possess uracil recognition pocket [9; 32], thus allowing the replication to be stopped when these DNA pols encounter a uracil in DNA template. Biochemical data provide strong support for the 'read-ahead' mechanism. Archaeal B family DNA polymerases, such as Pfu DNA pol, can recognize uracil in DNA template strand during DNA synthesis and will be stalled 4 bp upstream of a template uracil. Thereby preventing G:C→A:T mutation [33]. Although this PolB can bypass uracil in DNA template with low efficiency, the hyperthermoacidophilic crenarchaeon *Sulfolobus solfataricus* DNA polB1 (Sso PolB1) also has similar read-ahead behavior [34]. Furthermore, Pfu PolB displays much weaker ability to bypass uracil in DNA template than Sso PolB1 [34] . However, we demonstrated that Tga PolB can efficiently synthesize DNA despite encountering uracil in DNA template, which differs from other *Thermococcales* enzymes such as Pfu PolB, and Sso PolB1. Consistent with Sso PolB1, Tga PolB

cannot continue to replicate DNA when encountering an AP lesion in DNA template, consistent with other archaeal Family B DNA pols [34; 35].

As DNA polymerase substrate, deoxynucleotide triphosphates affect the exonuclease/polymerase balance of a DNA polymerase. It has been reported that dNTPs stimulate polymerase activity of a DNA polymerase and suppress its exonuclease activity. However, different DNA polymerases display distinct sensitivity to the dNTP inhibition. In this study, we demonstrate that Tga PolB is capable of extending primer at a low level (2 μM) of dNTPs, which is consistent with Sso polB1 [36]. In contrast to Sso PolB1, which retains its exonuclease activity up to 600 μM dNTPs, Tga PolB already lacks an exonuclease activity at 5 μM dNTPs [36].

A replicative DNA polymerase has polymerase activity and 3′-5′ exonuclease activity, and is in favor of exonuclease activity when encountering a mismatched primer end to ensure high fidelity of DNA replication. In this study, we show that Tga PolB is able to efficiently extend primer with a correct pair end at 20 nM. However, Tga PolB displays significantly reduced extension efficiencies for primers with incorrected pair ends at 20 nM, which may be caused by the exonuclease activity of Tga PolB that can remove the mismatched 3' nucleotide to generate a match base pair. Thus, Tga PolB might be in favorable for the exonuclease activity for removing incorrected nucleotide when encountering a mismatched pair end and then start to synthesize DNA after proofreading.

Last, we also show that Tga PolB displays higher affinity for binding primed DNA than for binding ssDNA at <300 nM. Furthermore, two bands corresponding to

primed DNA binding appeared while only band corresponding to ssDNA binding of was observed at both 200 nM and 300 nM Tga PolB. We proposed that one primed DNA molecular would be bound with two Tga PolB molecular whereas one Tga PolB molecular might bind one ssDNA molecular. Overall, Tga PolB has distinct binding patterns for primed DNA and ssDNA.

## Conclusion

In summary, we present the biochemical characteristics of the Family B DNA pol from the hyperthermophilic and radioresistant *T. gammatolerans*. We demonstrate that the recombinant Tga PolB possesses polymerase activity for DNA synthesis and exonuclease activity for removing misincorporated nucleotide. Surprisingly, Tga PolB can replicate DNA when encountering uracil in DNA template, which is sharply contrasted with other archaeal Family B DNA pols. However, this pol can be stalled by an AP site in DNA. Furthermore, dNTPs stimulate the polymerase activity and inhibit the exonuclease activity of Tga PolB. Tga PolB can bind to primed DNA with higher efficiencies than to ssDNA at low concentration, and also display distinct binding patterns for primed DNA and ssDNA. Last, Tga PolB can be used in routine PCR.

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## Author contributions

LZ and ZY designed experiments; DJ, HS, MW and QG performed experiments;

LZ, ZY, and PO analyzed data; LZ, ZY, and PO wrote and revised the paper.

## Conflict of interests

All authors declare that there is no conflict of interests regarding the publication of this paper.

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## Figure legends

**Fig. 1.** Tga PolB is able to synthesize DNA at 65$^{o}$C. A. the overexpression and purification of the recombinant Tga PolB protein. B. DNA synthesis of Tga PolB at 65$^{o}$C. The DNA synthesis reactions were performed by Tga PolB at 65$^{o}$C in the presence of varied concentrations. The synthesized DNA products were analyzed by running a denaturing polyacrylamide gel. CK: the reaction without the enzyme.

**Fig. 2.** Biochemical characterization of Tga PolB. A. the thermo-tolerance of the enzyme; B. the optimal pH of the enzyme activity; C. the effects of divalent cations on the enzyme activity; D. the effect of NaCl on the enzyme activity. The DNA synthesis reactions were performed at 65$^{o}$C. The synthesized DNA products were

analyzed by a denaturing polyacrylamide gel. CK in panels A, B and D, and CK1 in panels C: the reaction without the enzyme; CK2 in panel C: the reaction without a divalent cation.

**Fig. 3.** Inhibition of exonuclease activity of Tga PolB by dNTPs substrate. A. the exonuclease activity of Tga PolB. Tga PolB with varied concentrations was employed to perform exonuclease reactions by using the ssDNA as the substrate at 65$^{o}$C. The cleaved DNA products were analyzed by a denaturing polyacrylamide gel. B. the effect of dNTPs substrate on the polymerase and exonuclease activities of Tga PolB. The coupled polymerase and exonuclease activities of Tga PolB were performed at 65$^{o}$C in the presence of dNTPs with varied concentrations. The reaction products were analyzed by a denaturing polyacrylamide gel. CK: the reaction without the enzyme.

**Fig. 4.** Mismatch extension by Tga PolB. The match DNA (C:G) and mismatch DNA (A:G, G:G and T:G) were employed as the substrates in the DNA synthesis reactions catalyzed by Tga PolB (20, 50 and 200 nM) at 65$^{o}$C. The synthesized DNA products were analyzed by running a denaturing polyacrylamide gel. CK: the reaction without the enzyme.

**Fig. 5.** Bypass analysis of uracil and AP in DNA by Tga PolB. The uracil- and AP-containing primed DNA were employed as the substrates in the DNA synthesis reactions catalyzed by 200 nM Tga PolB at 65$^{o}$C. The synthesized DNA products were analyzed by running a denaturing polyacrylamide gel. CK: the reaction without the enzyme.

**Fig. 6.** DNA binding assays of Tga PolB. 200 nM ssDNA and primed DNA were incubated with Tga PolB with varied concentrations at room temperature, respectively. The retarded DNA products were analyzed by a native polyacrylamide gel. A. binding ssDNA; B. binding normal primed DNA; C. binding primed DNA with a mismatch (T/G). CK: the binding reaction without the enzyme.

**Fig. 7.** PCR amplification of Tga PolB. M: DNA marker; CK: the PCR reaction without enzyme.

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**Table 1** Sequences of the oligonucleotides used in this work

| Name | Sequence (5′-3′) |
|---|---|
| p22C* | CAG TGA ATT CGA GCT CGG TAC C |
| p22A* | CAG TGA ATT CGA GCT CGG TAC A |
| p22G* | CAG TGA ATT CGA GCT CGG TAC G |
| p22T* | CAG TGA ATT CGA GCT CGG TAC T |
| t59 | GCT TGC ATG CCT GCA GGT CGA CTC TAG AGG ATC CCC GGG TAC CGA GCT CGA ATT CAC TG |
| t59U | GC TTG CAT GCC TGC AGG UCGA CTC TAG AGG ATC CCC GGG TAC CGA GCT CGA ATT CAC TG |
| t59AP | GCT TGC ATG CCT GCA GGAPCGA CTC TAG AGG ATC CCC GGG TAC CGA GCT CGA ATT CAC TG |

*indicates the Cy3-labeling at 5′ end of primers (p22C, p22A, p22G and p22T).

The underlined base is used to prepare uracil (U)- and AP (Apurinc/apyrimidinic)-containing dsDNA.

**Supplemental Data**

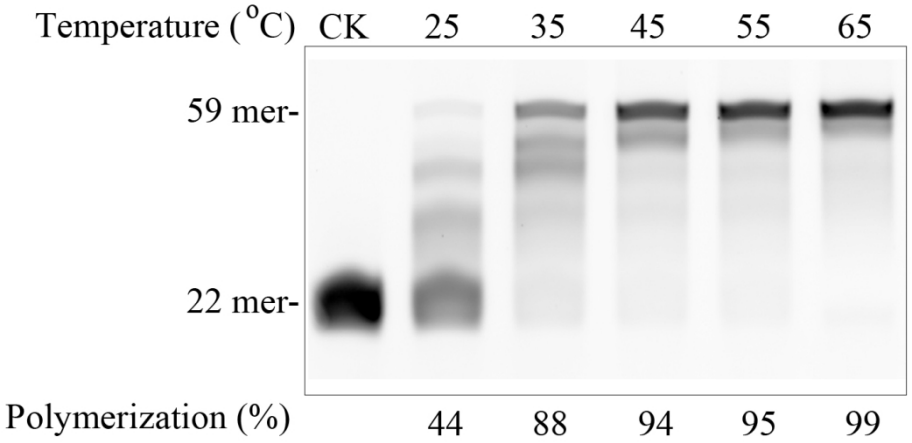
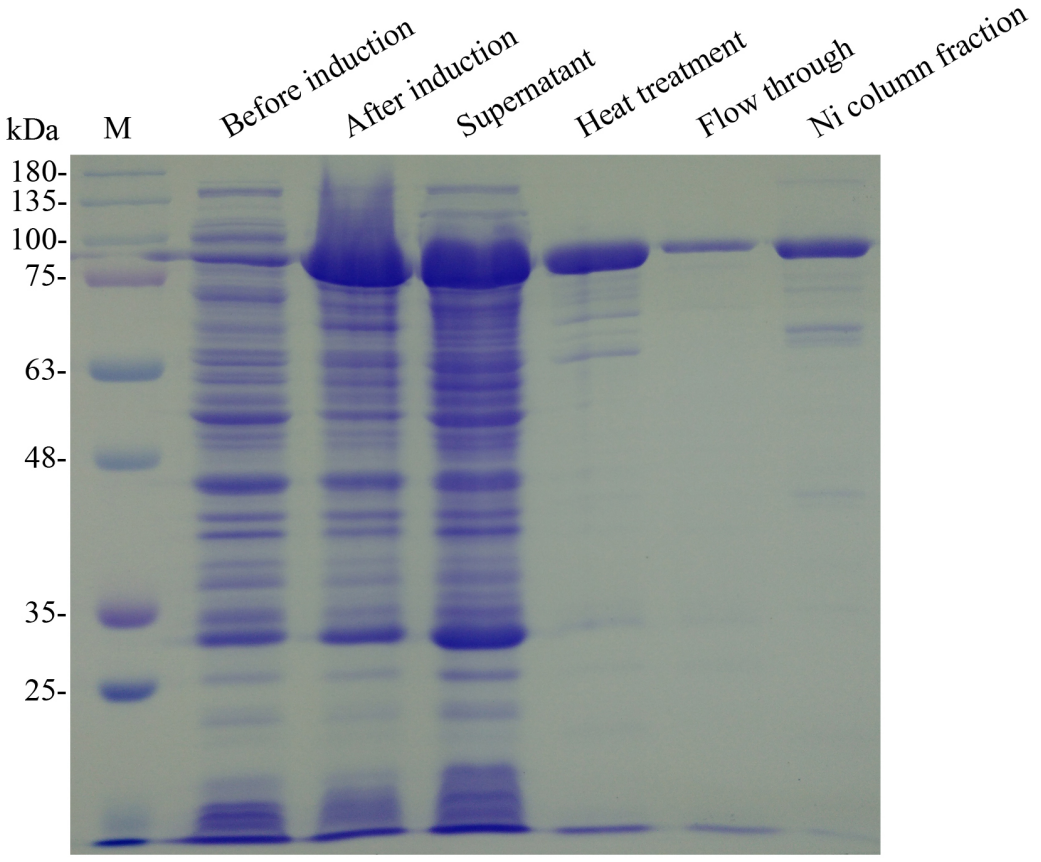
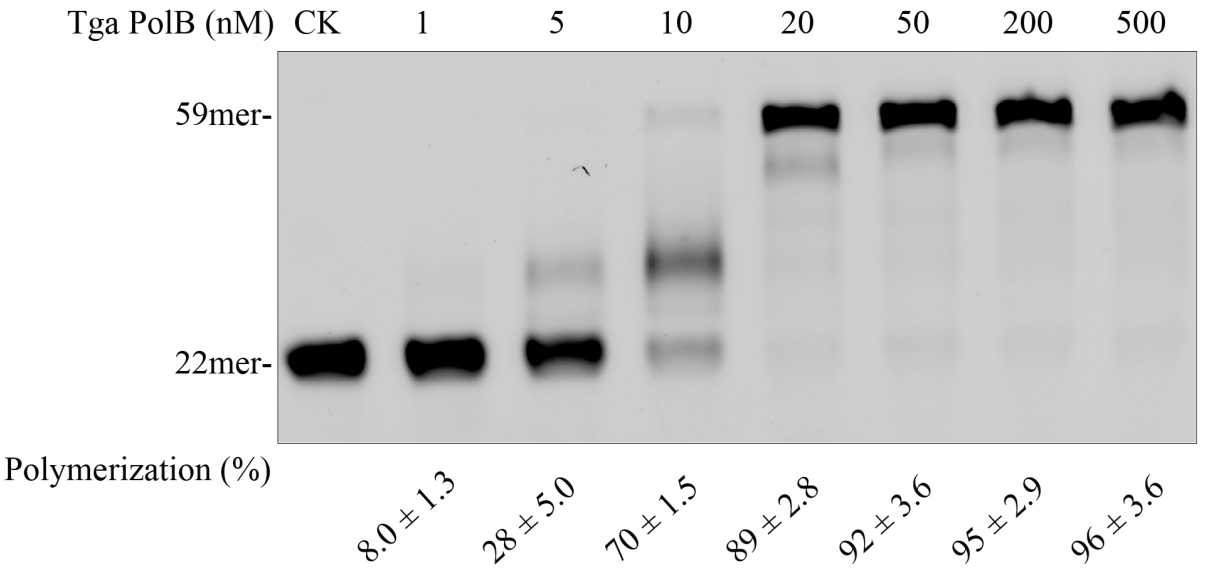


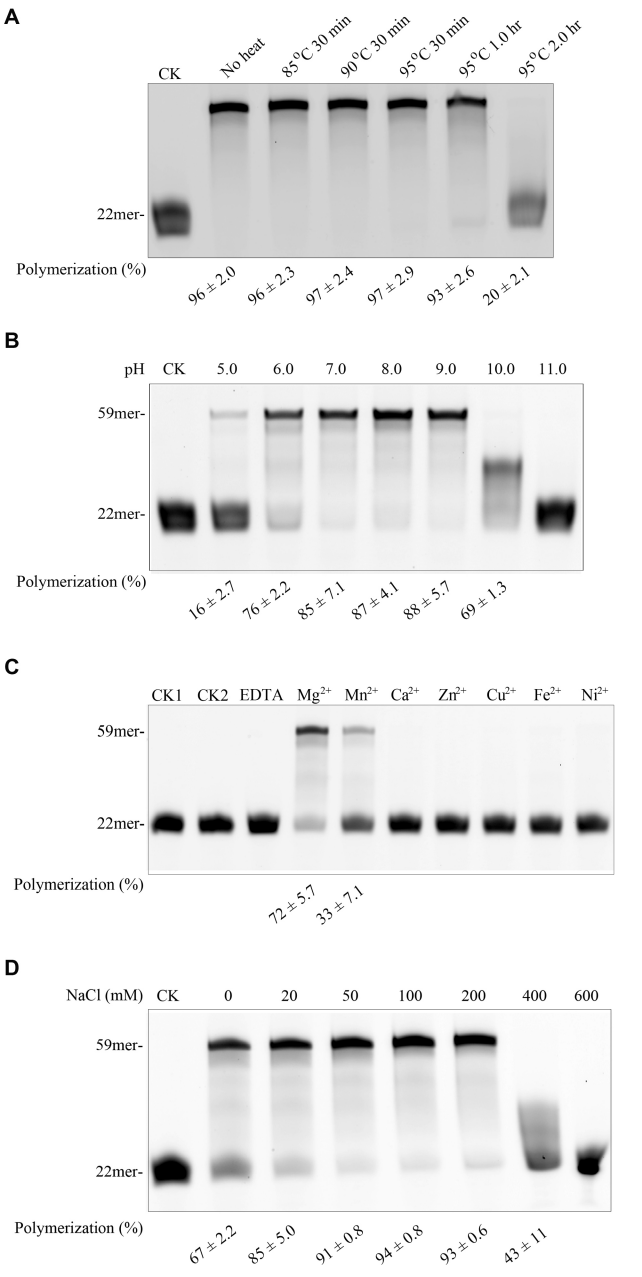

**Fig. s1.** DNA synthesis of Tga PolB at different temperatures. The DNA synthesis reactions were performed by 50 nM Tga PolB at different temperatures. The synthesized DNA products were analyzed by running a denaturing polyacrylamide gel. CK: the reaction without the enzyme.

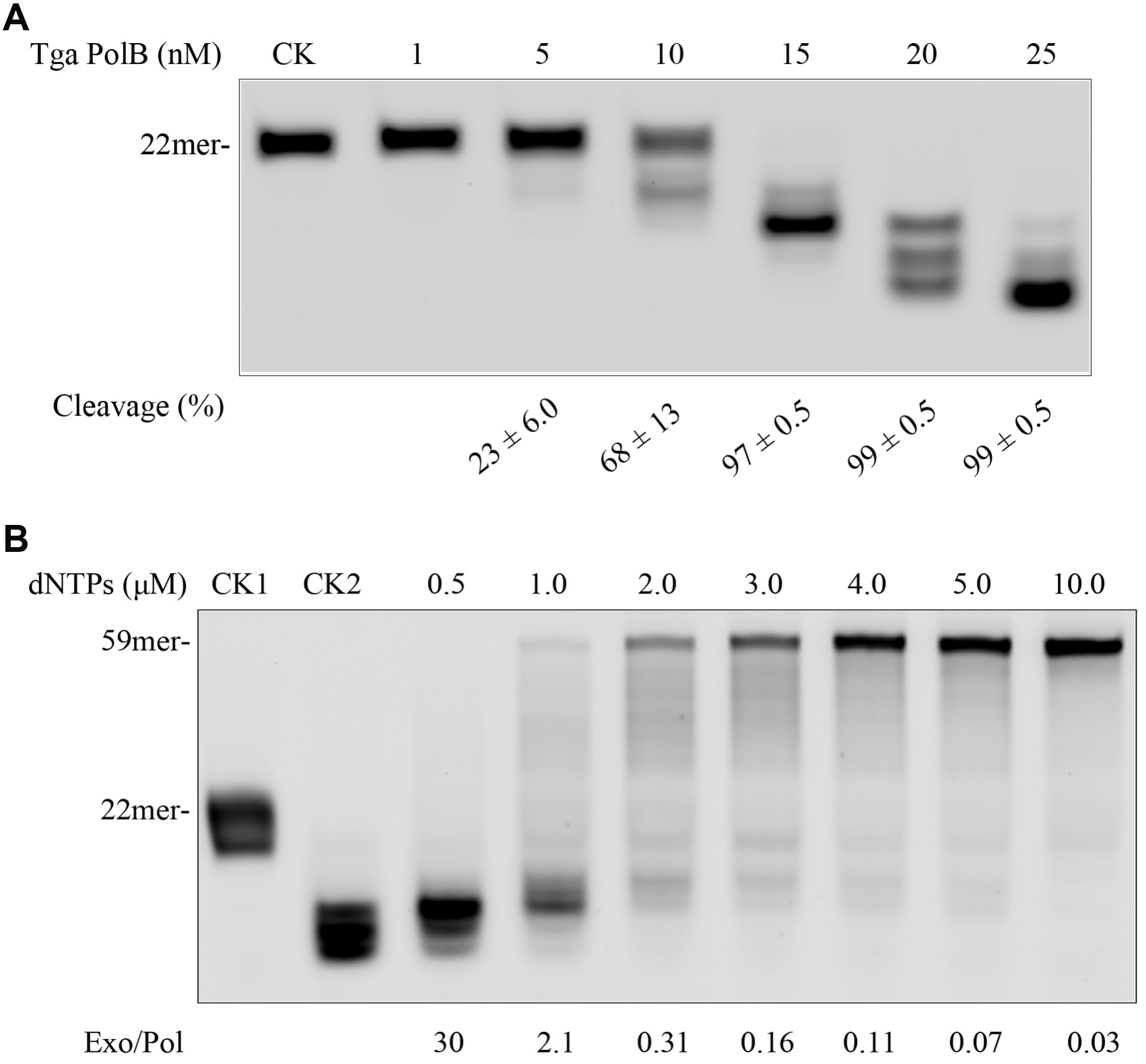
**A**

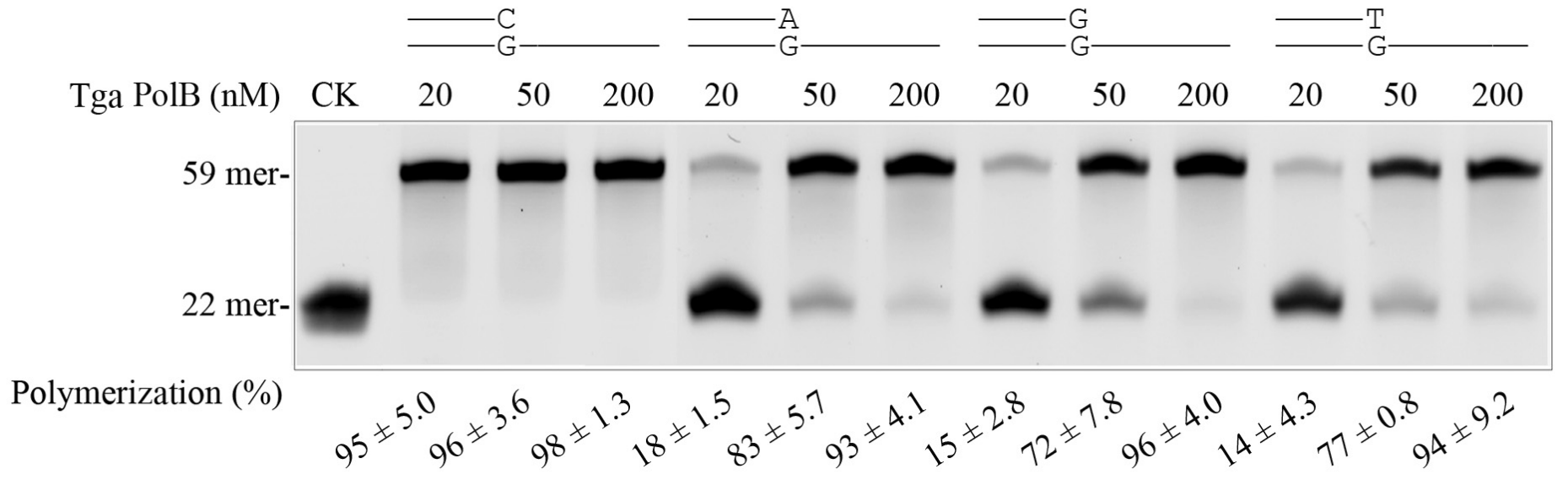

kDa
M
Before induction
After induction
Supernatant
Heat treatment
Flow through
Ni column fraction
180-
135-
100-
75-
63-
48-
35-
25-

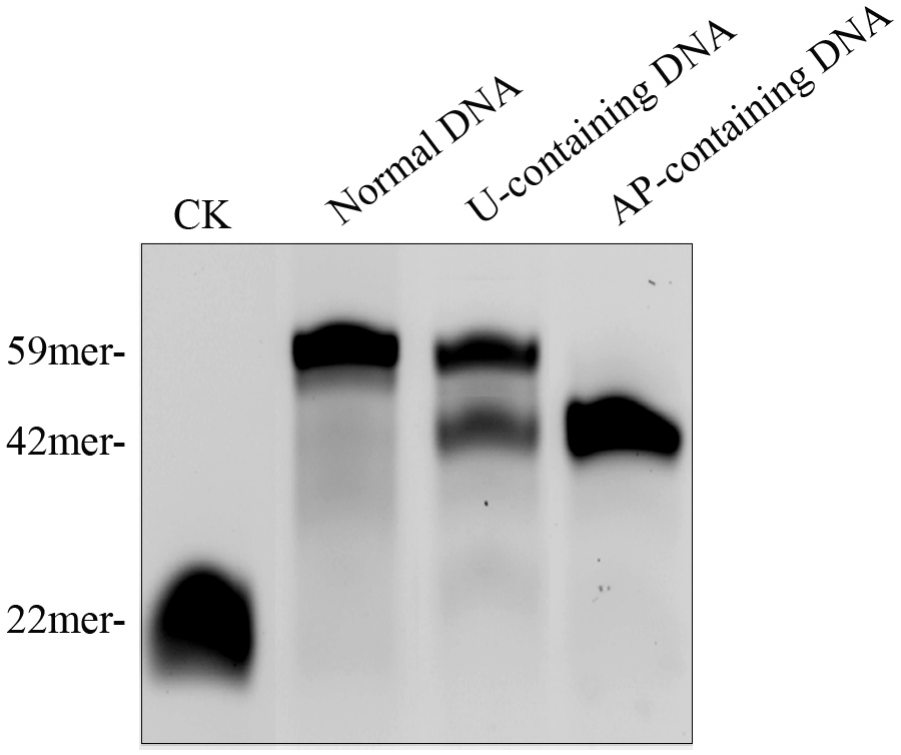
**B**

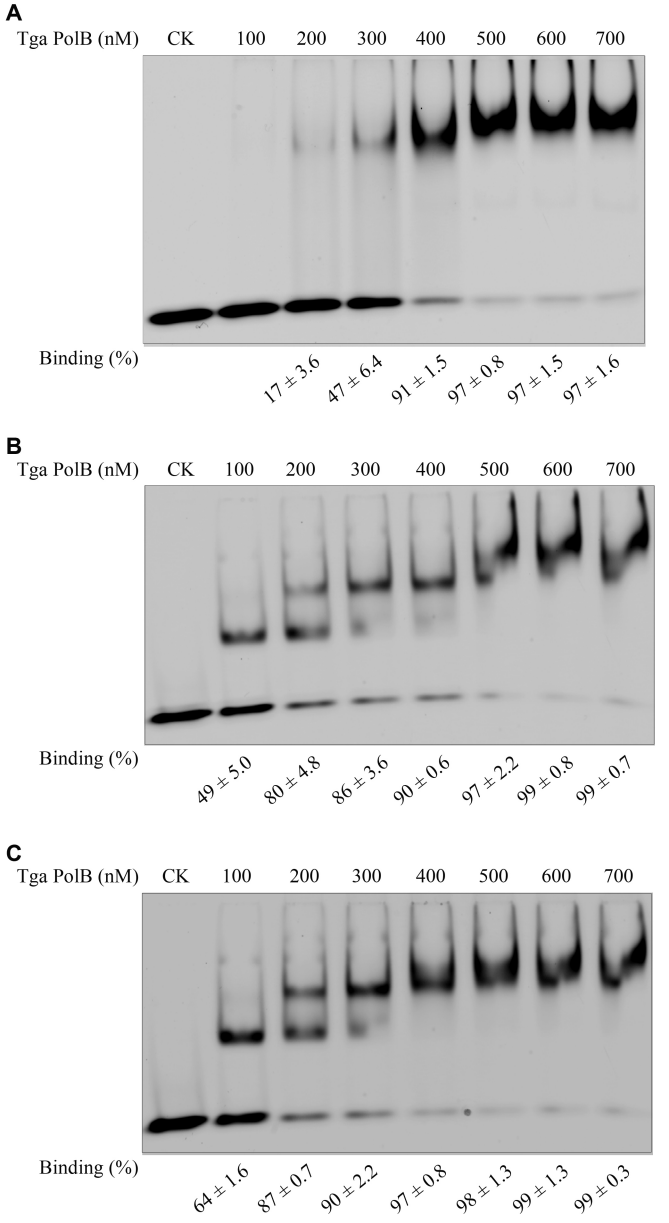

Tga PolB (nM)
CK
1
5
10
20
50
200
500
59mer-
22mer-
Polymerization (%)
8.0 ± 1.3
28 ± 5.0
70 ± 1.5
89 ± 2.8
92 ± 3.6
95 ± 2.9
96 ± 3.6

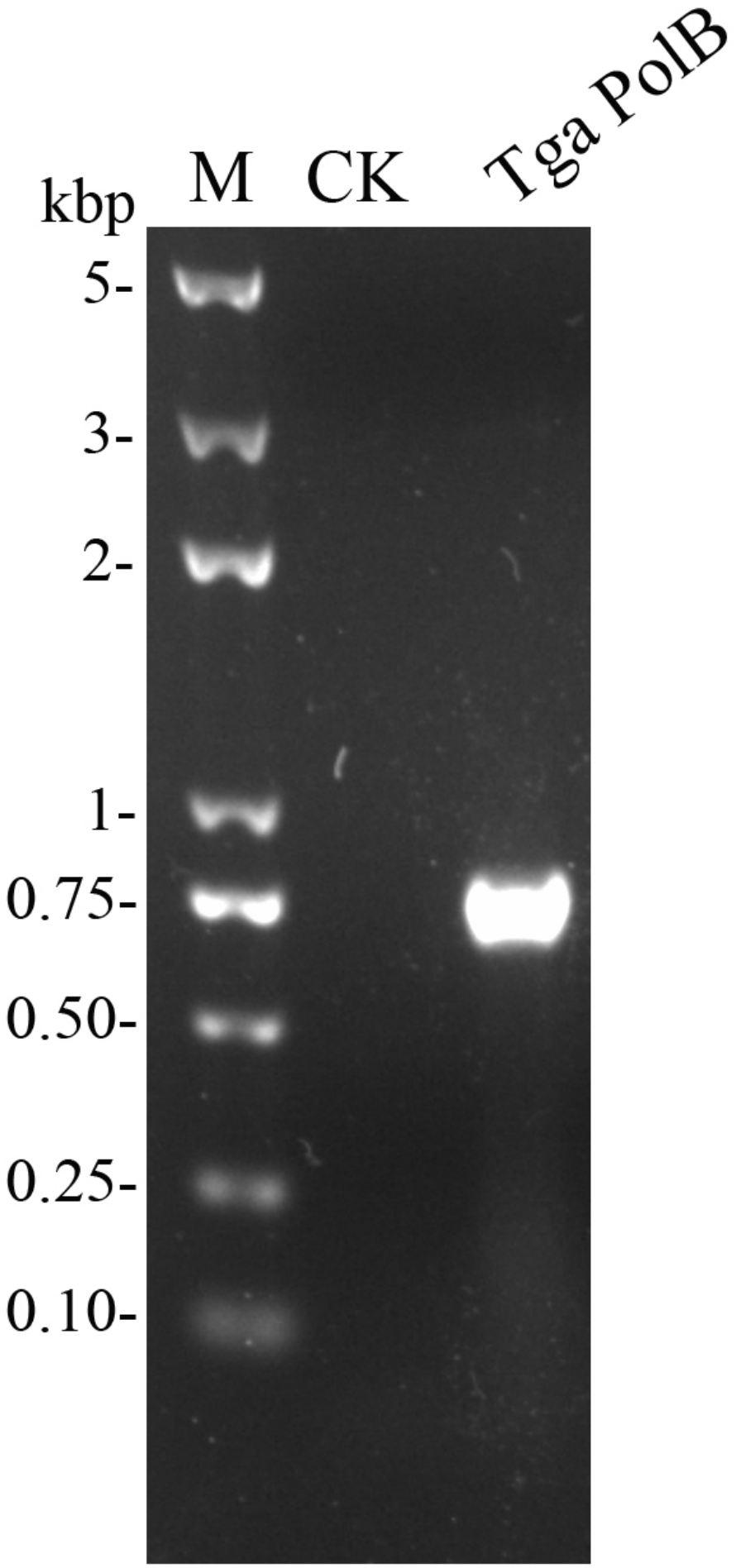

A
CK
No heat
85°C 30 min
90°C 30 min
95°C 30 min
95°C 1.0 hr
95°C 2.0 hr
22mer-
Polymerization (%)
96 ± 2.0
96 ± 2.3
97 ± 2.4
97 ± 2.9
93 ± 2.6
20 ± 2.1
B
pH
CK
5.0
6.0
7.0
8.0
9.0
10.0
11.0
59mer-
22mer-
Polymerization (%)
16 ± 2.7
76 ± 2.2
85 ± 7.1
87 ± 4.1
88 ± 5.7
69 ± 1.3
C
CK1
CK2
EDTA
Mg2+
Mn2+
Ca2+
Zn2+
Cu2+
Fe2+
Ni2+
59mer-
22mer-
Polymerization (%)
72 ± 5.7
33 ± 7.1
D
NaCl (mM)
CK
0
20
50
100
200
400
600
59mer-
22mer-
Polymerization (%)
67 ± 2.2
85 ± 5.0
91 ± 0.8
94 ± 0.8
93 ± 0.6
43 ± 11


A
Tga PolB (nM)
CK
1
5
10
15
20
25
22mer-
Cleavage (%)
23 ± 6.0
68 ± 13
97 ± 0.5
99 ± 0.5
99 ± 0.5
B
dNTPs (μM)
CK1
CK2
0.5
1.0
2.0
3.0
4.0
5.0
10.0
59mer-
22mer-
Exo/Pol
30
2.1
0.31
0.16
0.11
0.07
0.03


C
G
A
G
G
G
T
G
Tga PolB (nM)
CK
20
50
200
20
50
200
20
50
200
20
50
200
59 mer-
22 mer-
Polymerization (%)
95 ± 5.0
96 ± 3.6
98 ± 1.3
18 ± 1.5
83 ± 5.7
93 ± 4.1
15 ± 2.8
72 ± 7.8
96 ± 4.0
14 ± 4.3
77 ± 0.8
94 ± 9.2


CK
Normal DNA
U-containing DNA
AP-containing DNA
59mer-
42mer-
22mer-


A
Tga PolB (nM)
CK
100
200
300
400
500
600
700
Binding (%)
17 ± 3.6
47 ± 6.4
91 ± 1.5
97 ± 0.8
97 ± 1.5
97 ± 1.6
B
Tga PolB (nM)
CK
100
200
300
400
500
600
700
Binding (%)
49 ± 5.0
80 ± 4.8
86 ± 3.6
90 ± 0.6
97 ± 2.2
99 ± 0.8
99 ± 0.7
C
Tga PolB (nM)
CK
100
200
300
400
500
600
700
Binding (%)
64 ± 1.6
87 ± 0.7
90 ± 2.2
97 ± 0.8
98 ± 1.3
99 ± 1.3
99 ± 0.3


kbp
M
CK
Tga PolB
5-
3-
2-
1-
0.75-
0.50-
0.25-
0.10-

## Author Statement

All authors declare that they have no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

**SupplementalData**

# Characterization and application of a Family B DNA polymerase from the hyperthermophilic and radioresistant euryarchaeon *Thermococcus gammatolerans*

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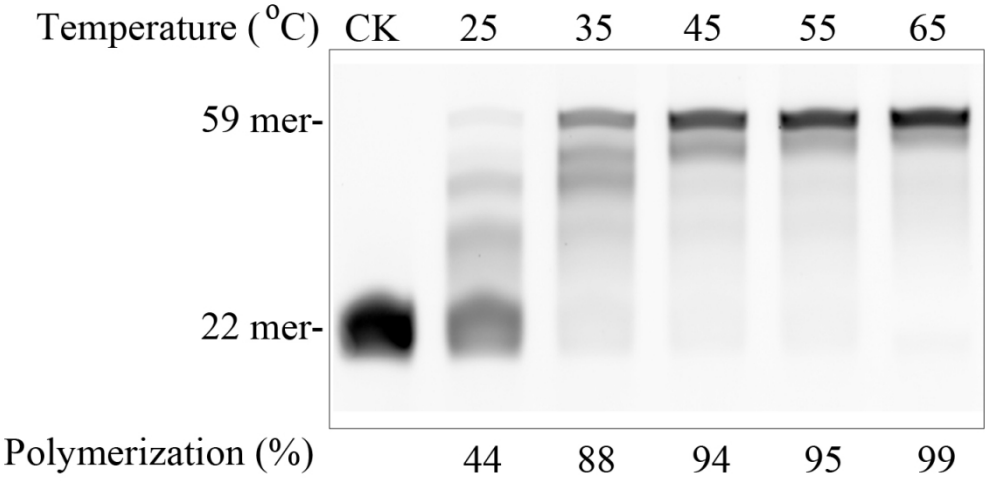



**Fig. s1.** DNA synthesis of Tga PolB atdifferent temperatures. The DNA synthesis reactions were performed by 50 nM Tga PolB at different temperatures. The synthesizedDNA products were analyzed by running a denaturing polyacrylamide gel. CK: the reaction without the enzyme.