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MINI-REVIEW

Strategies and procedures to generate chimeric DNA polymerases for improved applications

Zhuoxuan Yu[1] · **Jufang Wang**[1,2]

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Abstract

Chimeric DNA polymerase with notable performance has been generated for wide applications including DNA amplification and molecular diagnostics. This rational design method aims to improve specific enzymatic characteristics or introduce novel functions by fusing amino acid sequences from different proteins with a single DNA polymerase to create a chimeric DNA polymerase. Several strategies prove to be efficient, including swapping homologous domains between polymerases to combine benefits from different species, incorporating additional domains for exonuclease activity or enhanced binding ability to DNA, and integrating functional protein along with specific protein structural pattern to improve thermal stability and tolerance to inhibitors, as many cases in the past decade shown. The conventional protocol to develop a chimeric DNA polymerase with desired traits involves a Design-Build-Test-Learn (DBTL) cycle. This procedure initiates with the selection of a parent polymerase, followed by the identification of relevant domains and devising a strategy for fusion. After recombinant expression and purification of chimeric polymerase, its performance is evaluated. The outcomes of these evaluations are analyzed for further enhancing and optimizing the functionality of the polymerase. This review, centered on microorganisms, briefly outlines typical instances of chimeric DNA polymerases categorized, and presents a general methodology for their creation.

Key points

- *Chimeric DNA polymerase is generated by rational design method.*
- *Strategies include domain exchange and addition of proteins, domains, and motifs.*
- *Chimeric DNA polymerase exhibits improved enzymatic properties or novel functions.*

Keywords Chimeric enzyme · DNA polymerase · Domain exchange · Rational design · Thermal stability · Processivity

Introduction

As an essential component in DNA replication, DNA polymerase catalyzes the synthesis of a complementary DNA strand by adding nucleotides to the template strand. Since the discovery of DNA polymerase I capable of synthesizing DNA in vitro, and the unraveling of the complex mechanisms involved in DNA replication, we have been deepening our understanding of how genetic information is faithfully transmitted to the next generations (Lisova et al. 2022; Kuznetsova et al. 2022). This enhanced knowledge has laid the foundation for the development of numerous applications, including the polymerase chain reaction (PCR) (Mullis et al. 1986; Bhadra et al. 2020).

DNA polymerases, found in a wide ranges of organisms from eukaryotes and prokaryotes to viruses, were classified into seven families: A, B, C, D, X, Y and RT. Amazingly, enormous types of DNA polymerases in all seven families are rather conserved in structures, often described as a right-handed fold. This configuration includes distinct domains: the palm, which contains the polymerase's active site; the thumb, responsible for binding the replicated DNA; and the fingers, which interact with the incoming nucleotides (Kuznetsova et al. 2022). In addition to the right-hand-like structural configuration, some DNA polymerases also

✉ Jufang Wang
jufwang@scut.edu.cn

[1] School of Biology and Biological Engineering, South China University of Technology, Guangzhou 510006, China

[2] Guangdong Provincial Key Laboratory of Fermentation and Enzyme Engineering, South China University of Technology, Guangzhou 510006, China

possess additional domains such as the 3' to 5' exonuclease and/or 5' to 3' exonuclease domain (Zahurancik and Suo 2020; Dangerfield and Johnson 2023). Each domain within the DNA polymerase contributes uniquely to the enzyme's specific functional characteristics.

Currently, DNA polymerase is widely used in biotechnological fields, including DNA amplification, sequencing, and molecular diagnostics (Ordóñez and Redrejo-Rodríguez 2023; Akram et al. 2023; Chen et al. 2016). Nevertheless, wild-type polymerase fails to meet the growing demand of DNA replication under harsh conditions, such as extreme temperature and high salinity, or replicating templates with low concentration or from ancient fossil samples (Wang et al. 2022; Takahashi et al. 2018). Thus, there is an urgent need to generate engineered DNA polymerases with improved precision and efficiency to address those demands and to further maximize the potential of this promising protein.

A lot of engineering strategies have been applied to improve the performance of DNA polymerase, and many of them proved to be successful (Nikoomanzar et al. 2020; Kuznetsova and Kuznetsov 2023). One of the most effective strategies is directed evolution, a powerful technique to generate polymerases with desired properties or improvements. Numerous genetic mutants were obtained through mutagenesis, error-prone PCR, or DNA shuffling, followed by a screening process to identify variants with desired characteristics. The selected variants were subjected to further iterative cycles, gradually enhancing the desired characteristics such as polymerase activity (Ma et al. 2016; Ahmad et al. 2023), broadening the substrate spectrum (Dangerfield et al. 2022), and improving incorporation efficacy (Wang et al. 2017). This method links genotype and phenotype without revealing the underlying mechanism, facilitating the creation of active variants with beneficial gain-of-function mutations.

Another significant strategy is rational design. Previous studies have uncovered the structure of DNA polymerases and the role of different domains within a polymerase (Kuznetsova et al. 2022). It provided detailed information about which site may be critical for conformation changes or catalytic activity. Rational design built a solid foundation for achieving the desired properties or applications such as the incorporation of modified nucleotide (Gardner et al. 2019; Wang et al. 2022), exonuclease silencing (Motré et al. 2008), and PCR (Bhadra et al. 2020).

Recently, some events of progress have been made by integrating specific heterologous regions or domains to create chimeric DNA polymerases, which is one of the rational design strategies. These chimeric polymerases can exhibit advantages from both of the parent polymerases, or enhanced specific functions to compensate for their drawbacks.

This review provides a concise overview of the latest developments in chimeric DNA polymerases for diverse applications, categorized based on their fusion strategies. It outlines the key strategies and general procedures used for creating these polymerases, as well as their potential for improved performance in various applications.

Current progress of generating chimeric DNA polymerases

Typically, to generate chimeric DNA polymerases with improved functions, amino acid sequences from two or more distinct proteins are attached together to form an integral polymerase, which exhibits a notable improvement of some enzymatic characteristics.

Over the past decades, detailed studies of crucial enzymatic characteristics have been carried out to comprehensively evaluate the effectiveness of the modified DNA polymerase. Moreover, an in-depth analysis of the polymerase structure and kinetics has revealed the domains associated with these enzymatic features, providing insights for rational design (Kuznetsova et al. 2022). The activity of the polymerase, generally referring to the rate of elongating new DNA strands from 5' to 3', is vital for DNA replication, usually defined as processivity, which is known as how much nucleotides can be added to the strand by polymerase per single binding event to the primers. It is also described as amplification efficiency when it comes to applications. For the polymerase itself, it is essential as well to have thermal stability and the capability to maintain amplification at specific temperatures (Śpibida et al. 2017) as DNA polymerase is widely applied in PCR and other isothermal amplification techniques. Many studies also focus on fidelity, which is the precision of base insertion, as well as inhibitor tolerance, indicating the capacity to maintain robustness and functionality against inhibitors such as dyes or ions in buffers. Additionally, considering the diverse potential application of DNA polymerase, characteristics like specificity, proofreading activity, and the ability to incorporate unnatural nucleotides are of significant interest (Table 1).

Improved characteristics or development of novel functions in chimeric DNA polymerases are achieved by the introduction of different domains or proteins to the original polymerase, including homologous domains of polymerases from various species, certain proteins for various functions like DNA binding, and particular patterns of protein structure.

Homologous domain exchange

DNA polymerases share a great similarity in palm domains with relatively conserved catalytic residues, while the thumb and finger domains may vary considerably (Braithwaite and

Table 1 Description of enzymatic characteristics of interested DNA polymerases

Enzymatic properties	Description	Domain(s) related
Activity	General rate of elongating new DNA strands from 5' to 3'	Palm
Processivity	Number of nucleotides added by polymerase per single binding event	Palm, thumb, finger
Thermal stability	Ability to resist high temperature without losing activity	All
Fidelity	Accuracy of base insertion	Finger
Inhibitor tolerance	Ability to remain functional against inhibitors (e.g., EDTA, heparin, Nacl or ions)	All
Substrate specificity	Ability to distinguish template with specific sequences or modification modifications	Thumb, finger
Proofreading activity	Activity of 3' to 5' exonuclease	Exonuclease

Ito 1993). It provides extensive potential for polymerases to significantly improve certain characteristics or acquire novel functions without losing the original catalytic ability. A highly effective strategy is to conduct homologous domain exchange, wherein a specific domain of the native polymerase is replaced with a corresponding domain or a similar sequence from another polymerase.

There are many examples of generating chimeric bacterial DNA polymerases through homologous domain exchange. For instance, the segment from amino acids 292 to 423 in Taq polymerase was substituted with the sequence from amino acids 327 to 519 of *E. coli* pol I, which is also recognized as the 3'-5' exonuclease domain of the Klenow fragment. This exchange endowed the Taq polymerase with both 3'-5' exonuclease activity and proofreading capabilities (Villbrandt et al. 2000). A chimeric reverse transcriptase was constructed by fusing the 5'-3' nuclease domain of *T.* Z05 DNA polymerase from *Thermus* species Z05 with the 3'-5' exonuclease and polymerase domains of Tma polymerase from *Thermotoga maritima*. This enzyme could be utilized to perform RT-PCR for long templates in low concentration and quality instead of using a mixture of polymerase (Schönbrunner et al. 2006). Besides, a thermostable chimeric Tth-Taq DNA polymerase was constructed by fusing the N-terminal of Tth polymerase (amino acids 4 to 600) from *Thermus thermophilus* with the C-terminal of Taq DNA polymerase (amino acids 556 to 834). The resulting chimeric enzyme not only showed a fivefold increase in activity over Taq polymerase, but also displayed a much greater specificity in amplification compared to Tth polymerase, combining advantages from both original DNA polymerases (Ignatov et al. 2009). By replacing the finger and palm domains of KOD DNA polymerase from *Thermococcus kodakarensis* with those of Pfu DNA polymerase from *Pyrococcus furiosus*, the chimeric DNA polymerase exhibited processivity and thermal stability similar to those of KOD, while also possessing high fidelity derived from Pfu. The result indicated that the finger and palm domains could be the structural foundation for the fidelity of base insertion, while the thumb domain appears to be more critical for maintaining processivity (Faurholm et al. 2012).

In another case, helix N and helix P, which are critical regions of the nucleotide-binding sites in the RB69 DNA polymerase (gp43), were substituted with corresponding segments of DNA polymerase from the human cytomegalovirus (HCMV UL54). The chimeric gp43-UL54 polymerase exhibited increased sensitivity against foscarnet and acyclovir, facilitating further study to identify major determinants for the efficacy of these drugs (Tchesnokov et al. 2009).

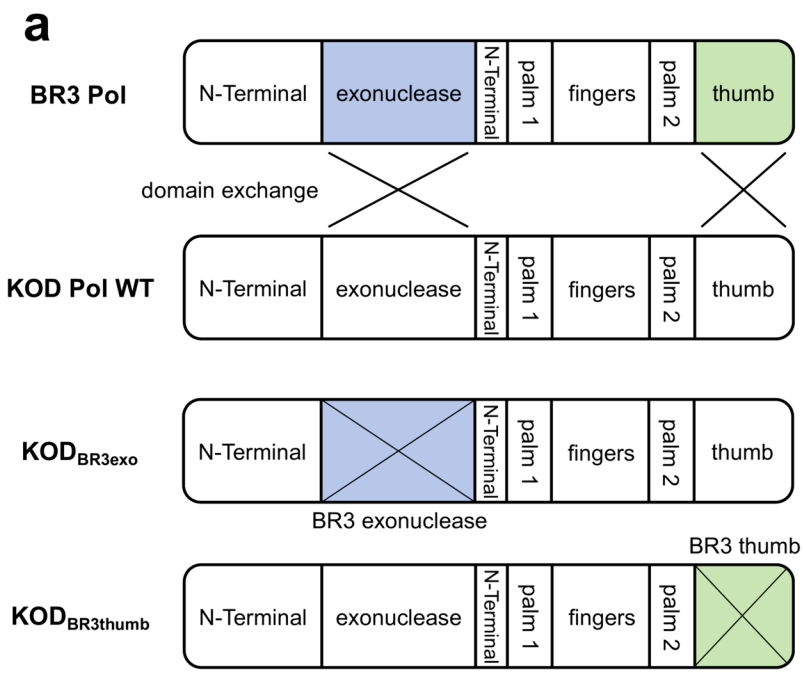
A recent study has created a halophilic version of KOD DNA polymerase based on the latest finding about BR3 polymerase, which was explored from archaea that flourish in deep-sea brine environments. The exonuclease or thumb region of KOD was substituted with its homologous domain in BR3 (Fig. 1a), leading to a threefold enhancement in the chimera polymerase's tolerance to NaCl, without compromising its thermal stability (Takahashi et al. 2018) (Fig. 1b).

The aforementioned examples serve as compelling evidence that domain exchange would be a highly effective strategy for the creation of novel characteristics in DNA polymerases (Table 2).

Integration of functional domain

Given the extensive sequence similarity among numerous DNA polymerases, a common strategy is to incorporate an extra domain with a specific function either at the terminus or in the middle of the polymerase sequence (Table 3), in addition to exchanging domains between polymerases.

Many attempts have been done to improve the processivity or other enzymatic properties of DNA polymerases. A famous example of this approach involves the insertion of a 76-amino-acid-residue thioredoxin binding domain (TBD) derived from bacteriophage T7 DNA polymerase. This domain is inserted between the helices H and H1, located at the tip of the thumb domain of the Klenow DNA polymerase. In contrast to the original Klenow DNA polymerase, the chimeric Klenow-TBD displayed an approximately eightfold higher activity due to the presence of thioredoxin, while the activity of Klenow DNA polymerase itself stayed unchanged (Bedford et al. 1997). Another case also showed



b

	K_d (nM)	
	100mM NaCl	300mM NaCl
BR3 Pol	15.1	4.4
KOD Pol WT	3.5	NA
KOD_{BR3exo}	6.5	3800
$KOD_{BR3thumb}$	12.5	221.4

Fig. 1 Chimeric KOD DNA polymerase by homologous domain exchange (Takahashi et al. 2018). **a** Schematic of BR3 Pol, KOD Pol WT and chimeric polymerases by exchanging homologous domains between KOD Pol and BR3 Pol. The exonuclease and thumb domains of BR3 Pol are highlighted in blue and green, respectively. **b** The dissociation constants K_d (nM) of BR3 Pol, KOD Pol WT, KOD_{BR3exo}, and $KOD_{BR3thumb}$ for binding to primer/template strands in buffers with either 100 mM or 300 mM NaCl. The maximum response units observed were fitted using the steady-state affinity model to calculate the equilibrium dissociation constant (K_d) for primer/template strands

Table 2 Progress of chimeric DNA polymerases by applying homologous domain exchange

Chimeric DNA polymerase	Properties optimized/applications	References
KOD + BR3 exonuclease	Increased tolerance to NaCl	(Takahashi et al. 2018)
KOD + BR3 thumb	Increased tolerance to NaCl	(Takahashi et al. 2018)
KOD + Pfu finger & palm	Increased fidelity	(Faurholm et al. 2012)
RB69 + HCMV UL54 helix P	Obtained sensitivity to foscarnet and acyclovir	(Tchesnokov et al. 2009)
RB69 + HCMV UL54 helix N	Obtained sensitivity to foscarnet and acyclovir	(Tchesnokov et al. 2009)
Taq + Klenow exonuclease	Improved processivity, 3'–5' exonuclease activity and proof-reading function	(Villbrandt et al. 2000)
Tma + T. Z05 5′-nuclease	Improved protein expression, RT-PCR	(Schönbrunner et al. 2006)
Tth + Taq	Improved activity and specificity of amplification	(Ignatov et al. 2009)

Table 3 Progress of chimeric DNA polymerases by integrating functional domain

Chimeric DNA polymerase	Properties optimized/applications	References
Klenow + T7 TBD	Improved processivity	(Bedford et al. 1997)
Taq + T3 TBD	Improved processivity and fidelity	(Davidson et al. 2003)
Sau-LF + T7 TBD	Improved processivity	(Zhai et al. 2019)
Stoffel + Pfu DBD	Improved processivity and thermal stability, increased tolerance to lactoferrin and heparin, amplification efficiency of GC-rich template	(Śpibida et al. 2018)
Bst-LF + Pab DBD	Improved processivity and thermal stability, increased tolerance to NaCl, heparin, and ethanol, *S. typhimurium* detection	(Li et al. 2023)
Gss + Pab DBD	Improved processivity, whole genome amplification	(Oscorbin et al. 2017)

that the insertion of TBD from the T3 DNA polymerase enhanced the processivity and fidelity of Taq DNA polymerase (Davidson et al. 2003). Likewise, the addition of T7 DNA polymerase TBD adjacent to the N-terminus of *Staphylococcus aureus* DNA polymerase large fragment (Sau-LF) led to a more than twofold enhancement in processivity with the addition of thioredoxin. In contrast, the wild-type Sau-LF showed no significant enhancement (Zhai et al. 2019).

DNA-binding domain (DBD), another functional domain identified in many thermophilic organisms, has been extensively investigated and utilized for creation of chimeric DNA polymerase due to the notable binding affinity for both single-stranded and double-stranded DNA. In a particular application, the DBD from DNA ligase of *Pyrococcus abyssi* was fused with the N-terminus of *Geobacillus* sp. 777 DNA polymerase (Gss). This modification led to a threefold increase in processivity, which subsequently led to approximately a fourfold increase in the quantity of DNA product during whole genome amplification (Oscorbin et al. 2017). A recently developed hybrid enzyme, generated by fusing the identical DBD from *Pyrococcus abyssi* DNA ligase with the K431E variant of *Bacillus stearothermophilus* DNA polymerase large fragment (Bst-LF) (Fig. 2a), allows for real-time loop-mediated isothermal amplification (LAMP) at temperatures as high as 73 °C. This enzyme maintains its activity after exposure to 70 °C for 8 h (Li et al. 2023) (Fig. 2b and c). Additionally, fusion of DBD from *Pyrococcus furiosus* DNA ligase with Taq Stoffel fragment—a 62.7 kDa fragment of the thermostable Taq DNA polymerase—has demonstrated approximately a threefold increase in its processivity. This hybrid polymerase exhibits a significantly higher resistance to PCR inhibitors such as heparin or lactoferrin. Remarkably, it is capable of amplifying GC-rich DNA templates even as high as 78% GC contents (Śpibida et al. 2018).

Integration of functional protein

Enormous types of proteins within cells are vital for their survival and proper functioning, such as enzymatic catalysis, structural support, transportation, signaling, and regulation of gene expression (Morris et al. 2022). Beyond polymerases themselves, proteins that can help polymerase maintain stable formation, reduce clashes within domains, or facilitate polymerase-DNA interactions may be of great potential to improve the performance of current DNA polymerases.

Significant advancements have been achieved in improved processivity of DNA polymerase through the use of functional domains as previously outlined (Table 3). However,

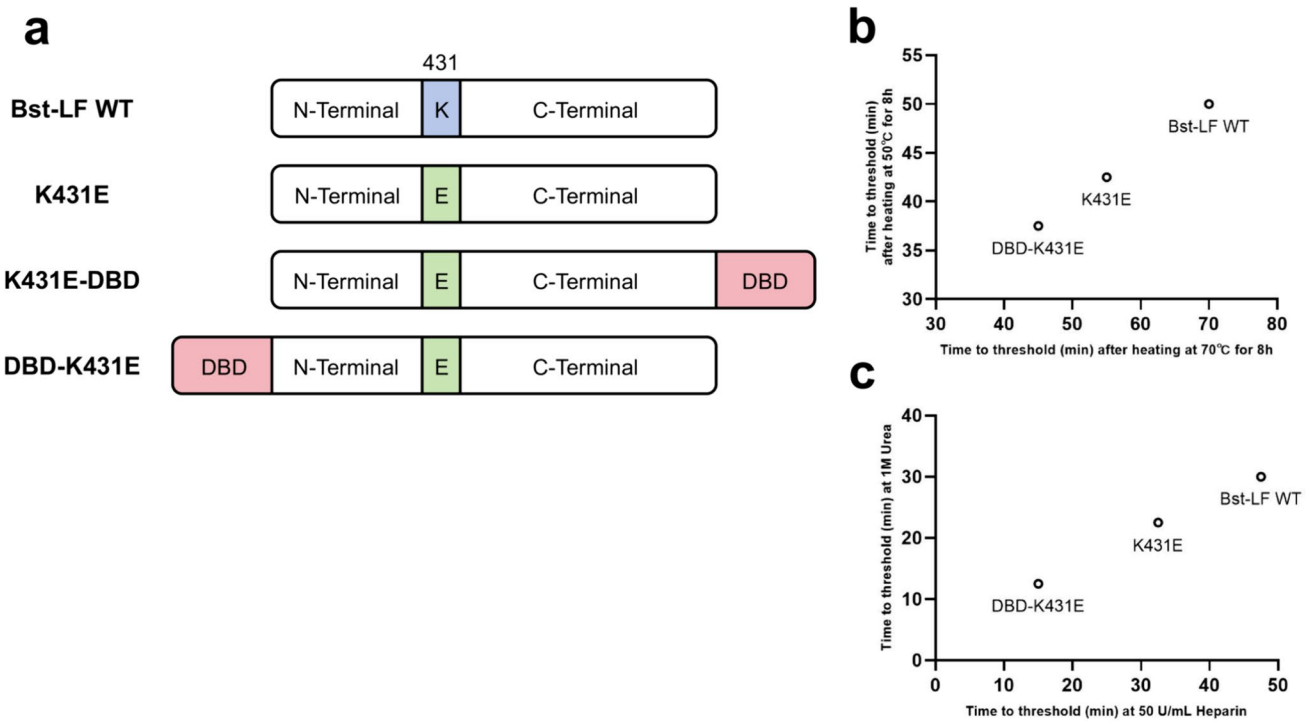


Fig. 2 Chimeric Bst DNA polymerase by integrating functional domain DBD (Li et al. 2023). **a** Schematic of Bst-LF WT, K431E variant of Bst-LF and chimeric polymerases by fusion with DBD from *Pyrococcus abyssi* DNA ligase at N-terminal or C-terminal of K431E. The lysine (K) and glutamate (E) at the 431 residue of Bst-LF and DBD domain are marked in blue, green, and red, respectively. **b** Thermal stability of Bst DNA polymerases variants. **c** Inhibitor tolerance of Bst DNA polymerase variants

this approach is restricted to a limited number of polymerases since it highly depends on specific protein–protein interactions for maintaining the stability of the polymerase. Hence, there is a pressing need for an effective strategy for a broader range of polymerases. The incorporation of additional functional protein could offer a solution, as indicated by numerous studies (Table 4).

DNA-binding protein Sso7d, originating from *Sulfolobus solfataricus*, is a monomeric, globular protein characterized by its surface rich in hydrophobic amino acids. It has drawn much attention due to its capability to bind to double-strand DNA in a sequence-independent manner. This characteristic implies that Sso7d could potentially provide a more broadly applicable approach to enhance the processivity of DNA polymerases by binding with non-specific DNA sequences (Guagliardi et al. 2002). The fusion of the heterologous Sso7d to Taq DNA polymerase, as depicted in Fig. 3a, has successfully increased its processivity without any negative effects on its catalytic activity or enzyme stability, thereby exhibiting significant benefits in PCR applications. The

Table 4 Progress of chimeric DNA polymerases by integrating functional protein

Chimeric DNA polymerase	Properties optimized/applications	References
Taq + Sso7d	Improved processivity	(Wang 2004)
Pfu + Sso7d	Improved processivity	(Wang 2004)
Stoffel + Sso7d	Improved processivity	(Wang 2004)
Sau-LF + Sso7d	Improved processivity, increased tolerance to KAc	(Wang 2004)
Tpa + Sso7d	Improved processivity	(Lee et al. 2010)
Gss + Sto7d	Improved processivity, increased tolerance to NaCl, heparin, urea, and blood	(Oscorbin et al. 2017)
M-MuLV RT + Sto7d	Improved cDNA synthesis, increased tolerance to NaCl, heparin, formamide, and blood	(Oscorbin et al. 2020)
RB69 + RB69 SSB	Improved processivity	(Sun et al. 2006)
Tzi + *Sso* SSB	Improved processivity and fidelity	(Lee et al. 2013)
Stoffel + *Neq* SSB-like protein	Improved processivity and thermal stability, increased tolerance to lactoferrin, heparin, and blood	(Olszewski et al. 2017)
Br512 + vHP47	Improved processivity and thermal stability	(Paik et al. 2023)
Br512 variants + vHP47	Improved processivity and thermal stability, increased tolerance to urea, LAMP	(Paik et al. 2022)

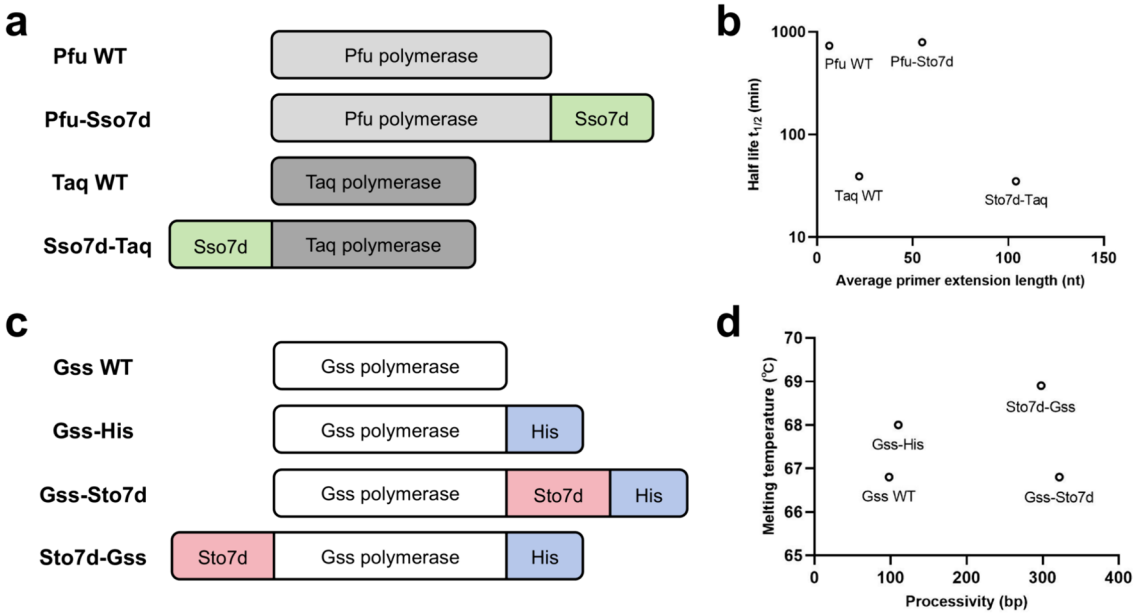


Fig. 3 Chimeric Pfu, Taq, Gss DNA polymerase by integrating functional protein Sso7d and Sto7d (Wang 2004; Oscorbin et al. 2017). **a** Schematic of Pfu WT, Taq WT and their corresponding chimeric polymerases by adding Sso7d at N-terminal or C-terminal of the polymerase. The Sso7d protein is shown in green. **b** Processivity of Pfu and its derivatives of Taq-polymerase. **c** Schematic of Gss WT and its chimeric polymerases by adding Sto7d at N-terminal and C-terminal of polymerase with the His-tag. The His-tag and Sto7d protein are shown in blue and red, respectively. **d** Thermal stability and processivity of Gss-polymerase derivatives

resulting chimeric enzymes, Sso7d-Taq and Pfu-Sso7d, displayed a ~ fivefold and ~ eightfold increase in processivity, respectively, while preserving comparable thermal stability (Wang 2004) (Fig. 3b). Furthermore, Sso7d has been successfully integrated with the Pfu polymerase and Taq Stoffel fragment (Wang 2004), as well as Tpa polymerase from *Thermococcus pacificus* and Sau-LF (Zhai et al. 2019), demonstrating its versatility in enhancing processivity for various DNA polymerases.

A similar effect has been observed in the fusion of the Sso7d counterpart from *Sulfolobus tokodaii*, known as Sto7d protein, to Gss DNA polymerase (Oscorbin et al. 2017) (Figure 3c and d), and subsequently to Moloney murine leukemia virus (M-MuLV) reverse transcriptase (Oscorbin et al. 2020). This chimeric reverse transcriptase, with Sto7d fused to its C-terminus, has shown a threefold increase in processivity during cDNA synthesis, as well as remarkable tolerance to various inhibitors, including NaCl, urea, formamide, and human whole blood (Oscorbin et al. 2020).

Potentials for using single-stranded DNA-binding protein (SSB), an auxiliary protein present at the replication fork, has garnered attention (Antony and Lohman 2019). SSB plays a crucial role in DNA replication by binding to single-stranded DNA, thereby preventing the formation of secondary structures and protecting ssDNA from exonuclease-induced degradation. Consequently, its strong DNA affinity could be beneficial for improving the DNA recognition capability of polymerases. In a study, the core domain of the C-terminus from the bacteriophage RB69 SSB was fused to the N-terminus of the corresponding DNA polymerase via a short, flexible linker Gly-Thr-Gly-Ser-Gly-Thr. The subsequent analysis revealed that this chimeric polymerase exhibited a substantial sevenfold improvement in processivity compared to the native RB69 DNA polymerase. This was evidenced by a significant increase in the average length of primer extension from 45.5 to 314.4 nucleotides, as well as maintaining the enzyme's fidelity (Sun et al. 2006). In a subsequent study, SSB from *Sulfolobus solfataricus* was fused to the N-terminus of the Tzi DNA polymerase from *Thermococcus zilligi* by a linker composed of amino acids in the sequence Gly-Ser-Gly-Gly-Val-Asp. This modification led to a notable increase in both processivity and fidelity compared to the original Tzi DNA polymerase (Lee et al. 2013).

Some proteins similar to SSB have also been identified and employed in the creation of fusion proteins. A thermally stable protein from *Nanoarchaeum equitans* showed a surprising ability to bind to ssDNA, dsDNA, or mRNA without any structure-specific preference (Olszewski et al. 2015). This magical *Neq* SSB-like protein was soon integrated at the N-terminus of the Taq Stoffel fragment, leading to a significantly elevated extension rate and processivity. It also exhibited excellent thermal stability and tolerance to PCR inhibitors including lactoferrin, heparin, and whole blood, making this chimeric DNA polymerase a promising candidate for PCR applications (Olszewski et al. 2017).

The research for potential proteins that can be aligned with the existing polymerases has never been limited to the polymerase's own components. One representative is the actin-binding protein villin, which plays a key role in the morphogenesis of microvilli, membrane protrusions of epithelial cells (Friederich et al. 1999). The C-terminus of the villin headpiece (vHP47) characterized by fast-folding kinetics due to strong hydrophobic interactions has been fused to polymerases; this fusion has shown improved amplification efficiency (Paik et al. 2022). Combined with sequence substitution predicted by machine learning, a comprehensive approach has been developed to generate polymerases that can withstand high temperatures. In this case, vHP47 was connected to several Br512 variants, and the resulting chimeric polymerase showed improved thermal stability, with a denaturation temperature up to 2.5 °C higher than the original Br512 variants. This enhancement allows isothermal amplification at 73 °C, a condition under which all commercially available Bst DNA polymerases are rendered inactive (Paik et al. 2023).

Integration of particular pattern of structure

Proteins function with precision and efficiency due to their specific three-dimensional configurations and conformations. These are determined by the organization of the helices, sheets, bulges, and residues within the protein's structure, referred to as the primary and secondary structures. Any variations in these structures may lead to a drastic change in the properties, as many studies have confirmed (Sotomayor-Vivas et al. 2022). Therefore, adding a particular structural pattern to the polymerase based on existing knowledge could improve the performance of the DNA polymerase, and some progress in this area has been achieved, as indicated in Table 5.

Helix-hairpin-helix (HhH) motif is a common structural element that plays a role in sequence-non-specific DNA binding, as exemplified by human DNA polymerase β (Pelletier et al. 1996). These motifs are typically observed as single HhH or several $(HhH)_2$ domains, such as DNA topoisomerase V (Topo V) which has been identified to contain 24 HhH motifs (Osterman and Mondragón 2022). Subsequent research has demonstrated that the HhH motifs of Topo V can improve the processivity of the Taq Stoffel fragment and Pfu DNA polymerase (Pavlov et al. 2002), φ29 DNA polymerase (de Vega et al. 2010), Bst-LF (Pavlov et al. 2012), etc. It was also found that the addition of different numbers of the HhH motif into catalytic domains of polymerase resulted in more or less an increase in thermal stability and inhibitor tolerance (Pavlov et al. 2012; Gao et al.

Table 5 Progress of chimeric DNA polymerases by integrating structural pattern

Chimeric DNA polymerase	Properties optimized/applications	References
Stoffel + Topo V HhH	Improved processivity and thermal stability, increased tolerance to NaCl	(Pavlov et al. 2002)
Pfu + Topo V HhH	Improved processivity and thermal stability, increased tolerance to NaCl	(Pavlov et al. 2002)
φ29 + Topo V HhH	Improved processivity, *B. subtilis* genomic DNA detection	(de Vega et al. 2010)
Bst-LF + Topo V HhH	Improved processivity and thermal stability	(Pavlov et al. 2012)
M-MuLV RT + 8xHis-Strep	Improved cDNA synthesis and thermal stability	(Yano et al. 2019)
φ29 + Topo V $(HhH)_2$	Improved processivity, increased tolerance to KGlc, nanopore sequencing	(Gao et al. 2021)

2021), as illustrated in Fig. 4. Even though HhHs motifs from different domains within the same polymerase shares a similar structure pattern, they showed a different effect on the thermal stability of the polymerase due to distinct partial charges at the same pH (Gao et al. 2021). Furthermore, chimeric φ29 DNA polymerase, which exhibits high salt tolerance t, has broadened its applications in genomic DNA detection (de Vega et al. 2010) and nanopore sequencing (Gao et al. 2021).

Protein tags have been widely used for facilitating the purification of the recombinant expression of various proteins. In addition, some studies indicate that these tags could also improve the enzymatic characteristics of DNA polymerases. A His-Tag with 8 histidine residues and a Streptavidin Tag were fused to either the N- or C- terminal of the M-MuLV RT, and the chimeric polymerase expressed in silkworm larvae exhibited higher thermal stability and cDNA synthesis activity at 55 °C, a temperature at which the original M-MuLV RT loses its activity (Yano et al. 2019).

General procedure to generate chimeric DNA polymerase

Subsequent paragraphs detailed the standard for creating chimeric DNA polymerases with desired properties, suitable for a range of molecular biology applications to address specific challenges or enhance established techniques. The process follows the Design-Build-Test-Learn (DBTL) cycle, as depicted in the following steps (Fig. 5).

Design of the chimeric DNA polymerase

This section includes choosing parent polymerases, identifying related domains, and deciding in which way the domains will be connected. The first step is the selection of parent polymerases. Parent DNA polymerases with desired characteristics should be identified for subsequent integration. For example, if the goal is for enhanced thermal stability of the polymerase, it would be beneficial to explore DNA

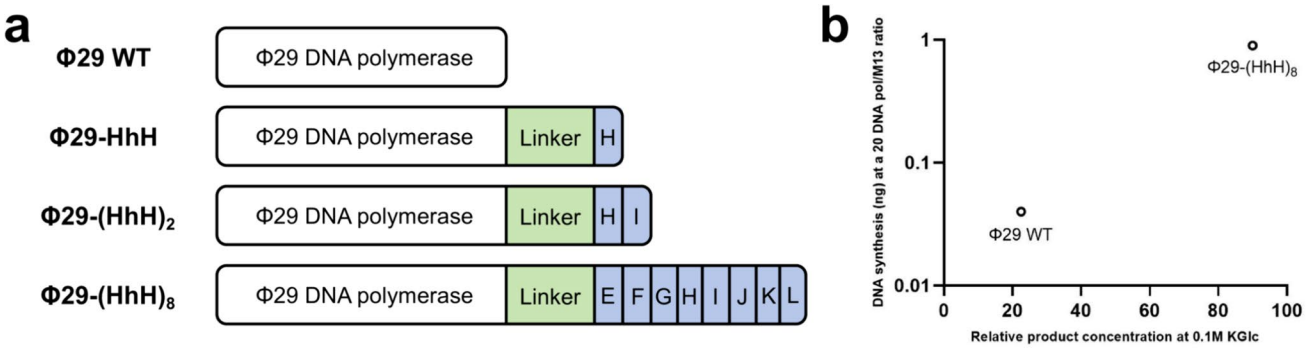


Fig. 4 Chimeric Φ29 DNA polymerase by integrating structural pattern Helix-hairpin-Helix and its repeats (Gao et al. 2021). **a** Schematic of Φ29 WT and its chimeric polymerases by adding different copies of Helix-hairpin-Helix motif at C-terminal of the wild-type polymerase. The flexible Gly-Thr-Gly-Ser-Gly-Ala linker is shown in green. The Topo V $(HhH)_2$ domain H (residues 696–751), H and I (residues 696–802), and E to L (residues 518–964) are shown in blue. **b** Processivity and tolerance of KGlc of Φ29 DNA polymerase and its derivative

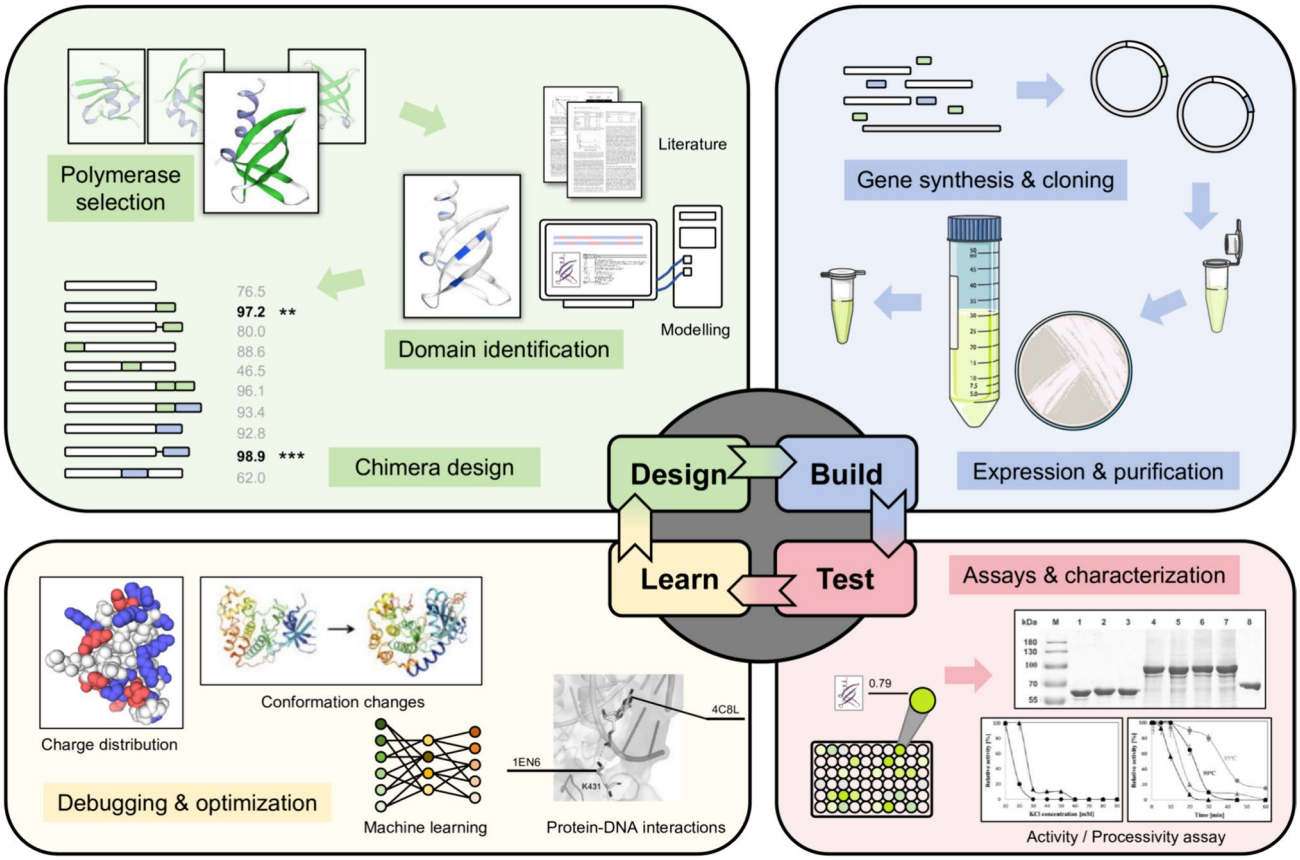


Fig. 5 Schematic of general procedure for developing novel type of chimeric DNA polymerases. Applying the Design-Build-Test-Learn cycle inspired by synthetic biology, the procedure shown in the figure outlines the most significant steps in this procedure briefly

polymerase from thermophilic organisms. This approach is consistent with the principle of Form Follows Function (FFF). Besides, selected species that are closely related evolutionarily can increase the likelihood of successful recombination due to the higher homology between domains. The second step involves pinpointing the specific areas of interest within the proteins. DNA polymerases possess similar palm domains with necessary active sites, but their thumb and finger domains show quite different, potentially accounting for their distinct characteristics (Braithwaite and Ito 1993). It is crucial to determine which regions of the parent polymerases are responsible for the desired activity. You can opt for a rational design strategy on the basis of published literature, or, alternatively, employ a semi-rational design strategy with the help of computational models. The third step is the recombination design. This step is to decide how to integrate those domains in a manner that ensures they are organized in a structurally and functionally compatible way. Optional methods include tandem fusion, linker fusion, and domain insertion. Tandem fusion involves linking domains sequentially, either at N or C terminus. However, this method is not universally effective, given that the N- or C-termini of the peptide often play a pivotal role in achieving the desired protein conformation (Patel et al. 2022). Consequently, a linker is typically required to ensure adequate spacing between domains. Flexible or helical linkers, which are rich in small or hydrophilic amino acids, facilitate interactions between domains. For instance, the most widely used flexible linker is composed of a sequence of $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$, and Gly-Thr-Gly-Ser-Gly-Thr or Gly-Ser-Gly-Gly-Val-Asp was used in aforementioned cases (Sun et al. 2006; Lee et al. 2013; Arai 2021). In contrast, rigid linker preserves the distance between domains through the use of helical structures or prolines residues. Typical motifs include the sequence $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_n$ and the pattern $(aa\text{-}Pro)_n$, as summarized in a review (Patel et al. 2022). Domain insertion was carried out in a few cases (Bedford et al. 1997), probably due to the challenge of identifying a suitable ligation site within a domain.

Build up the chimeric DNA polymerase

The chimeric gene is synthesized using molecular biology techniques including restriction digestion and ligation, overlap

extension PCR, and Gibson assembly. Then, it is cloned into an appropriate expression vector for expression and purification. The recombinant plasmid is transformed into suitable host cells, such as *Escherichia coli* or *Pichia pastoris*. Cells are cultured in an appropriate medium and polymerase protein is induced to express. Then, the chimeric DNA polymerase is purified from cell extracts using protein purification techniques, such as centrifugation, dialysis, and chromatography. Note that it may take several trials to decide the most efficient host and cloning method, but carefulness is always needed to maintain the activity of the polymerase as it is crucial for following characterization.

Test the characteristics of the chimeric DNA polymerase

The successful expression and purification of the chimeric DNA polymerase should be confirmed using techniques like SDS-PAGE and western blotting. The chimeric DNA polymerase can be characterized regarding its biochemical properties including activity, thermal stability, processivity, and fidelity. Briefly, the processivity of the polymerase is evaluated in single-stranded primer synthesis extension (Perumal 2023), and the activity of the DNA polymerase is monitored in double-stranded DNA synthesis with fluorescence dye (Yano et al. 2019) or radiolabeled nucleotide (Oscorbin et al. 2022). Thermal stability or resistance to inhibitors is tested by comparing the activity of the chimeric polymerase with the wild type in a gradient of temperature or some chemicals (Li et al. 2023). Note that different assay methods may result in contradicting conclusions as data might not be described in the same way (Oscorbin et al. 2017; Pavlov et al. 2012); thus, protocols and parameters, which may be found in the method section of published works in literature, should be confirmed carefully.

Learn from the result for debugging and optimization

If the initial design and construct fail to get the anticipated characteristics, check if there is anything wrong with the expression conditions that inactivated the chimeric DNA polymerase. Subsequent efforts involve altering some design, such as domain boundaries, linker sequences, homology of parent polymerases, or alleviating steric hindrance. Recent reports have also provided some novel points of view to deeply understand what makes a successful design, including analysis of charge distribution on the surface of the protein (Paik et al. 2022), analysis of the structure of the polymerase-DNA complex (Li et al. 2023), and the manipulation of conformation changes of the polymerase (Antony and Lohman 2019; Evans et al. 2022). Machine learning has been used to assist in evaluating the performance of the chimeras as well (Paik et al. 2023).

Challenges and perspectives

The numerous examples previously discussed have demonstrated the feasibility of optimizing DNA polymerases through the strategy of creating chimeric polymerases. However, there are still several challenges which are restricting the broader application of this approach.

Predicting the domains responsible for certain properties from parent polymerases can be challenging in the absence of a comprehensive understanding of the structure and the interactions between the domains. This endeavor necessitates insights from the field of structural biology, as well as the exploration of novel polymerases derived from organisms that inhabit extreme environments with high or low temperature, zero gravity, or strong radiation. Such efforts are crucial for further expanding the library of polymerase available for study (Dorawa et al. 2022; Ahlqvist et al. 2022).

Another important factor is the unpredictable interactions when various proteins or domains are fused with existing DNA polymerase. Identifying the most suitable chimeric polymerases from so many possibilities can be time-consuming and resource-intensive, especially when we lack the understanding of the factors that confer the desired characteristics. But there is an encouraging development that artificial intelligence (AI) powered by machine learning–based can prominently facilitate determining trials with the greatest potential by using computing models. It has an innate ability to uncover subtle relationships that may be imperceptible to human analysis, by processing vast amounts of data (Ferruz et al. 2023; Kouba et al. 2023).

It is also vital to consider the structural compatibility of the chimeric polymerase. The fusion of domains from different polymerases can lead to steric clashes or voids, potentially compromising the stability or activity of the chimeric polymerase to some extent. Hence, the design of a proper linker with optimal length and sequence to balance flexibility and rigidity of the chimera is highly significant (Patel et al. 2022; Huang et al. 2021). Moreover, charge engineering, which involves analyzing the effect of the surface charges on protein domains, may yield unexpected results (Paik et al. 2022; Martínez Del Río et al. 2023).

In some instances, fusing genes of the domains before translation, as mentioned earlier, may lead to detrimental domain interactions or reduced protein expression (Yu et al. 2015). To address this, a post-translational fusion strategy may be applied. This post-translational conjugation technique by fusing protein after ribosomal translation has also been reported as effective in some cases, which was summarized in a recent review by Taylor et al. (2022). Although this approach has not yet been tested with DNA polymerases, it presents an intriguing opportunity and is worth a try.

Despite facing these challenges, progress in the fields of protein engineering, structural biology, molecular evolution,

and AI has empowered researchers to surmount these obstacles and develop chimeric DNA polymerases that exhibit innovative characteristics and improved performance. In conclusion, the ongoing research in these areas is anticipated to deepen our understanding of DNA polymerases. Consequently, this intensive knowledge will lay the groundwork for a more extensive spectrum of applications across diverse domains.

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Declarations

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References

Ahlqvist J, Linares-Pastén JA, Jasilionis A, Welin M, Håkansson M, Svensson LA, Wang L, Watzlawick H, Ævarsson A, Friðjónsson ÓH, Hreggviðsson GÓ, Ketelsen Striberny B, Glomsaker E, Lanes O, Al-Karadaghi S, Nordberg Karlsson E (2022) Crystal structure of DNA polymerase I from Thermus phage G20c. Acta Crystallogr D Struct Biol 78:1384–1398. https://doi.org/10.1107/S2059798322009895

Ahmad S, Ali SF, Iftikhar S, Rashid N (2023) Engineering a DNA polymerase from Pyrobaculum calidifontis for improved activity, processivity and extension rate. Int J Biol Macromol 233:123545. https://doi.org/10.1016/j.ijbiomac.2023.123545

Akram F, Shah FI, Ibrar R, Fatima T, Haq IU, Naseem W, Gul MA, Tehreem L, Haider G (2023) Bacterial thermophilic DNA polymerases: a focus on prominent biotechnological applications. Anal Biochem 671:115150. https://doi.org/10.1016/j.ab.2023.115150

Antony E, Lohman TM (2019) Dynamics of *E. coli* single stranded DNA binding (SSB) protein-DNA complexes. Semin Cell Dev Biol 86:102–111. https://doi.org/10.1016/j.semcdb.2018.03.017

Arai R (2021) Design of helical linkers for fusion proteins and protein-based nanostructures. Methods Enzymol 647:209–230. https://doi.org/10.1016/bs.mie.2020.10.003

Bedford E, Tabor S, Richardson CC (1997) The thioredoxin binding domain of bacteriophage T7 DNA polymerase confers processivity on *Escherichia coli* DNA polymerase I. Proc Natl Acad Sci USA 94:479–484. https://doi.org/10.1073/pnas.94.2.479

Bhadra S, Maranhao AC, Paik I, Ellington AD (2020) One-enzyme reverse transcription qPCR using Taq DNA polymerase. Biochemistry 59. https://doi.org/10.1021/acs.biochem.0c00778

Braithwaite DK, Ito J (1993) Compilation, alignment, and phylogenetic relationships of DNA polymerases. Nucleic Acids Res 21:787–802. https://doi.org/10.1093/nar/21.4.787

Chen T, Hongdilokkul N, Liu Z, Adhikary R, Tsuen SS, Romesberg FE (2016) Evolution of thermophilic DNA polymerases for the recognition and amplification of C2'-modified DNA. Nat Chem 8:556–562. https://doi.org/10.1038/nchem.2493

Dangerfield TL, Johnson KA (2023) Kinetics of DNA strand transfer between polymerase and proofreading exonuclease active sites regulates error correction during high-fidelity replication. J Biol Chem 299:102744. https://doi.org/10.1016/j.jbc.2022.102744

Dangerfield TL, Kirmizialtin S, Johnson KA (2022) Substrate specificity and proposed structure of the proofreading complex of T7 DNA polymerase. J Biol Chem 298:101627. https://doi.org/10.1016/j.jbc.2022.101627

Davidson JF, Fox R, Harris DD, Lyons-Abbott S, Loeb LA (2003) Insertion of the T3 DNA polymerase thioredoxin binding domain enhances the processivity and fidelity of Taq DNA polymerase. Nucleic Acids Res 31:4702–4709. https://doi.org/10.1093/nar/gkg667

de Vega M, Lázaro JM, Mencía M, Blanco L, Salas M (2010) Improvement of φ29 DNA polymerase amplification performance by fusion of DNA binding motifs. Proc Natl Acad Sci USA 107:16506–16511. https://doi.org/10.1073/pnas.1011428107

Dorawa S, Werbowy O, Plotka M, Kaczorowska A-K, Makowska J, Kozlowski LP, Fridjonsson OH, Hreggvidsson GO, Aevarsson A, Kaczorowski T (2022) Molecular Characterization of a DNA polymerase from *Thermus thermophilus* MAT72 phage vB_Tt72: a novel type-a family enzyme with strong proofreading activity. Int J Mol Sci 23:7945. https://doi.org/10.3390/ijms23147945

Evans GW, Craggs T, Kapanidis AN (2022) The rate-limiting step of DNA synthesis by DNA polymerase occurs in the fingers-closed conformation. J Mol Biol 434:167410. https://doi.org/10.1016/j.jmb.2021.167410

Faurholm B, McEwan P, Bourn W, Rush G (2012) Chimeric dna polymerases. Google Patents. https://patents.google.com/patent/US20120115188A1/en. Accessed 21 Feb 2024

Ferruz N, Heinzinger M, Akdel M, Goncearenco A, Naef L, Dallago C (2023) From sequence to function through structure: deep learning for protein design. Comput Struct Biotechnol J 21:238–250. https://doi.org/10.1016/j.csbj.2022.11.014

Friederich E, Vancompernolle K, Louvard D, Vandekerckhove J (1999) Villin function in the organization of the actin cytoskeleton: correlation of in vivo effects to its biochemical activities in vitro*. J Bio Chem 274:26751–26760. https://doi.org/10.1074/jbc.274.38.26751

Gao YP, He Y, Chen LY, Liu X, Ivanov I, Yang XR, Tian H (2021) Chimeric Phi29 DNA polymerase with helix-hairpin-helix motifs shows enhanced salt tolerance and replication performance. Microb Biotechnol 14:1642–1656. https://doi.org/10.1111/1751-7915.13830

Gardner AF, Jackson KM, Boyle MM, Buss JA, Potapov V, Gehring AM, Zatopek KM, Corrêa IR, Ong JL, Jack WE (2019)

Therminator DNA polymerase: modified nucleotides and unnatural substrates. Front Mol Biosci 6:28. https://doi.org/10.3389/fmolb.2019.00028

Guagliardi A, Cerchia L, Rossi M (2002) The Sso7d protein of *Sulfolobus solfataricus*: in vitro relationship among different activities. Archaea 1:87–93. https://doi.org/10.1155/2002/313147

Huang Z, Zhang C, Xing X-H (2021) Design and construction of chimeric linker library with controllable flexibilities for precision protein engineering. Methods Enzymol 647:23–49. https://doi.org/10.1016/bs.mie.2020.12.004

Ignatov K, Kramarov V, Billingham S (2009) Chimeric dna polymerase. Google Patents. https://patents.google.com/patent/US20090209005A1/en. Accessed 21 Feb 2024

Kouba P, Kohout P, Haddadi F, Bushuiev A, Samusevich R, Sedlar J, Damborsky J, Pluskal T, Sivic J, Mazurenko S (2023) Machine learning-guided protein engineering. ACS Catal 13:13863–13895. https://doi.org/10.1021/acscatal.3c02743

Kuznetsova AA, Kuznetsov NA (2023) Direct enzyme engineering of B family DNA polymerases for biotechnological approaches. Bioengineering (Basel) 10:1150. https://doi.org/10.3390/bioengineering10101150

Kuznetsova AA, Fedorova OS, Kuznetsov NA (2022) Structural and molecular kinetic features of activities of DNA polymerases. Int J Mol Sci 23:6373. https://doi.org/10.3390/ijms23126373

Lee JI, Cho SS, Kil E-J, Kwon S-T (2010) Characterization and PCR application of a thermostable DNA polymerase from *Thermococcus pacificus*. Enzyme Microb Technol 47:147–152. https://doi.org/10.1016/j.enzmictec.2010.06.003

Lee JE, Potter RJ, Mandelman D (2013) Ssb - polymerase fusion proteins. Google Patents. https://patents.google.com/patent/EP1934372B1/en. Accessed 22 Feb 2024

Li JX, Li Y, Li YM, Ma Y, Xu W, Wang JF (2023) An enhanced activity and thermostability of chimeric Bst DNA polymerase for isothermal amplification applications. Appl Microbiol Biot. https://doi.org/10.1007/s00253-023-12751-6

Lisova AE, Baranovskiy AG, Morstadt LM, Babayeva ND, Tahirov TH (2022) Human DNA polymerase α has a strong mutagenic potential at the initial steps of DNA synthesis. Nucleic Acids Res 50:12266–12273. https://doi.org/10.1093/nar/gkac1101

Ma Y, Zhang B, Wang M, Ou Y, Wang J, Li S (2016) Enhancement of polymerase activity of the large fragment in DNA polymerase I from *Geobacillus stearothermophilus* by site-directed mutagenesis at the active site. Biomed Res Int 2016:2906484. https://doi.org/10.1155/2016/2906484

Martínez Del Río J, López-Carrobles N, Mendieta-Moreno JI, Herrera-Chacón Ó, Sánchez-Ibáñez A, Mendieta J, Menéndez-Arias L (2023) Charge engineering of the nucleic acid binding cleft of a thermostable HIV-1 reverse transcriptase reveals key interactions and a novel mechanism of RNase H inactivation. J Mol Biol 435:168219. https://doi.org/10.1016/j.jmb.2023.168219

Morris R, Black KA, Stollar EJ (2022) Uncovering protein function: from classification to complexes. Essays Biochem 66:255. https://doi.org/10.1042/EBC20200108

Motré A, Li Y, Kong H (2008) Enhancing helicase-dependent amplification by fusing the helicase with the DNA polymerase. Gene 420:17–22. https://doi.org/10.1016/j.gene.2008.04.017

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H (1986) Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol 51(Pt 1):263–273. https://doi.org/10.1101/sqb.1986.051.01.032

Nikoomanzar A, Chim N, Yik EJ, Chaput JC (2020) Engineering polymerases for applications in synthetic biology. Q Rev Biophys 53:e8. https://doi.org/10.1017/S0033583520000050

Olszewski M, Balsewicz J, Nowak M, Maciejewska N, Cyranka-Czaja A, Zalewska-Piątek B, Piątek R, Kur J (2015) Characterization of a single-stranded DNA-binding-like protein from Nanoarchaeum equitans–a nucleic acid binding protein with broad substrate specificity. PLoS ONE 10:e0126563. https://doi.org/10.1371/journal.pone.0126563

Olszewski M, Śpibida M, Bilek M, Krawczyk B (2017) Fusion of Taq DNA polymerase with single-stranded DNA binding-like protein of *Nanoarchaeum equitans* - expression and characterization. PLoS ONE 12:e0184162. https://doi.org/10.1371/journal.pone.0184162

Ordóñez CD, Redrejo-Rodríguez M (2023) DNA polymerases for whole genome amplification: considerations and future directions. Int J Mol Sci 24:9331. https://doi.org/10.3390/ijms24119331

Oscorbin IP, Belousova EA, Boyarskikh UA, Zakabunin AI, Khrapov EA, Filipenko ML (2017) Derivatives of Bst-like Gss-polymerase with improved processivity and inhibitor tolerance. Nucleic Acids Res 45:9595–9610. https://doi.org/10.1093/nar/gkx645

Oscorbin IP, Wong PF, Boyarskikh UA, Khrapov EA, Filipenko ML (2020) The attachment of a DNA-binding Sso7d-like protein improves processivity and resistance to inhibitors of M-MuLV reverse transcriptase. FEBS Lett 594:4338–4356. https://doi.org/10.1002/1873-3468.13934

Oscorbin IP, Novikova LM, Filipenko ML (2022) Comparison of reverse transcriptase (RT) activities of various M-MuLV RTs for RT-LAMP assays. Biology (Basel) 11:1809. https://doi.org/10.3390/biology11121809

Osterman A, Mondragón A (2022) Structures of topoisomerase V in complex with DNA reveal unusual DNA-binding mode and novel relaxation mechanism. Elife 11:e72702. https://doi.org/10.7554/eLife.72702

Paik I, Bhadra S, Ellington AD (2022) Charge engineering improves the performance of Bst DNA polymerase fusions. ACS Synth Biol 11:1488–1496. https://doi.org/10.1021/acssynbio.1c00559

Paik I, Ngo PHT, Shroff R, Diaz DJ, Maranhao AC, Walker DJF, Bhadra S, Ellington AD (2023) Improved Bst DNA polymerase variants derived via a machine learning approach. Biochemistry 62:410–418. https://doi.org/10.1021/acs.biochem.1c00451

Patel DK, Menon DV, Patel DH, Dave G (2022) Linkers: a synergistic way for the synthesis of chimeric proteins. Protein Expr Purif 191:106012. https://doi.org/10.1016/j.pep.2021.106012

Pavlov AR, Belova GI, Kozyavkin SA, Slesarev AI (2002) Helix-hairpin-helix motifs confer salt resistance and processivity on chimeric DNA polymerases. Proc Natl Acad Sci USA 99:13510–5. https://doi.org/10.1073/pnas.202127199

Pavlov AR, Pavlova NV, Kozyavkin SA, Slesarev AI (2012) Cooperation between catalytic and DNA binding domains enhances thermostability and supports DNA synthesis at higher temperatures by thermostable DNA polymerases. Biochemistry 51:2032–2043. https://doi.org/10.1021/bi2014807

Pelletier H, Sawaya MR, Wolfle W, Wilson SH, Kraut J (1996) A structural basis for metal ion mutagenicity and nucleotide selectivity in human DNA polymerase beta. Biochemistry 35:12762–12777. https://doi.org/10.1021/bi9529566

Perumal SK (2023) A real-time fluorescent gp32 probe-based assay for monitoring single-stranded DNA-dependent DNA processing enzymes. Biochem Biophys Rep 35:101518. https://doi.org/10.1016/j.bbrep.2023.101518

Schönbrunner NJ, Fiss EH, Budker O, Stoffel S, Sigua CL, Gelfand DH, Myers TW (2006) Chimeric thermostable DNA polymerases with reverse transcriptase and attenuated 3'-5' exonuclease activity. Biochemistry 45:12786–12795. https://doi.org/10.1021/bi0609117

Sotomayor-Vivas C, Hernández-Lemus E, Dorantes-Gilardi R (2022) Linking protein structural and functional change to mutation using amino acid networks. PLoS ONE 17:e0261829. https://doi.org/10.1371/journal.pone.0261829

Śpibida M, Krawczyk B, Olszewski M, Kur J (2017) Modified DNA polymerases for PCR troubleshooting. J Appl Genetics 58:133–142. https://doi.org/10.1007/s13353-016-0371-4

Śpibida M, Krawczyk B, Zalewska-Piątek B, Piątek R, Wysocka M, Olszewski M (2018) Fusion of DNA-binding domain of *Pyrococcus furiosus* ligase with Taq Stoffel DNA polymerase as a useful tool in PCR with difficult targets. Appl Microbiol Biotechnol 102:713–721. https://doi.org/10.1007/s00253-017-8560-6

Sun SY, Geng L, Shamoo Y (2006) Structure and enzymatic properties of a chimeric bacteriophage RB69 DNA polymerase and single-stranded DNA binding protein with increased processivity. Proteins 65:231–238. https://doi.org/10.1002/prot.21088

Takahashi M, Takahashi E, Joudeh LI, Marini M, Das G, Elshenawy MM, Akal A, Sakashita K, Alam I, Tehseen M, Sobhy MA, Stingl U, Merzaban JS, Di Fabrizio E, Hamdan SM (2018) Dynamic structure mediates halophilic adaptation of a DNA polymerase from the deep-sea brines of the Red Sea. FASEB J 32:3346–3360. https://doi.org/10.1096/fj.201700862RR

Taylor RJ, Geeson MB, Journeaux T, Bernardes GJL (2022) Chemical and enzymatic methods for post-translational protein-protein conjugation. J Am Chem Soc 144:14404–14419. https://doi.org/10.1021/jacs.2c00129

Tchesnokov EP, Obikhod A, Schinazi RF, Götte M (2009) Engineering of a chimeric RB69 DNA polymerase sensitive to drugs targeting the cytomegalovirus enzyme. J Biol Chem 284:26439–26446. https://doi.org/10.1074/jbc.M109.012500

Villbrandt B, Sobek H, Frey B, Schomburg D (2000) Domain exchange: chimeras of *Thermus aquaticus* DNA polymerase, *Escherichia coli* DNA polymerase I and *Thermotoga neapolitana* DNA polymerase. Protein Eng 13:645–654. https://doi.org/10.1093/protein/13.9.645

Wang Y (2004) A novel strategy to engineer DNA polymerases for enhanced processivity and improved performance in vitro. Nucleic Acids Res 32:1197–1207. https://doi.org/10.1093/nar/gkh271

Wang L, Liang C, Wu J, Liu L, Tyo KEJ (2017) Increased processivity, misincorporation, and nucleotide incorporation efficiency in *Sulfolobus solfataricus* Dpo4 thumb domain mutants. Appl Environ Microbiol 83:e01013-e1017. https://doi.org/10.1128/AEM.01013-17

Wang G, Du Y, Ma X, Ye F, Qin Y, Wang Y, Xiang Y, Tao R, Chen T (2022) Thermophilic nucleic acid polymerases and their application in xenobiology. Int J Mol Sci 23:14969. https://doi.org/10.3390/ijms232314969

Yano T, Lee JM, Xu J, Morifuji Y, Masuda A, Hino M, Morokuma D, Fujita R, Takahashi M, Kusakabe T, Mon H (2019) Expression of the thermostable Moloney murine leukemia virus reverse transcriptase by silkworm-baculovirus expression system. J Asia Pac Entomol 22:453–457. https://doi.org/10.1016/j.aspen.2019.02.008

Yu K, Liu C, Kim B-G, Lee D-Y (2015) Synthetic fusion protein design and applications. Biotechnol Adv 33:155–164. https://doi.org/10.1016/j.biotechadv.2014.11.005

Zahurancik WJ, Suo Z (2020) Kinetic investigation of the polymerase and exonuclease activities of human DNA polymerase ε holoenzyme. J Biol Chem 295:17251–17264. https://doi.org/10.1074/jbc.RA120.013903

Zhai B, Chow J, Cheng Q (2019) Two approaches to enhance the processivity and salt tolerance of *Staphylococcus aureus* DNA polymerase. Protein J 38:190–198. https://doi.org/10.1007/s10930-019-09818-7