Recombinant Expression of *Taq* DNA Polymerase Using pBluescript SK(+) and Chromatography-Free Purification: A Cost-Effective Strategy for Low-Resource Laboratories

ABSTRACT

Taq DNA polymerase is a cornerstone of molecular biology, yet its production typically relies on proprietary vectors and affinity-based purification systems, limiting access in educational and resource-constrained environments. In this study, we demonstrate the first experimentally validated expression of thermostable *Taq* DNA polymerase using the widely available pBluescript SK(+) cloning vector—traditionally used for blue-white screening—as a protein expression platform in *E. coli*. Expression was paired with a simplified purification strategy involving heat denaturation and ammonium sulfate precipitation. This chromatography-free strategy requires no affinity tags or proprietary reagents and yielded enzymatically active Taq DNA polymerase with a mean purity of 90.1 ± 2.1%, as determined by SDS-PAGE densitometry. Functional validation through PCR amplification and thermostability assays confirmed that the enzyme is functionally equivalent to commercial Ex Taq (Takara Bio). We also assessed the workflow's scalability, reproducibility, and compatibility with basic laboratory infrastructure. Compared to commercial enzyme, our protocol reduced the cost-per-reaction by 70–85%, depending on enzyme input. These findings demonstrate that pBluescript SK(+)—typically reserved for cloning—can support effective recombinant protein production when paired with optimized conditions. This study introduces a low-cost, open-access alternative to proprietary enzyme production, offering a viable solution for decentralized, budget-conscious molecular biology training in teaching labs and under-resourced research environments.

Keywords: Taq DNA polymerase, protein expression, pBluescript SK(+), heat purification, ammonium sulfate precipitation, low-cost biotechnology, chromatography-free purification, resource-limited laboratories

1. INTRODUCTION

Thermostable DNA polymerase from *Thermus aquaticus* (*Taq*) has been one of the most impactful enzymes in molecular biology, enabling the polymerase chain reaction (PCR) through its ability to catalyze DNA synthesis at high temperatures (Chien et al., 1976; Saiki et al., 1988). Its heat resistance allows for repeated denaturation of DNA strands without enzyme degradation, an essential feature for reliable PCR amplification (Saiki et al., 1988). Current industrial-scale *Taq* DNA polymerase production typically relies on high-expression systems such as pET vectors with T7 promoters in *Escherichia coli* and purification via affinity chromatography using His-tags and nickel-based matrices (Engelke et al., 1990, Olszewski et al., 2017; Samman et al., 2023). These systems offer high yields but come with high costs—both in terms of proprietary vectors and specialized equipment, making them inaccessible to many teaching laboratories and under-funded academic programs (Engelke et al., 1990, Olszewski et al., 2017; Samman et al., 2023). However, hands-on recombinant protein work is often restricted in teaching laboratories due to cost and infrastructure constraints, despite its pedagogical value in training students in core molecular biology techniques (Gomes et al., 2022).

Early studies explored simpler systems for expressing and purifying *Taq* DNA polymerase. Lawyer et al. (1989) cloned the *Taq* DNA polymerase gene into general cloning vectors and demonstrated functional expression in *E. coli* (Lawyer et al., 1989). Shortly after, Engelke et al. (1990) purified active enzyme using only heat-denaturation, indicating feasibility but with limited optimization (Engelke et al., 1990). These foundational Taq protocols, while groundbreaking, were not easily transferable to undergraduate or resource-limited teaching labs, often requiring extensive troubleshooting and specialized equipment to achieve reproducible results (Carroll and Keller, 2014). More recent workflows continued to prioritize engineered variants—such as Stoffel fragments and fusion proteins—to enhance specificity, processivity, or inhibitor resistance; examples include fusion constructs with thermostable Single-Stranded DNA-Binding (SBB) proteins and polymerase variants optimized for hot-start PCR (Olszewski et al., 2017; Samman et al., 2023). Although not used in this study, codon-optimized systems under the control of a rhaBAD promoter have achieved yields of approximately 8.5 mg/L culture—while still relying on affinity resins and chromatographic purification (Laksmi et al., 2024).

Simplified purification strategies combining heat denaturation with ammonium sulfate precipitation have been shown to yield active Taq DNA polymerase with >90% purity (Pluthero, 1993). Although heat-based purification strategies are effective in principle, they have rarely been adapted for cost-sensitive or infrastructure-limited instructional laboratories, which often lack chromatographic equipment and the technical support needed for reproducible results (Carroll and Keller, 2014; Bhadra et al., 2022). Moreover, such strategies seldom integrate fully open-access cloning systems or provide detailed, reproducible strategies suitable for low-resource environments. In this context, a clear knowledge gap remains: the need for a streamlined, reproducible, chromatography-free purification that empowers academic and teaching labs without compromising enzymatic performance.

Despite widespread access to pBluescript SK(+), it has not been validated as a viable platform for recombinant protein production—limiting its use to cloning. Moreover, existing expression and purification workflows often rely on T7-driven vectors, engineered tags, or chromatography, placing them out of reach for many instructional labs. As a result, there remains

a lack of simple, low-cost, reproducible workflows that integrate widely available vectors with functional protein expressions.

This study addresses that gap by demonstrating the feasibility of using pBluescript SK(+), a classical high-copy cloning vector, as an expression system for a thermostable enzyme. We inserted the *Taq* DNA polymerase gene between *EcoRI* and *SalI* sites under the *lac* promoter, expressed it in *E. coli*, and employed a purification strategy using only heat-denaturation and ammonium sulfate precipitation. We systematically evaluated multiple aspects of the workflow, including the yield of purified enzyme, purity assessed by SDS-PAGE, thermostability, and PCR performance in comparison to commercial Taq DNA polymerase. A cost analysis was also performed, along with identification of key troubleshooting steps to ensure reproducibility. These assessments confirmed that the strategy consistently yields Taq DNA polymerase with $90.1 \pm 2.1\%$ purity and PCR fidelity comparable to commercial formulations. Notably, the workflow reduces reagent costs by approximately 65% relative to commercial Ex Taq (Takara Bio) and remains fully compatible with standard laboratory equipment, eliminating the need for affinity chromatography or specialized systems. By repurposing a classical cloning vector within a streamlined, expression and chromatography-free purification strategy, this work offers a practical, scalable, and affordable alternative tailored to the needs of undergraduate and resource-limited laboratory settings. It bridges the gap between proprietary commercial systems and the realities of academic teaching environments, promoting decentralized biotechnology training while reaffirming the utility of foundational molecular biology tools.

2. MATERIALS AND METHODS

2.1 PCR amplification and gel confirmation of the *Taq* DNA polymerase gene

Genomic DNA was extracted from *Thermus aquaticus* strain ATCC 25104 (Brock and Freeze, 1969) (American Type Culture Collection, Manassas, VA, USA) using the DNeasy Blood & Tissue Kit (Fisher Scientific, Waltham, MA, USA). The *Taq* DNA polymerase gene was amplified using gene-specific primers incorporating restriction sites for directional cloning. The forward primer (5′-CGCGAA**GTCGAC**ATGAGGGGGATGCTGCCCCTC-3′) included a SalI site (**GTCGAC**) and an overhang (CGCGAA); the reverse primer (5′-AAGCTT**GAATTC**TTAGGTGGTATCACTCCTTGGCGG-3′) included an EcoRI site (**GAATTC**) and an overhang (AAGCTT). Primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA).

PCR reactions (50 µL) included 10 ng genomic DNA, 0.5 µM of each primer, 200 µM dNTPs, 1× Ex Taq buffer (Mg^{2+} plus), and 1.25 U Ex Taq DNA Polymerase (Takara Bio USA, San Jose, CA, USA; Cat. #RR001A). Thermocycling conditions were: 95 °C for 3 min; 30 cycles of 95 °C for 30 s, 68 °C for 30 s, and 72 °C for 2.5 min; final extension at 72 °C for 7 min. Amplicons were resolved on 1% agarose gels in 1× TAE containing ethidium bromide alongside 1 Kb DNA ladder (NEB, Ipswich, MA, USA), and visualized using a Gel Doc XR+ System (Bio-Rad, Hercules, CA, USA). A 2523 bp (2499 bp gene + 24 bp overhangs with restriction sites) band confirmed successful amplification.

2.2 Cloning, transformation, colony PCR, and sequencing

The PCR product and pBluescript SK(+) vector were double-digested with SalI-HF and EcoRI-HF (New England Biolabs, Ipswich, MA, USA) in CutSmart buffer at 37 °C for 2 h. Digested DNA fragments were gel-purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research, Irvine, CA, USA). Ligation was performed overnight at 16 °C using T4 DNA Ligase (NEB, Ipswich, MA, USA), and 5 µL of ligation mix was transformed into 100 µL chemically competent *E. coli* DH5α cells (Made in-house) using a standard heat-shock method.

Transformants were plated on LB agar supplemented with 100 µg/mL ampicillin, 40 µg/mL X-gal, and 0.5 mM IPTG (GoldBio, St. Louis, MO, USA), and incubated at 37 °C overnight. White colonies were screened by colony PCR using the M13 forward primer (5′-GTAAAACGACGGCCAGT-3′) and a *Taq* DNA polymerase gene-specific reverse primer (5′-GGCGGGAAGCCCTAGCTCGTC-3′). A ~1.5 kb product confirmed insertion. Plasmid DNA was isolated using the QIAprep Spin Miniprep Kit (Qiagen, Germantown, MD, USA), and Sanger sequencing (Eurofins Genomics) was performed using T7 (5′-TAATACGACTCACTATAGGG-3′) and T3 (5′-ATTAACCCTCACTAAAGGGA-3′) primers. Sequences were aligned to the reference gene (GenBank: J04639.1) using SnapGene v6.0.2 to verify orientation and fidelity.

2.3 Expression and purification of *Taq* DNA polymerase

The confirmed plasmid was transformed into *E. coli* BL21 (DE3) cells (Thermo Fisher, C600003). A single colony was cultured overnight in LB with 100 µg/mL ampicillin, then diluted 1:100 into 200 mL LB + ampicillin and grown to $OD_{600} \approx 0.6$. Protein expression was induced with 1 mM IPTG and continued for 6 h at 30 °C. Cells were pelleted at 4,000 × g for 20 min at 4 °C. Cell pellets were resuspended in Buffer A (500 mM Tris-HCl, pH 8.0; 50 mM glucose; 1 mM EDTA; 4 mg/mL lysozyme) and incubated 20 min at room temperature. Buffer B (10 mM Tris-HCl, pH 8.0; 50 mM KCl; 1 mM EDTA; 0.5% Triton X-100; ~0.5% IGEPAL CA-630) was added, and the lysate was incubated at 75 °C for 90 min in a hybridization oven. Following centrifugation (12,000 × g, 30 min, 4 °C), the clarified lysate was treated with ammonium sulfate to 60% saturation (390 g/L) and stirred at 4 °C for 1 h. Precipitated proteins were pelleted (12,000 × g, 30 min, 4 °C), and resuspended in cold storage buffer (50 mM Tris-HCl, pH 8.0; 150 mM NaCl; 1 mM EDTA; 0.1% Triton X-100; 1 mM DTT).

Dialysis was performed using a 10 kDa MWCO membrane (Spectra/Por 7; Repligen) against 1 L storage buffer with three buffer changes (2 h × 2, then overnight), followed by centrifugation at 15,000 × g for 20 min to remove aggregates. The supernatant was mixed with sterile-filtered 80% glycerol to a final concentration of 40%, aliquoted, and stored at –20 °C.

2.4 SDS-PAGE and purity analysis

Protein purity was assessed by SDS-PAGE using a 10% resolving gel and 4% stacking gel in a Mini-PROTEAN Tetra Cell system (Bio-Rad, Hercules, CA, USA). Samples were prepared in 2× Laemmli buffer with 5% β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA), heated at 95 °C for 5 min, and electrophoresed alongside a Thermo Scientific PageRuler Prestained Protein Ladder (Thermo Fisher Scientific, Waltham, MA, USA). Gels were stained with Bio-Safe Coomassie G-250 and imaged on a Gel Doc XR+ system. Band intensities were analyzed using Image Lab Software (v6.1; Bio-Rad). The ~94 kDa band corresponding to Taq DNA polymerase was selected as the region of interest (ROI) for each sample. Purity was calculated as the intensity of the Taq DNA polymerase band divided by the total lane intensity,

expressed as a percentage. This value reflects the relative abundance of Taq DNA polymerase within the sample. Preparations achieving ≥90% purity were classified as high quality.

2.5 Functional and thermostability validation

Protein concentration was determined by Bradford assay using bovine serum albumin (BSA) standards and absorbance at 595 nm measured with a SpectraMax M3 Microplate Reader (Molecular Devices, San Jose, CA, USA). A standard curve was generated from known concentrations of Bovine Serum Albumin (BSA), and the concentration of the purified Taq enzyme was interpolated from the linear regression equation. For functional validation, PCR amplification of an 813 bp fragment from λ DNA (10 ng) was performed in 25 µL reactions containing 0.2 mM dNTPs and 0.5 µM each of the forward (5′-GCCCGTTCTGCGAGGCGG-3′) and reverse (5′-CACGAATAGTCGGCTCAACGTGGG-3′) primers. Reactions were carried out using decreasing volumes of purified Taq polymerase enzyme (1.0, 0.8, 0.7, 0.6, 0.5, 0.4, and 0.3 µL). For comparison, a parallel set of reactions was performed using decreasing volumes of commercial Ex Taq (Takara Bio) enzyme. PCR conditions were: 95 °C for 2 min; 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; final extension at 72 °C for 7 min. Products were analyzed alongside a 1 Kb DNA ladder (NEB, Ipswich, MA, USA) on 1% agarose gel stained with ethidium bromide. Band intensities were quantified using ImageJ software by selecting regions of interest (ROIs) corresponding to the 813 bp bands. Intensities were normalized to the highest value (Ex Taq enzyme at 1.0 µL) and averaged across three independent biological replicates. Data were expressed as mean ± standard deviation, and differences between in-house and commercial enzyme intensities at each titration point were evaluated using an unpaired two-tailed t-test.

To evaluate thermostability, 5 µL aliquots of purified Taq polymerase enzyme (1 U/µL) were subjected to repeated heat stress. Since we did not directly measure nucleotide incorporation, unit equivalency for the in-house Taq enzyme was estimated by PCR titration. Specifically, we compared the amplification efficiency of serially diluted in-house enzyme with commercial Ex Taq enzyme under identical conditions and designated the volume that matched 1 U of Ex Taq (as defined by Takara Bio) as equivalent to 1 U of in-house enzyme. This value was used to standardize enzyme concentrations in subsequent thermostability assays. An untreated aliquot was used as a control. Enzymatic activity after heat treatment was assessed by PCR under the same reaction conditions described above, using 25 cycles. Band intensity of the 813 bp product was used to evaluate thermal resilience. Following PCR, band intensities of the 813 bp products were measured using ImageJ software. For each heat treatment condition, the intensity of the corresponding PCR band was quantified and normalized to the untreated control (set as 100%). The resulting values were expressed as percentage residual activity. Data from three independent experiments were averaged, and the mean ± standard deviation (SD) was plotted as a bar graph to represent the relative enzymatic activity after thermal stress.

2.6 Scalability assessment of the *Taq* DNA polymerase expression and purification

To evaluate the scalability of the expression and purification strategy, recombinant *E. coli* BL21(DE3) cells harboring the *pBluescript SK(+)–Taq* construct were cultured in 5 mL, 50 mL, and 300 mL volumes of LB broth with 100 µg/mL ampicillin. Cultures were grown, induced with IPTG, and processed following the same expression and purification strategy described in Sections 2.3 and 2.4. Final protein concentrations were quantified using the Bradford assay as detailed in Section 2.5, and enzyme activity was validated by PCR amplification of an 813 bp λ

DNA fragment using the conditions outlined in the same section. Three biological replicates were performed per scale. Mean yield (µg) and standard deviation were calculated and plotted to assess the scalability of the workflow.

2.7 Cost analysis

To assess the economic feasibility of the in-house Taq polymerase production workflow, a detailed cost analysis was performed based on actual reagent usage and enzyme yield from a representative 300 mL culture. Only direct material costs were included—such as growth media, restriction enzymes, PCR reagents, buffers, IPTG, lysozyme, detergents, ammonium sulfate, and glycerol. Costs for durable equipment, consumables (e.g., pipette tips, centrifuge tubes), and labor were excluded to reflect conditions typical of academic and teaching laboratories. Academic list prices were obtained from suppliers including Fisher Scientific, NEB, Bio-Rad, and Zymo Research.

Total reagent costs and individual item breakdowns for the 300 mL scale are provided in Supplementary Table 4, which details all materials used from cloning through final storage. The average yield of purified Taq polymerase (1620 µg per 300 mL culture) was calculated from three independent preparations, and the cost per microgram was computed by dividing total reagent cost by total protein yield. A direct cost comparison between in-house enzyme and commercial Ex Taq (Takara Bio) is presented in Supplementary Table 3, which highlights an estimated ~65% cost reduction.

Supplementary Table 2 summarizes total yield and cost-efficiency data across the 50 mL and 300 mL cultures, supporting the scalability of the strategy. Supplementary Table 1 provides a full itemized list of reagent and consumable costs used in both scales. These values are visualized in Table 1 (side-by-side cost/yield comparison) and Figure 6 (cost-per-µg bar graph), confirming the workflow's cost-effectiveness and scalability.

3. RESULTS

3.1 Cloning and restriction-based validation of the *Taq* DNA polymerase gene

The cloning and expression strategy for *Taq* DNA polymerase is outlined in Figure 1A. The full-length *Taq* gene (2499 bp) was PCR-amplified from *Thermus aquaticus* genomic DNA using primers engineered with 5′ SalI and 3′ EcoRI restriction sites and short overhangs, resulting in a 2523 bp amplicon. As shown in Figure 1B, successful amplification was confirmed by a 2523 bp (2499 bp gene + 24 bp restriction sites with overhang) band in lanes 2 and 3. No product was detected in the negative control (lane 4), indicating high specificity of the primers.

For directional cloning, the amplified insert and the pBluescript SK(+) vector were double-digested with SalI-HF and EcoRI-HF. Gel electrophoresis (Figure 1C) revealed the expected 2499 bp *Taq* gene (lane 2), linearized pBluescript vector (~2.9 kb, lane 3), and undigested supercoiled vector (lane 4), confirming digestion efficiency. The digested products were gel-purified, ligated, and transformed into chemically competent *E. coli* DH5α for initial propagation and screening. The ligation product was then used to create expression-ready plasmids, which were transformed into *E. coli* BL21(DE3). Protein expression was driven by the

lac promoter in the pBluescript SK(+) vector—demonstrating its underappreciated capacity to support thermostable enzyme expression beyond its typical cloning role.

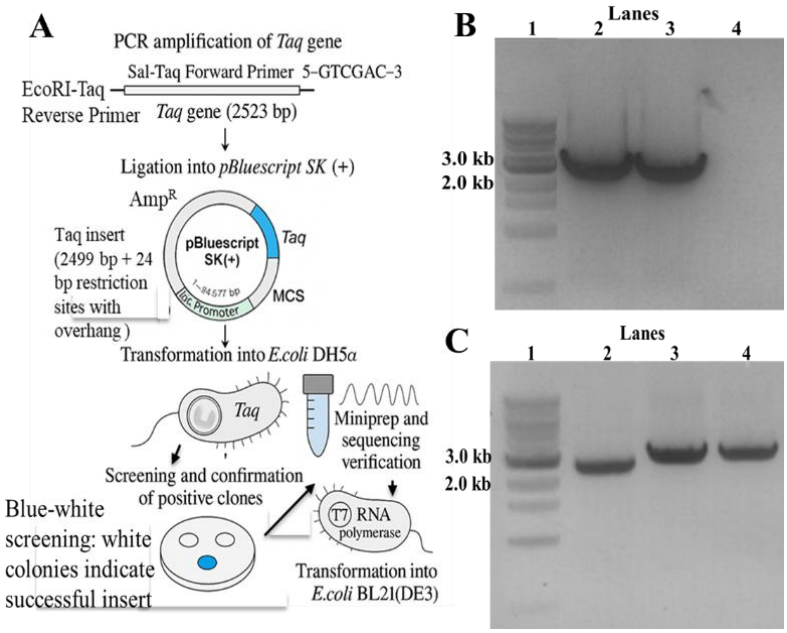


Figure 1. Cloning workflow and molecular verification of the *Taq* DNA polymerase gene.
(A) *Cloning and expression workflow for recombinant Taq DNA polymerase using pBluescript SK(+).* The full-length *Taq* DNA polymerase gene (2499 bp) was PCR amplified from *Thermus aquaticus* genomic DNA using primers engineered with *SalI* and *EcoRI* restriction sites and additional overhangs, resulting in a total amplicon size of 2523 bp. The PCR product was ligated into the multiple cloning site (MCS) of the pBluescript SK(+) vector downstream of the *lac* promoter. The recombinant plasmid was introduced into chemically competent *E. coli* DH5α cells. Positive clones were identified through white/blue colony screening on X-gal/IPTG plates and confirmed by plasmid miniprep and Sanger sequencing. Verified plasmids were subsequently transformed into *E. coli* BL21(DE3) cells for inducible expression under the lac-inducible expression via IPTG. **(B) *Agarose gel validation of Taq DNA polymerase amplification and vector-insert preparation.*** Agarose gel (1%) analysis of PCR product. Lane 1: 1 Kb DNA ladder (NEB); Lanes 2 and 3: 2523 bp (2499 bp gene + 24 bp overhangs with restriction sites) PCR product; Lane 4: negative control (no template).
(C) *Restriction digest of the Taq polymerase PCR product and pBluescript SK(+) vector for ligation*. Lane 1: 1 Kb DNA ladder (NEB); Lane 2: digested *Taq* gene (2499 bp); Lane 3: linearized vector (~2.9 kb); Lane 4: undigested pBluescript SK(+) (supercoiled).

3.2 Colony screening and PCR-based confirmation of insert-positive clones

To screen for recombinant plasmids, transformants were plated on LB agar supplemented with ampicillin, X-gal, and IPTG. Blue-white screening yielded a high density of white colonies (Figure 2A), consistent with disruption of the *lacZ* α-fragment due to insertion of the *Taq* gene into the multiple cloning site (MCS) of pBluescript SK (+). Blue colonies, indicative of empty vectors, served as negative controls. White colonies were further screened via colony PCR using the M13 forward primer and a *Taq*-specific reverse primer. As shown in Figure 2B, four independent white colonies (lanes 2–5) yielded the expected ~1.5 kb PCR product, confirming successful insertion of the *Taq* gene. The blue colony (lane 6) did not produce any amplification product, verifying the absence of insert in non-recombinant clones.

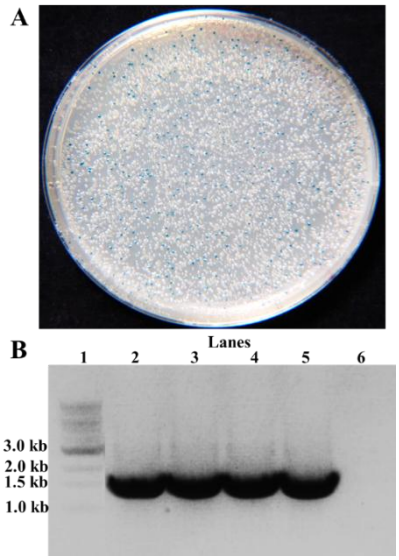


Figure 2. Colony screening and insertion verification of the *Taq* gene.
(A) *Blue-white screening for identification of recombinant E. coli DH5α colonies.* Blue-white selection of *E. coli*

DH5α transformants on LB/ampicillin/IPTG/X-gal plates. White colonies indicate disruption of the *lacZ* gene by successful *Taq* gene insertion into the *lacZ* locus.
(B) ***Colony PCR screening of E. coli DH5α transformants for Taq insert verification*****.** Colony PCR using M13 forward and a *Taq* gene-specific reverse primer. Agarose gel (1%) analysis of PCR product. Lane 1: 1 Kb DNA ladder (NEB); Lanes 2, 3, 4, 5: positive clones with ~1.5 kb product; Lane 6: negative control (blue colony).

Following PCR validation, plasmid DNA was isolated from confirmed colonies and sequenced using T7 and T3 primers to assess insert orientation and sequence fidelity. Full-length sequence alignment with the reference *Taq* polymerase gene (GenBank: J04639.1) revealed 100% identity and an intact open reading frame with the *SalI* and *EcoRI* restriction sites (Supplementary Figure 1).

Together, these results confirmed the successful cloning and sequence verification of the full-length *Taq* gene into the pBluescript SK(+) plasmid backbone.

3.3 Expression, purification, and purity assessment of Taq DNA polymerase

To evaluate the expression and purification efficiency of recombinant Taq polymerase, SDS-PAGE was performed across key stages of the workflow. As shown in Figure 3A, a prominent band at ~94 kDa, corresponding to the expected molecular weight of Taq DNA polymerase, was absent in the uninduced lysates (lanes 2 and 3) but strongly induced following IPTG treatment (lane 4). This confirms robust recombinant expression in *E. coli* BL21(DE3) under control of the lac-inducible expression via IPTG. Heat treatment at 75 °C for 90 min selectively enriched thermostable Taq polymerase in the soluble fraction (lane 5), eliminating many heat-labile bacterial proteins. The final dialyzed preparation, following ammonium sulfate precipitation, yielded a single dominant band at ~94 kDa (lane 6), indicating successful purification and minimal contamination.

Densitometric analysis of band intensities (Figure 3B) quantitatively tracked purity progression. Taq polymerase accounted for <5% of total protein pre-induction, increasing to ~40% post-induction. Following heat treatment, the Taq band comprised ~85% of total lane intensity, and the final ammonium sulfate dialyzed preparation achieved an average purity of 90.1 ± 2.1% (n = 3), surpassing the high-purity threshold (dashed line at 90%). These results demonstrate that the non-affinity purification strategy was sufficient to achieve high-quality enzyme preparations suitable for functional assays.

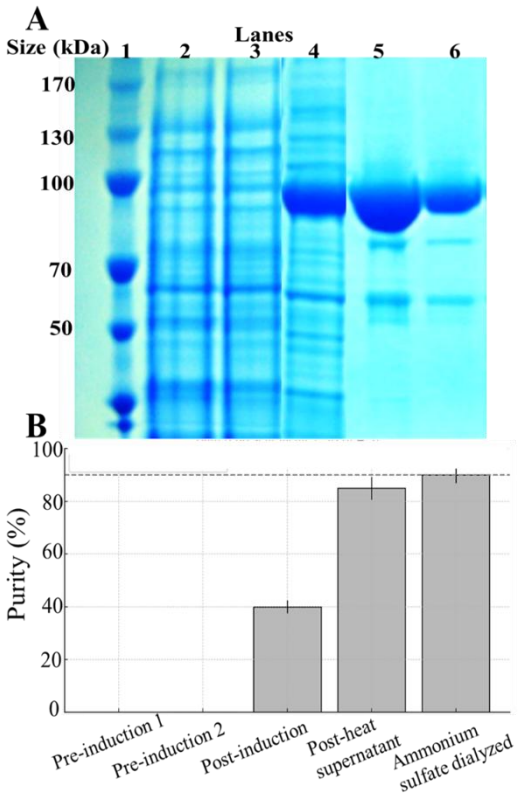


Figure 3. Expression, purification, and purity assessment of recombinant Taq DNA polymerase.
(A) ***SDS-PAGE analysis of Taq DNA polymerase expression and purification workflow.*** Protein fractions from various stages of the purification workflow were separated by SDS-PAGE (10% resolving, 4% stacking gel) and visualized using Coomassie Brilliant Blue staining. Lane 1: Thermo Scientific PageRuler Prestained Protein Ladder (10–170 kDa); Lanes 2–

3: Uninduced *E. coli* BL21(DE3) lysate (negative control); Lane 4: Cell lysate following IPTG induction, prior to heat treatment, showing a prominent ~94 kDa band corresponding to Taq polymerase; Lane 5: Supernatant after heat treatment at 75 °C for 90 min, demonstrating thermal enrichment of soluble Taq polymerase; Lane 6: Final purified Taq polymerase following ammonium sulfate precipitation and dialysis, appearing as a single dominant ~94 kDa band with estimated purity 90.1 ± 2.1%. **(B) *Quantification of purified Taq polymerase purity across purification steps***. Band intensities from the SDS-PAGE gel in (A) were quantified by densitometry. The ~94 kDa band corresponding to Taq polymerase was measured and expressed as a percentage of the total lane intensity. Purity increased following IPTG induction and heat treatment, with the final ammonium sulfate dialyzed sample reaching 90.1 ± 2.1% purity. Bars represent the mean estimated purity at each step. The dashed line marks the 90% threshold commonly used to define high-quality enzyme preparations. Data represent mean ± SD (n = 3).

3.4 Functional validation and thermostability of purified Taq DNA polymerase

Functional equivalence of the in-house purified Taq DNA polymerase was assessed through titration-based PCR amplification of an 813 bp λ DNA fragment. As shown in Figure 4A, robust amplification was achieved across decreasing enzyme volumes (1.0 to 0.3 µL) for both commercial Ex Taq polymerase (lanes 2–8) and the in-house purified Taq enzyme (lanes 9–15). Band intensities declined with reduced enzyme input, but even the lowest volume tested (0.3 µL) yielded detectable PCR product in both cases, confirming that the purified enzyme retains high catalytic activity. These results demonstrate that the in-house Taq DNA polymerase is functionally equivalent to Ex Taq (Takara Bio) in PCR amplification performance.

Quantification of PCR product intensities from this titration experiment is shown in Figure 4B. Normalized intensities declined with decreasing enzyme volume for both commercial and in-house Taq. Importantly, corresponding lanes showed highly similar intensity values, with no statistically significant differences observed between the two enzyme sources at any titration point ($p > 0.05$, unpaired two-tailed t-test). These data confirm that the in-house Taq polymerase is functionally equivalent to commercial Ex Taq in PCR amplification efficiency.

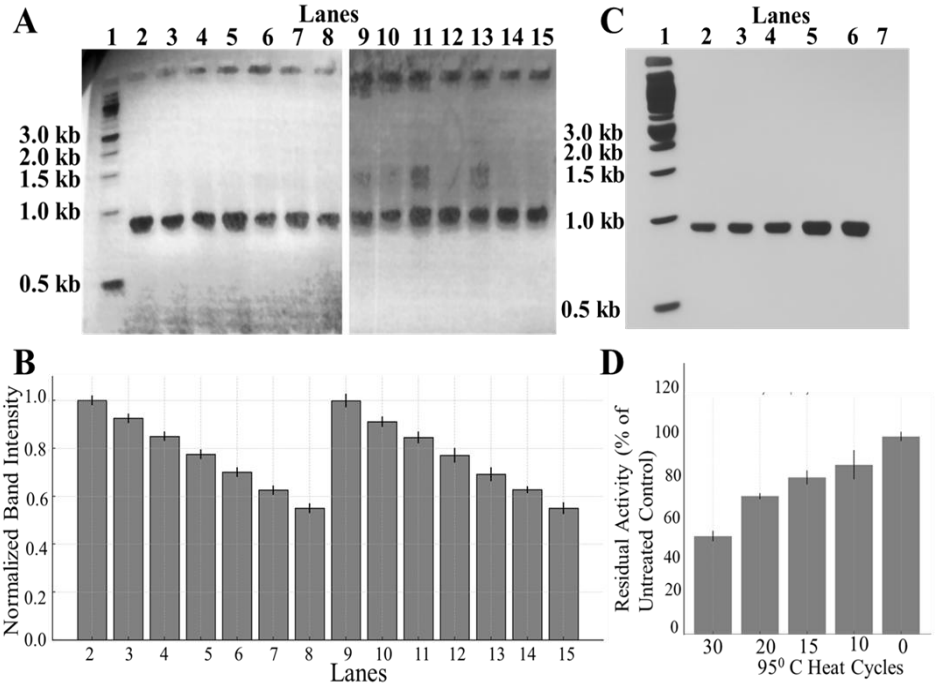


Figure 4. PCR titration and thermostability analysis of purified Taq DNA polymerase. (A) *PCR titration to determine the functional equivalence of purified Taq polymerase.* Agarose gel electrophoresis (1%) of 813 bp λ DNA PCR products generated using decreasing volumes of Taq polymerase enzymes (1.0, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3 µL). Lane 1: 1 kb DNA ladder (NEB); Lanes 2–8: PCR products amplified with decreasing volumes of commercial Ex Taq enzyme; Lanes 9–15: PCR products amplified with decreasing volumes of purified in-house Taq enzyme. **(B) *Quantification of PCR product band***

intensities from Figure 4A. Intensities were normalized to the brightest band (lane 2: Ex Taq enzyme at 1.0 µL) and measured using ImageJ software. Bars represent mean ± SD (n = 3) from three independent biological replicates. No statistically significant differences were observed between purified and commercial Taq at corresponding enzyme volumes (unpaired two-tailed t-test, $p > 0.05$). These results confirm the functional equivalence of the in-house enzyme to Ex Taq (Takara Bio). **(C)** ***Thermostability assay of purified in-house Taq DNA polymerase.*** PCR products amplified with heat-treated and untreated purified in-house Taq polymerase were separated on a 1% agarose gel. Lane 1: 1 kb DNA ladder; Lanes 2–5: purified in-house Taq treated for 30, 20, 15, and 10 cycles at 95 °C, respectively; Lane 6: untreated control Taq; Lane 7: negative control (no template). Band intensity reflects residual polymerase activity following thermal stress. **(D)** ***Quantification of Taq polymerase thermostability***. Graph shows the mean residual activity of the Taq polymerase enzyme after each heat treatment, expressed as a percentage of the untreated control (set as 100%). Band intensity was quantified using ImageJ software, and values represent mean ± SD (n = 3) from three independent experiments.

To further test thermal robustness, aliquots of the purified in-house Taq enzyme were subjected to repeated 95 °C heat stress cycles (0, 10, 15, 20, 30 cycles, 30 s each) followed by PCR amplification of the same target. As shown in Figure 4C, untreated enzyme (lane 6) produced strong amplicons, while enzyme subjected to 10–30 heat cycles (lanes 2–5) displayed progressive but partial loss of activity. No amplification was observed in the negative control (lane 7). Quantitative analysis of residual enzymatic activity is shown in Figure 4D. The purified Taq enzyme retained 85.7 ± 4.3%, 79.6 ± 6.1%, and 70.4 ± 5.8% of its activity after 10, 15, and 20 thermal cycles, respectively. Even after 30 cycles, residual activity remained at 50.2 ± 3.7%, underscoring the inherent thermostability of the enzyme. These findings confirm that the simplified purification strategy preserves the core thermal resilience and catalytic competence of the Taq polymerase enzyme.

3.5 Scalability of *Taq* polymerase expression and purification

To assess the scalability of the simplified expression and purification workflow, recombinant E. coli BL21(DE3) cultures were grown at three different volumes (5 mL, 50 mL, and 300 mL) under identical conditions, and the total yield of purified Taq polymerase was quantified via Bradford assay. As shown in Figure 5, the Taq enzyme yield increased proportionally with culture volume, demonstrating a strong linear correlation and confirming the robustness of the workflow across small to moderately large batch sizes. From 5 mL cultures, an average yield of 24.5 ± 2.2 µg of purified Taq was obtained, while 50 mL and 300 mL cultures yielded 265.1 ± 12.4 µg and 1612.3 ± 63.9 µg, respectively.

This ~40-fold increase in yield from 5 mL to 300 mL cultures closely aligns with the expected 60-fold increase in input volume, suggesting minimal loss in expression efficiency or protein recovery at larger scales. Moreover, all scaled preparations consistently produced active enzyme, as confirmed by PCR amplification (data not shown), indicating that neither protein integrity nor catalytic activity was compromised during upscaling.

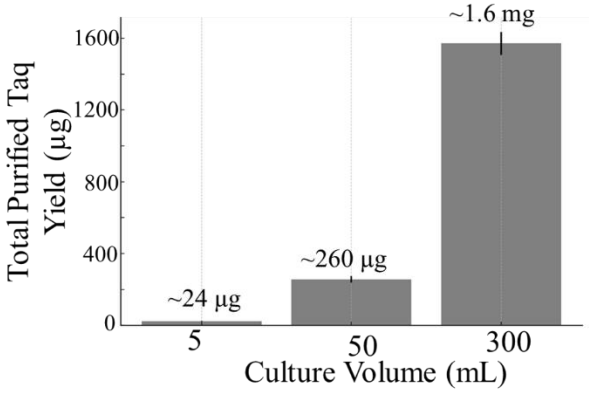


Figure 5. Scalability of *Taq* DNA polymerase expression and purification.

Graph showing total yield of the purified in-house Taq polymerase enzyme (µg) obtained from 5 mL, 50 mL, and 300 mL LB cultures, using the same IPTG-inducible expression and heat-based purification strategy. Protein concentrations were determined by Bradford assay, and

values represent the mean ± SD (n = 3) from three biological replicates. Yields increased proportionally with culture volume, confirming linear scalability of the production workflow.

These results validate the scalability of the pBluescript SK(+)-based system for producing high-yield, functionally active Taq polymerase enzyme using only basic molecular biology infrastructure. The workflow is thus suitable for flexible implementation in educational or resource-limited laboratory settings without the need for specialized fermenters or affinity purification systems.

3.6 Cost analysis and scalability of in-house Taq DNA polymerase production

To evaluate the cost-effectiveness of our workflow, we compared enzyme yields and material costs from 50 mL and 300 mL cultures (Table 1). The 300 mL culture yielded 1,620 µg of purified Taq polymerase enzyme at a cost of $13.62, while the 50 mL culture produced 260 µg for $12.20. This scale-up reduced the cost-per-microgram from $0.0469 to $0.0086, representing a substantial improvement in cost-efficiency.

Table 1. Purified in-house Taq polymerase production cost and yield from a 300 mL culture

Averaged data from three independent preparations used for final cost-effectiveness calculations.

Replicate	Total Yield (µg)	Total Cost (USD)	Cost per µg (USD)
1	1,570	13.62	0.0087
2	1,640	13.62	0.0083
3	1,650	13.62	0.0083
Mean ± SD	**1,620 ± 40**	**13.62**	**0.0086 ± 0.0002**

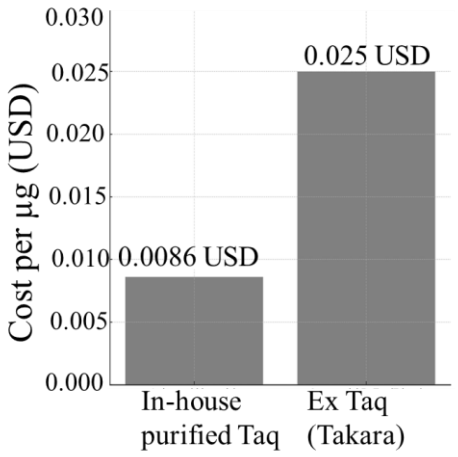
Figure 6 visualizes this comparison, highlighting a ~65% cost reduction relative to commercial Ex Taq polymerase. Supplementary Table 3 provides the pricing data used in this comparison. Supplementary Table 1 lists all reagents, enzymes, and consumables used from gene amplification through enzyme storage. Supplementary Table 2 summarizes yield and cost outcomes across the two tested volumes, confirming consistent improvements with scale-up. Supplementary Table 4 presents the full line-item breakdown for the 300 mL benchmark, including media, enzymes, buffers, and additives.

Although capital equipment and labor costs were excluded, this conservative estimate strongly supports the affordability and accessibility of the strategy for instructional or independent laboratory use. Based on typical usage volumes of 0.5–1.0 U per 25 µL PCR reaction—as estimated from titration-based activity analysis (Figure 4A)—the cost of purified in-house Taq DNA polymerase per reaction ranges from $0.004–$0.009, depending on enzyme input. By comparison, commercial Ex Taq polymerase typically costs $0.03–$0.05 per reaction.

This ~70–85% reduction in per-reaction cost further supports the practical affordability of our in-house strategy for instructional PCR applications.

Collectively, these findings confirm that our in-house production approach is scalable, cost-effective, and functionally equivalent to Ex Taq (Takara Bio), making it ideal for educational or resource-limited settings.

Figure 6. Cost comparison of in-house versus commercial Taq polymerase production.
Graph comparing the cost per microgram (µg) of Taq DNA polymerase produced using the simplified in-house strategy described in this study versus the cost of commercially available Ex Taq DNA polymerase (Takara Bio). In-house production from a 300 mL culture yielded 1,620 µg of enzyme at an estimated cost of $0.0086/µg, compared to $0.0250/µg for the commercial preparation—reflecting a ~65% cost reduction. Cost estimates were based on actual reagent usage, yield per batch, and current academic pricing (see Supplementary Table 3). Bars represent mean ± SD (n = 3) based on three independent preparations. These results demonstrate the economic viability of in-house enzyme production using only standard laboratory reagents and equipment.



4. DISCUSSION

This study establishes that the pBluescript SK(+) plasmid—a vector traditionally used for cloning and blue-white screening—can be effectively repurposed for the expression of thermostable Taq DNA polymerase when paired with heat-based purification and ammonium sulfate precipitation. The approach eliminates the need for affinity tags, column chromatography, or proprietary expression systems (Engelke et al., 1990; Pluthero, 1993) and consistently yields enzymatically active Taq polymerase with high purity (90.1 ± 2.1%). These findings address a critical gap in molecular biology education and resource-limited environments, especially in settings where commercial enzymes are prohibitively expensive or unavailable, and access to expression systems like T7 vectors is limited (Carroll and Keller, 2014; Bhadra et al., 2022). This gap has also been widely acknowledged in broader reviews of diagnostic infrastructure limitations in low-income settings (Rodrigues et al., 2016; Chakraborty, 2024).

The performance of the recombinant enzyme was validated through multiple functional assays, confirming that the simplified purification strategy retains both catalytic activity and thermal stability. Titration-based PCR amplification using an 813 bp target fragment showed that the in-house enzyme performs comparably to commercial Ex Taq across a range of input volumes. No statistically significant differences in PCR product yield were observed ($p > 0.05$), confirming functional equivalence to Ex Taq (Takara Bio). Thermostability assays further demonstrated that the enzyme retains >70% residual activity after 20 repeated 95 °C heat cycles, and ~50% after 30 cycles at 95 °C, levels consistent with commercial thermostable polymerases (Lawyer et al., 1989; Ghadessy et al., 2001). Thermal stability of family A DNA polymerases has been closely correlated with structural adaptations in both theoretical and experimental studies (Borhani and Arab, 2023; Bulygin et al., 2023), supporting the mechanistic basis for heat-based purification.

The purification strategy—based solely on heat denaturation at 75 °C and 60% ammonium sulfate precipitation—achieved high purity without the need for chromatography or resin-based enrichment. This strategy takes advantage of Taq polymerase's intrinsic thermostability to selectively enrich the enzyme while denaturing most host proteins (Pluthero, 1993; Zhou et al., 2017). The rationale for this approach dates back to the earliest characterization of Taq polymerase stability and utility in PCR workflows (Gelfand, 1989). Recent innovations have further validated the feasibility of chromatography-free workflows for Taq polymerase using auto-inducible expression systems (Menacho-Melgar et al., 2021) and low-cost, open-access purification strategies adaptable to resource-limited labs (Mendoza-Rojas et al., 2021). Additional work has explored expression of thermostable polymerases from *Pyrococcus furiosus* and *Geobacillus stearothermophilus*, offering further models for scalable, low-cost production (Nuryana et al., 2023; Agustriana et al., 2023; Khaerunnisa et al., 2025). Our findings are consistent with earlier work on simplified purification (Pluthero, 1993; Zhou et al., 2017) but expand significantly on prior reports by incorporating functional validation, reproducibility testing, scalability, and cost analysis in an educational context. Scalability testing further demonstrated the robustness of this workflow across 5 mL, 50 mL, and 300 mL culture volumes. The ~40-fold increase in yield from 5 mL to 300 mL closely mirrors the increase in culture volume, indicating minimal loss during purification. This yield compares favorably to more complex systems using codon-optimized constructs and T7-driven vectors, which typically generate 2–8 mg/L but rely on costly affinity purification (Olszewski et al., 2017; Zhou et al., 2017; Samman et al., 2023). These expression levels are consistent with reports from recombinant protein factories and high-cell density systems (Rosano and Ceccarelli, 2014; Choi et al., 2006; Ferrer-Miralles et al., 2009).

A key finding of this study is the substantial reduction in material costs. The cost-per-microgram of purified Taq DNA polymerase was reduced by approximately 65% compared to commercial Ex Taq (Figure 6, Table 1), with the 300 mL culture yielding 1,620 µg of enzyme for $13.62. More strikingly, the per-reaction cost—estimated at $0.004–$0.009 for 0.5–1.0 U per 25 µL PCR—represents a 70–85% reduction relative to the $0.03–$0.05 per reaction cost of commercial enzyme. These values are consistent with previous educational or diagnostic efforts that reported higher per-unit costs for student-prepared or recombinant Taq polymerase, ranging from $0.05 to $0.50 per unit depending on strategy complexity and expression system (Ferralli et al., 2007; Zhra et al., 2024). Recent theoretical and cost-focused studies support the viability of in-house enzyme production over commercial kits, especially in decentralized or teaching labs (Wingfield, 2015; Chakraborty, 2024). These savings are particularly impactful in instructional settings where enzyme cost is often a limiting factor for hands-on experimentation. Educationally, this strategy reinforces classical molecular biology skills—including restriction enzyme digestion, blue-white screening, colony PCR, protein expression, and non-affinity purification—all without reliance on proprietary kits. These features make the workflow ideal for biotechnology instruction and align with global initiatives to democratize molecular biology education, including platforms like BioBuilder and DIYBio (Bernstein et al., 2015; Vilanova and Porcar, 2014). The open-source framework is particularly aligned with new trends in decentralized biotech training and resource-sharing initiatives (Ferrer-Miralles et al., 2009; Chakraborty, 2024). By using reagents and vectors already available in most teaching labs, the strategy lowers barriers to entry for resource-limited institutions. This is particularly relevant for laboratories in low- and middle-income countries—such as those in Africa, South America, and parts of Asia—where infrastructure and reagent costs often prohibit routine molecular biology

applications. The strategy's reliance on standard reagents and licensing-free tools ensures global accessibility and adaptability.

A key novelty of this study lies in experimentally validating the repurposing of the classical pBluescript SK(+) cloning vector for expression of a thermostable polymerase—a use that, to our knowledge, has not been demonstrated in published experimental workflows. Unlike traditional T7-driven systems, the pBluescript SK(+) plasmid is widely available in teaching labs and provides a truly open-access, licensing-free platform for protein expression—a novel application for a vector typically reserved for cloning. When paired with a chromatography-free purification strategy, this strategy provides a streamlined alternative to proprietary workflows. While individual elements of this strategy —such as heat denaturation and salt precipitation— have been described in earlier studies (e.g., Pluthero, 1993), they were not previously optimized or validated in combination with a non-expression vector like pBluescript SK(+) for enzyme production in low-resource settings. Critically, our approach achieves a ~65% reduction in cost-per-microgram and up to 85% reduction in cost-per-reaction relative to commercial enzymes. The inclusion of quantitative benchmarking, thermostability profiling, and reagent-level cost analysis further distinguishes this work as a practical and reproducible solution. To our knowledge—and based on available literature—our study is the first to experimentally validate the use of pBluescript SK(+) for functional expression of *Taq* DNA polymerase. By centering accessibility, we address an urgent need in molecular biology training and promote broader adoption of core tools in underfunded teaching and research environments. This aligns with broader calls for expanded access to core molecular technologies via DIY science, synthetic biology education, and local enzyme manufacturing (Vilanova and Porcar, 2014; Nuryana et al., 2023).

This work builds on foundational efforts to express and purify Taq polymerase (Lawyer et al., 1989; Engelke et al., 1990), extending their utility into the modern landscape of open science and decentralized biotechnology education. By integrating widely available molecular tools with rigorous functional validation, scalability testing, and cost analysis, we provide a sustainable framework for enzyme production that is accessible, reproducible, and instructionally relevant. Our approach contributes to a growing ecosystem of low-cost, open-source workflows that empower resource-limited teaching and research laboratories.

Specifically, this study: (1) repurposes the pBluescript SK(+) vector—traditionally reserved for cloning—as a functional expression platform for a thermostable polymerase; (2) eliminates the need for affinity tags and chromatography by relying on heat denaturation and salt precipitation; (3) delivers a reproducible, low-cost strategy validated across culture scales, performance assays, and cost comparisons; and (4) supports decentralized enzyme production using tools already common in academic labs. To our knowledge, no prior study integrates all these elements into a single, experimentally validated, instructional-grade workflow. As enzyme access remains a persistent barrier to practical molecular biology, this strategy offers a viable model for expanding hands-on training and enabling community-based life science innovation.

5. CONCLUSION

This study presents a validated, cost-effective, and scalable workflow for the expression and purification of thermostable Taq DNA polymerase using the pBluescript SK(+) plasmid and

a non-chromatographic purification strategy. By combining IPTG-inducible expression in E. coli with heat denaturation and ammonium sulfate precipitation, we achieved high-yield enzyme production with 90.1 ± 2.1% purity and robust functional activity across a range of PCR titrations. The purified enzyme retained thermostability and performed equivalently to commercial Ex Taq (Takara Bio), maintaining >70% residual activity after 20 heat cycles and ~50% after 30 cycles at 95 °C. Critically, the workflow reduced the material cost per microgram of enzyme by ~65% and lowered the cost per PCR reaction by 70–85% compared to commercial Ex Taq—without reliance on proprietary vectors, affinity resins, or specialized equipment. These cost savings, along with the strategy's reproducibility across multiple culture volumes, make it particularly well-suited for teaching laboratories, outreach programs, and research environments with limited infrastructure.

In addition to its technical merits, this work introduces a fully open-access alternative to conventional Taq polymerase production by repurposing pBluescript SK(+), a widely available vector not previously validated for thermostable enzyme expression. This strategy reinforces essential molecular biology skills and provides a turnkey, low-barrier solution for decentralized biotechnology education and research. Importantly, its reliance on standard reagents and accessible tools makes it especially valuable for low-resource settings globally. Overall, our findings offer a practical model for sustainable enzyme production and underscore the broader potential of classical molecular tools in advancing accessible, hands-on science.

Supplementary Materials

The following supplementary data are available:

Supplementary Figure 1. Sequence verification of the recombinant *pBluescript-Taq* plasmid.

Supplementary Table 1. Itemized reagent and consumable costs for 50 mL and 300 mL cultures

Supplementary Table 2. Yield and cost summary by culture volume

Supplementary Table 3. Comparison of production costs for in-house Taq DNA polymerase and commercial Ex Taq (Takara Bio).

Supplementary Table 4. Detailed Cost Breakdown (300 mL scale)

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models and text-to-image generators have been used during the writing or editing of this manuscript.

REFERENCES

Chien, A., Edgar, D. B., & Trela, J. M. (1976). Deoxyribonucleic acid polymerase from the extreme thermophile Thermus aquaticus. *Journal of Bacteriology, 127*(3), 1550–1557.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., & Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science, 239*(4839), 487–491.

Engelke, D. R., Krikos, A., Bruck, M. E., & Ginsburg, D. (1990). Purification of Thermus aquaticus DNA polymerase expressed in Escherichia coli. *Analytical Biochemistry, 191*(2), 396–400.

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*(9), e0184162.

Samman, N., Al-Muhalhil, K., & Nehdi, A. (2023). A simple and efficient method for Taq DNA polymerase purification based on heat denaturation and affinity chromatography. *Journal of King Saud University – Science, 35*(3), 102565.

Gomes, L. C., Ferreira, C., & Mergulhão, F. J. (2022). Implementation of a practical teaching course on protein engineering. *Biology, 11*(3), 387.

Lawyer, F. C., Stoffel, S., Saiki, R. K., Myambo, K., Drummond, R., & Gelfand, D. H. (1989). Isolation, characterization, and expression in Escherichia coli of the DNA polymerase gene from Thermus aquaticus. *Journal of Biological Chemistry, 264*(11), 6427–6437.

Carroll, C. W., & Keller, L. C. (2014). An adaptable investigative graduate laboratory course for teaching protein purification. *Biochemistry and Molecular Biology Education, 42*(6), 486–494.

Laksmi, F. A., Dewi, K. S., Nuryana, I., Yulianti, S. E., Ramadhan, K. P., Hadi, M. I., & Nugraha, Y. (2024). High-level expression of codon-optimized Taq DNA polymerase under the control of rhaBAD promoter. *Analytical Biochemistry, 692*, 115581.

Pluthero, F. G. (1993). Rapid purification of high-activity Taq DNA polymerase. *Nucleic Acids Research, 21*(20), 4850.

Bhadra, S., Paik, I., Torres, J. A., Fadanka, S., Gandini, C., Akligoh, H., Molloy, J., & Ellington, A. D. (2022). Preparation and use of cellular reagents: a low-resource molecular biology reagent platform. *Current Protocols, 2*(3), e387.

Brock, T. D., & Freeze, H. (1969). Thermus aquaticus gen. n. and sp. n., a nonsporulating extreme thermophile. *Journal of Bacteriology, 98*(1), 289–297.

Rodrigues, C., Desai, N., & Fernandes, H. (2016). Molecular diagnosis in resource-limited settings. *Clinical Microbiology Newsletter, 38*(7), 51–56.

Chakraborty, S. (2024). Democratizing nucleic acid-based molecular diagnostic tests for infectious diseases at resource-limited settings–from point of care to extreme point of care. *Sensors & Diagnostics, 3*(4), 536–561.

Ghadessy, F. J., Ong, J. L., & Holliger, P. (2001). Directed evolution of polymerase function by compartmentalized self-replication. *Proceedings of the National Academy of Sciences, 98*(8), 4552–4557.

Borhani, S., & Arab, S. S. (2023). Investigation of thermal stability characteristic in family A DNA polymerase—A theoretical study. *Progress in Biophysics and Molecular Biology, 182*, 15–25.

Bulygin, A. A., Kuznetsova, A. A., Fedorova, O. S., & Kuznetsov, N. A. (2023). Comparative analysis of Family A DNA-polymerases as a searching tool for enzymes with new properties. *Molecular Biology, 57*(2), 182–192.

Zhou, H., Zhang, Y., Hu, Z., Mu, A., & Gu, X. (2018). High-efficiency separation and purification of Taq DNA polymerase. In Liu, H., Song, C., & Ram, A. (Eds.), *Advances in Applied Biotechnology. ICAB 2016. Lecture Notes in Electrical Engineering, 444* (pp. 663–672). Singapore: Springer. https://doi.org/10.1007/978-981-10-4801-2_68

Gelfand, D. H. (1989). Taq DNA polymerase. In *PCR Technology: Principles and Applications for DNA Amplification* (pp. 17–22). London: Palgrave Macmillan UK.

Menacho-Melgar, R., Yang, T., & Lynch, M. D. (2021). Instant Taq: Rapid autoinducible expression and chromatography-free purification of Taq polymerase. *bioRxiv*. https://doi.org/10.1101/2021.09.25.461831

Mendoza-Rojas, G., Sarabia-Vega, V., Sanchez-Castro, A., Tello, L., Cabrera-Sosa, L., Nakamoto, J. A., Peñaranda, K., Adaui, V., Alcántara, R., & Milón, P. (2021). A low-cost and open-source protocol to produce key enzymes for molecular detection assays. *STAR Protocols, 2*(4), 100899.

Nuryana, I., Laksmi, F. A., Dewi, K. S., Akbar, F. R., & Harmoko, R. (2023). Codon optimization of a gene encoding DNA polymerase from Pyrococcus furiosus and its expression in Escherichia coli. *Journal of Genetic Engineering and Biotechnology, 21*(1), 129.

Agustriana, E., Nuryana, I., Laksmi, F. A., Dewi, K. S., Wijaya, H., Rahmani, N., Yudiargo, D. R., Ismadara, A., Helbert, H., Hadi, M. I., & Purnawan, A. (2023). Optimized expression of large fragment DNA polymerase I from Geobacillus stearothermophilus in Escherichia coli expression system. *Preparative Biochemistry & Biotechnology, 53*(4), 384–393.

Khaerunnisa, L., Laksmi, F. A., Rahayuningsih, M., Amalia, A. R., Violando, W. A., Nuryana, I., Ertanto, Y., & Nugraha, Y. (2025). One-step rapid production of DNA polymerase from Pyrococcus furiosus in Escherichia coli system under optimized culture conditions. *Preparative Biochemistry & Biotechnology*. Advance online publication. https://doi.org/10.1080/10826068.2025.0000000

Rosano, G. L., & Ceccarelli, E. A. (2014). Recombinant protein expression in Escherichia coli: Advances and challenges. *Frontiers in Microbiology, 5*, 172.

Choi, J. H., Keum, K. C., & Lee, S. Y. (2006). Production of recombinant proteins by high cell density culture of Escherichia coli. *Chemical Engineering Science, 61*(3), 876–885.

Ferrer-Miralles, N., Domingo-Espín, J., Corchero, J. L., Vázquez, E., & Villaverde, A. (2009). Microbial factories for recombinant pharmaceuticals. *Microbial Cell Factories, 8*(1), 17.

Zhra, M., Al Saud, A., Alzayer, M., Okdah, L., Tamim, H., Fakhoury, H. M. A., & Aljada, A. (2024). Cost-effective in-house COVID-19 reverse transcription-polymerase chain reaction testing with yeast-derived Taq polymerase. *Annals of Thoracic Medicine, 19*(2), 165–171. https://doi.org/10.4103/atm.atm_180_23

Wingfield, P. T. (2015). Overview of the purification of recombinant proteins. *Current Protocols in Protein Science, 80*(1), 6.1–6.35.

Bernstein, R., Ingram, K., & Hart, K. M. (2015). *BioBuilder: Synthetic Biology in the Lab*. O'Reilly Media, Inc.

Vilanova, C., & Porcar, M. (2014). iGEM 2.0—Refoundations for engineering biology. *Nature Biotechnology, 32*(5), 420–424.

Ferralli, P., Egan, J. D., & Erickson, F. L. (2007). Making Taq DNA polymerase in the undergraduate biology laboratory. *Bios, 78*(2), 69–74.

Supplementary Figure 1. Sequence verification of the recombinant *pBluescript-Taq* plasmid.
In silico alignment of the cloned *Taq* sequence with the reference gene (GenBank: J04639.1), confirming 100% sequence identity and correct reading frame. Red boxes highlight restriction sites.

Supplementary Table 1. Itemized reagent and consumable costs for gene cloning, expression, purification, and activity validation workflows at 50 mL and 300 mL culture volumes.

All prices reflect 2024–2025 U.S. academic rates using standard vendors (e.g., NEB, Fisher Scientific, GenScript). Labor and equipment depreciation costs are excluded in accordance with standard academic costing.

Item / Reagent	Unit Cost (USD)	Total Cost - 50 mL (USD)	Total Cost - 300 mL (USD)	Notes
LB broth powder	0.20	0.20	0.60	
Ampicillin	0.10	0.10	0.30	
IPTG	0.25	0.25	0.75	
Restriction enzymes (SalI + EcoRI)	0.40	0.40	0.40	Used for cloning
T4 DNA Ligase	0.30	0.30	0.30	For ligation
Agarose + EtBr	0.25	0.25	0.25	1% gel
Competent *E. coli* BL21(DE3)	0.80	0.80	0.80	Commercial cells
X-gal + IPTG + plates	0.30	0.30	0.30	For blue-white selection
Ammonium sulfate	0.15	0.15	0.45	Heat precipitation
Tris/KCl/EDTA buffer	0.10	0.10	0.30	Buffer preparation
Dialysis tubing (MWCO 10 kDa)	0.30	0.30	0.30	
Bradford reagent + BSA	0.15	0.15	0.20	Protein quantification
Pipette tips, tubes, plasticware	0.20	0.20	0.30	General consumables
PCR primers (M13 + gene-specific)	0.10	0.10	0.10	Used in colony PCR
dNTPs (colony PCR)	0.05	0.05	0.05	One reaction

Miniprep spin columns	**1.00**	**1.00**	**1.00**	Assumes screening of a single clone
Sanger sequencing	**6.00**	**6.00**	**6.00**	Based on T7 and T3 primers
SOC media	0.20	0.20	0.20	Used after transformation
Homemade competent cell reagents	**0.10**	**0.10**	**0.10**	$CaCl_2$ and glycerol
Labor/technical time	**0.00**	**0.00**	**0.00**	Labor costs excluded as per academic standard practice
DNeasy kit (gDNA extraction)	1.00	1.00	1.00	From *T. aquaticus*
IGEPAL CA-630	0.05	0.05	0.05	Lysis buffer component
Triton X-100	0.05	0.05	0.05	Lysis buffer component
DTT	0.05	0.05	0.05	Storage buffer component
Glycerol	**0.05**	**0.05**	**0.05**	50% in storage buffer
λ DNA template	0.05	0.05	0.05	Used for PCR validation
β-mercaptoethanol	0.05	0.05	0.05	For SDS-PAGE
DNA ladder (1 kb)	**0.15**	**0.15**	**0.15**	NEB ladder used for gel analysis
PCR reaction dNTPs + buffer	**0.15**	**0.15**	**0.15**	For full λ DNA PCR validation
TOTAL (including optional items)	**—**	**$12.20**	**$13.62**	Confirmed after itemization

Supplementary Table 2. Yield and cost summary by culture volume

Total protein yield and cost efficiency from scaled-up culture volumes.

Prep Scale	Total Yield (µg)	Total Cost (USD)	Cost per µg (USD)
50 mL	260	12.20	0.0469
300 mL	1,620	13.62	0.0086

Supplementary Table 3. Comparison of production costs for in-house Taq DNA polymerase and commercial Ex Taq (Takara).

Source	Total Yield (μg)	Total Cost (USD)	Cost per μg (USD)
In-house (this study)	1,620	13.62	0.0086
Commercial Ex Taq	250	6.25	0.0250

Supplementary Table 4, Detailed Cost Breakdown (300 mL scale)

Culture Volume	Total Yield	Total Cost (USD)	Cost per μg (USD)
50 mL	260 μg	12.20	0.0469
300 mL	1,620 μg	13.62	0.0086