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A low-cost production method for Bst DNA polymerase (large fragment)

Manuel Victor San Pedro[1,2], Van Aldren Cañas[1], Joshua Roberto Garcia[1], Crisanto Lopez[1,2], Marjorie Zulueta[1], Edjohn Aaron Macauyag*[1]

[1]Integrated Protein Research and Development Center, Ateneo Research Institute of Science and Engineering, School of Science and Engineering, Ateneo de Manila University
[2]Department of Biology, Ateneo de Manila University

ABSTRACT

BstLF, the large fragment of Bst DNA polymerase, is a recombinant strand-displacing DNA polymerase derived from *Geobacillus stearothermophilus* and serves as the primary enzyme utilized in loop-mediated isothermal amplification (LAMP). Recently, the application of LAMP as a convenient and accessible diagnostic tool has gained considerable attention. However, the high cost of reagents presents a significant barrier to the development of LAMP diagnostic kits, limiting accessibility for laboratories in low-resource settings.

In this study, we report a low-cost production method for BstLF utilizing more readily available alternative reagents coupled with a simplified purification process. The resulting home-brewed BstLF demonstrated comparable amplification efficiency and maintained thermal and storage stability after 10 months, aligning closely with the performance of the commercially available Bst 2.0 DNA polymerase. Furthermore, we propose this alternative production method to democratize access to LAMP-based diagnostics research. A cost analysis reveals a remarkable 94.17% reduction in the cost per reaction when using the home-brewed BstLF compared to the commercial Bst 2.0 DNA polymerase. Overall, this research introduces a streamlined and cost-effective method for the small-scale production of BstLF, thus facilitating greater access to LAMP assays in areas with limited resources.

*Corresponding author
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INTRODUCTION

Loop-mediated isothermal amplification (LAMP) has emerged as a simple and convenient point-of-care (PoC) diagnostic technique widely utilized in both research and healthcare settings due to its high sensitivity, specificity, rapid result readout, and simplified protocols (Soroka et al., 2021). In contrast, conventional quantitative reverse transcription polymerase chain reaction (qRT-PCR) involves expensive equipment, such as thermocyclers, and requires trained personnel to conduct the assay (Das et al., 2022). LAMP assays alleviate the burden of high capital costs associated with diagnostic laboratories, as they only require an isothermal heat block instead of a costly real-time thermocycler. This attribute makes LAMP particularly suitable for disease detection in remote and resource-limited areas (Avendaño & Patarroyo, 2020). Moreover, LAMP is noted for its ease of use and shorter run times compared to qRT-PCR (Das et al., 2022).

LAMP employs isothermal amplification utilizing strand-displacing DNA polymerases, notably the large fragment of DNA polymerase I from Geobacillus stearothermophilus, known as Bst DNA polymerase large fragment (BstLF). For applications such as reverse transcription LAMP (RT-LAMP), BstLF can be supplemented with reverse transcriptases,

KEYWORDS

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including Human Immunodeficiency Virus 1 Reverse Transcriptase (HIV-RT) or Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT), allowing for the detection of RNA in samples (Nzelu et al., 2019). Customized LAMP diagnostic kits can target specific DNA and RNA sequences, making them suitable for diagnosing various diseases, including COVID-19, HIV, and various cancers (Valera et al., 2021).

LAMP, which uses BstLF, can also be used in combination with quenching of unincorporated amplification signal reporters (QUASR), which is a sequence-specific endpoint detection technique. This technique utilizes an additional short quencher probe in a closed-tube system, significantly reducing false-positive results in RT-LAMP assays (Ball et al., 2016). QUASR methods are packaged into qualitative endpoint detection kits that combine reverse transcriptase to offer rapid, accurate, and on-site diagnosis of diseases (Wu et al., 2021). Before integrating these kits with other sequence-specific detection methods, they are developed and optimized using quantitative reverse transcription loop-mediated isothermal amplification (qRT-LAMP). Once optimized, the reaction includes fluorescent primers and probes for QUASR, or dipsticks in a lateral flow device for colorimetric detection (Sagar et al., 2023). Packaged within a master mix, the amplification reaction employs isothermal equipment integrated with biosensors (Das et al., 2022). These kits are user-friendly, making them ideal for primary healthcare settings and facilitating transport close to patient locations (Atceken et al., 2023).

Despite their convenience, LAMP-based diagnostics face challenges due to the high costs of essential enzymes, which are typically produced in centralized manufacturing laboratories and sourced from biotechnology companies in the global north. This situation is particularly challenging in low- and middle-income countries (LMICs), where cold supply chain limitations and high importation costs hinder the establishment of PoC diagnostic kits. A significant portion of industrial enzymes is produced by companies such as Novozymes, DuPont, DSM, and Roche, which sell their products at premium prices (Fasim et al., 2021). Modified Bst DNA polymerases, including Bst 2.0 and Bst 3.0, are produced by companies like New England Biolabs, which have applied for patents to enhance enzyme efficacy. For instance, the cost of NEB Bst 2.0 DNA polymerase, the most commonly used RT-LAMP enzyme, is approximately $3.38 per reaction, excluding additional reagents. This reliance on international supply chains has made countries more vulnerable during public health crises (Luo et al., 2023).

Recent advancements in low-cost methods for producing diagnostic kits have been observed worldwide. In Peru, Alcántara et al. outlined their affordable SARS-CoV-2 detection techniques utilizing enzymes produced locally by Mendoza-Rojas et al. (Alcántara et al., 2021; Mendoza-Rojas et al., 2021). Similarly, Fomsgaard and Rosenstierne in Denmark investigated alternative SARS-CoV-2 detection methods that did not rely on commercial kits, addressing the international shortages experienced during the COVID-19 pandemic (Fomsgaard and Rosenstierne, 2020). Additionally, Graham et al. in the United States described their open-source approaches for SARS-CoV-2 detection, independent of commercial kits (Graham et al., 2021). Decentralized diagnostic techniques can be rapidly deployed, particularly in resource-limited settings or during fast-moving outbreaks. This underscores the necessity for flexible diagnostic solutions that can be adapted to different contexts, addressing local needs and utilizing available resources (Matthews et al., 2020).

Given the challenges in accessing reagents for diagnostics, particularly in low- and middle-income countries (LMICs), cost-effective manufacturing processes utilizing alternative materials provide a viable solution to meet the growing demand. Several studies have suggested using alternative materials, such as skim milk (Khani and Bagheri, 2020) and lactose (Y. Zhang et al., 2020), as inducers for recombinant protein production. These materials can serve as substitutes for isopropyl β-D-1-thiogalactopyranoside (IPTG) in inducing protein expression in Escherichia coli BL21(DE3). Additionally, there have been studies about cheaper substitutes for extraction components such as lysozyme. Lysozyme aids in cell lysis by cleaving the peptidoglycan cell wall of bacteria through the hydrolysis of 1,4-β-linkages between N-acetyl-muramic acid and N-acetyl-D-glucosamine residues (Callewaert and Michiels, 2010). A previous study found that approximately 3.50% of chicken egg whites consist of lysozyme (Alderton et al., 1944), which can be extracted and purified for protein extraction during cell lysis (Shahmohammadi, 2018).

In this study, we investigated low-cost and accessible alternative reagents, including locally sourced powdered milk as a substitute for IPTG and raw egg whites as an alternative to lysozyme. This approach also allowed us to develop a simpler purification protocol that eliminates the need for secondary heparin purification and employs a lower concentration of BstLF in the RT-LAMP assay. We compared the performance of the home-brewed BstLF produced using this alternative method with that of the commercial Bst 2.0 through functional assays. Additionally, we discussed the cost reductions achieved through these proposed methodologies.

MATERIALS AND METHODS

Expression and purification of BstLF using an alternative method

In this study, portions of the optimized production protocol for BstLF was modified by substituting alternative components for the induction, extraction, and purification processes. Additionally, optimizations to reduce the quantity of home-brewed BstLF used in the RT-LAMP assay were conducted.

Heat shock transformation and PCR verification
The expression vector pET15b_BstLF was used in this study with Addgene accession number 153313. The BstLF gene downstream of the 6x-His tag is controlled by the Lac1 promoter and T7 terminator. The sequence of BstLF (GenBank U23149, UniProt P52026) was obtained from the *G. stearothermophilus* Bst DNA polymerase. The amino acid sequence of BstLF covers amino acids (289-876) of the full-length protein. The plasmid was then used to transform ultracompetent *E. coli* Rosetta (DE3) through heat shock. PCR verification using gene-specific primers was performed to verify the plasmid.

Expression, extraction, and purification of BstLF
To produce BstLF, *Escherichia coli* BL21 (DE3) transformed with pET-15b-BstLF was cultivated at 18°C with shaking at 200 rpm (Rivera et al., 2020). Two brands of powdered full cream milk (PM1 and PM2) were used as an alternative to conventional IPTG, at a concentration of 1% (w/v). Cell cultures were incubated for 16 hours under the same conditions. Additionally, setups supplemented with 1 mM IPTG were prepared as positive controls during the induction process.

The protein extraction process was modified by utilizing egg whites instead of lysozyme. In this extraction method, 10% (v/v) egg white was incorporated into the lysis buffer (100 mM KCl, 25 mM Tris–Cl, pH 8.0). Lysozyme (0.2 mg/mL) was also

included in the lysis buffer as a positive control. BstLF was extracted enzymatically via sonication (Rivera et al., 2020).

For purification, BstLF was processed using Qiagen Nickel Nitrilotriacetic acid (Ni-NTA) agarose resin through affinity chromatography. The protein was eluted with 100 mM imidazole in a one-column volume fraction and characterized by SDS-PAGE. The purified fractions were pooled in a pre-storage buffer containing 50 mM KCl, 12.5 mM Tris-HCl, and 0.1 mM EDTA, and quantified using the Bradford assay with bovine serum albumin as the standard. Ultraviolet-visible (UV–Vis) spectrophotometry was employed to assess potential nucleic acid contamination by examining the A260/A280 nm ratios of the fractions. BstLF was subsequently prepared in a storage buffer composed of 50 mM KCl, 12.5 mM Tris-HCl, 0.1 mM EDTA, 50% (v/v) glycerol, 0.1% Triton® X-100, and 1 mM dithiothreitol (DTT, pH 8.0) (Rivera et al., 2020). Only pure BstLF, as confirmed by SDS-PAGE, was used in the RT-LAMP assays. This protocol established a purification method that does not require a heparin column. The proteins were stored at a higher concentration of approximately 500 ng/μL for scouting the minimum effective concentration (MEC) in the RT-LAMP assay, before dilution to the formulation concentration.

Fluorescent qRT-LAMP for Functional Assay and Scouting for MEC of BstLF in RT-LAMP

The functional assay for BstLF was conducted using an optimized LAMP protocol designed to amplify the nucleocapsid (N) gene of SARS-CoV-2 RNA. The N2 primer set designed by Zhang et al. (Y. Zhang et al., 2020) was employed, utilizing heat-inactivated hCoV-19 RNA from the Omicron variant (NR-56495) as the target sample.

The master mix for the RT-LAMP functional assay was prepared following the specified protocol for Bst 2.0 WarmStart® DNA Polymerase (NEB #M0538) and supplemented with 700 μM dUTP and 0.02 U/μL UDG to prevent carryover contamination (Andersson et al., 2018). For real-time monitoring of amplification, 700 mM betaine was included to enhance the integrity of RNA templates and improve the amplification efficiency of primer sets by minimizing secondary structure formation caused by GC-rich regions (Henke et al., 1997; Jensen et al., 2010). WarmStart® RTx Reverse Transcriptase (NEB #M0380) was added for cDNA synthesis of the SARS-CoV-2 RNA template, and Bst 2.0 WarmStart® DNA Polymerase (NEB #M0538) served as the positive enzyme control. The concentration of BstLF was optimized to establish a lower minimum effective concentration (MEC) that would yield good amplification curves and corresponding cycle threshold (Cq) values. This optimization involved testing different concentrations of BstLF in RT-LAMP, specifically 15.70, 12.0, 9.0, and 6.0 ng/μL.

The reaction was performed using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad #1855195) at an optimal temperature of 65°C for 60 cycles, with each cycle lasting 30 seconds and an initial cDNA synthesis step at 58°C for 10 minutes. Following amplification, the polymerase was inactivated by heating at 90°C for 5 minutes. A melt curve analysis was conducted by gradually increasing the temperature from 65°C to 95°C at 0.5°C/s intervals.

The amplification and melt curve profiles were monitored using CFX Maestro™ (Bio-Rad #12013758). Endpoint detection was performed via electrophoresis on a 2% agarose gel using a 1× Tris–acetate–EDTA buffer, running for 45 minutes at 100 V. The amplification curve of BstLF in fluorescent RT-LAMP, along with the Cq value and agarose gel electrophoresis (AGE) profile of isothermal amplification, were utilized to assess the MEC.

Structural characterization of BstLF and stability analysis

Circular dichroism spectroscopy

Circular dichroism spectroscopy (CD-Spec) analysis was performed using a JASCO J-1000 CD spectropolarimeter from the Protein, Proteomics, and Metabolomics Core Facility of the Philippine Genome Center. The samples were run in CD-Spec using a 1-mM quartz cuvette. The settings for the CD run were 0.50 nm data pitch, 10.0 nm bandwidth, 50 nm/min scanning speed, 0.50 s digital integration time (D.I.T.), and 200–240 nm wavelength range. The collected spectra were processed using the Spectral Manager software. Secondary structure estimation was also performed using the same software with Yang's reference spectra (Sreerama and Woody 2000), a deconvolution standard for proteins.

Homology modeling of the BstLF and secondary structure composition calculation

The modeled structure of BstLF was predicted through iterative threading assembly refinement (I-TASSER) by the Zhang lab (Zhao et al. 2012). The model with the lowest root mean square deviation or RMSD was used as the modelled structure of BstLF. The proportions of alpha helices, beta sheets, and random coils were calculated from the predicted model, and these were used as references to the secondary structure calculated from the CD spectra of the home-brewed BstLF.

Assessment of the home-brewed BstLF storage stability

The storage stability of BstLF was assessed by producing different batches of BstLF, storing the aliquots in −20°C, and then using the stored BstLF in fluorescent real-time qRT-LAMP assay to evaluate any changes in function by assessing C_q values and the AGE profile of produced amplification products.

Cost Analysis

Cost of materials were obtained via quotations obtained from various local suppliers in the Philippines during the year 2023 which were certified by the Ateneo de Manila University.

RESULT AND DISCUSSION

Production of a simpler and lower cost home-brewed BstLF

Conventional protein production workflows typically involve standard reagents, such as isopropyl β-D-1-thiogalactopyranoside (IPTG) for protein induction and lysozyme for protein extraction. However, these components can be quite costly, with IPTG priced at approximately $28.21 per liter for batch expression and lysozyme costing $75.50 for a 0.5 mL stock at a concentration of 50 mg/mL.

The expense of these crucial components often becomes a bottleneck in laboratories, particularly in low-resource settings. Recent efforts have focused on developing protocols to reduce the costs associated with routine protein expression and molecular biology assays (Mendoza-Rojas et al., 2021). For instance, Khani and Bagheri (2020) demonstrated that 1.00% (w/v) skim milk could successfully replace 1 mM IPTG for inducing recombinant FliC protein. Their study found that skim milk not only serves as a viable substitute but may also be a superior alternative due to the toxicity associated with IPTG (Khani and Bagheri, 2020).

In this study, we investigated the potential of powdered full cream milk, which is both cheaper and more accessible than skim milk, to induce protein expression. Initially, we evaluated

the ability of powdered full cream milk to induce expression and compared its effectiveness to that of IPTG. Using Rosetta (DE3) *E. coli* cells transformed with sfGFP, we observed that PM1, while showing a slower initial induction time, achieved comparable induction levels to IPTG after 12 hours (Fig. 1A). Successful induction of BstLF with PM1 and PM2 was confirmed by the appearance of a prominent ~69 kDa band in the SDS-PAGE analysis of the crude lysate from induced cells, similar to the lysates of IPTG-induced cells (Figure 1B and Supplementary Fig. 1). Notably, a cold induction step at 18°C was introduced to enhance the yield of functional BstLF and promote proper protein folding (Bartolo-Aguilar et al., 2022).

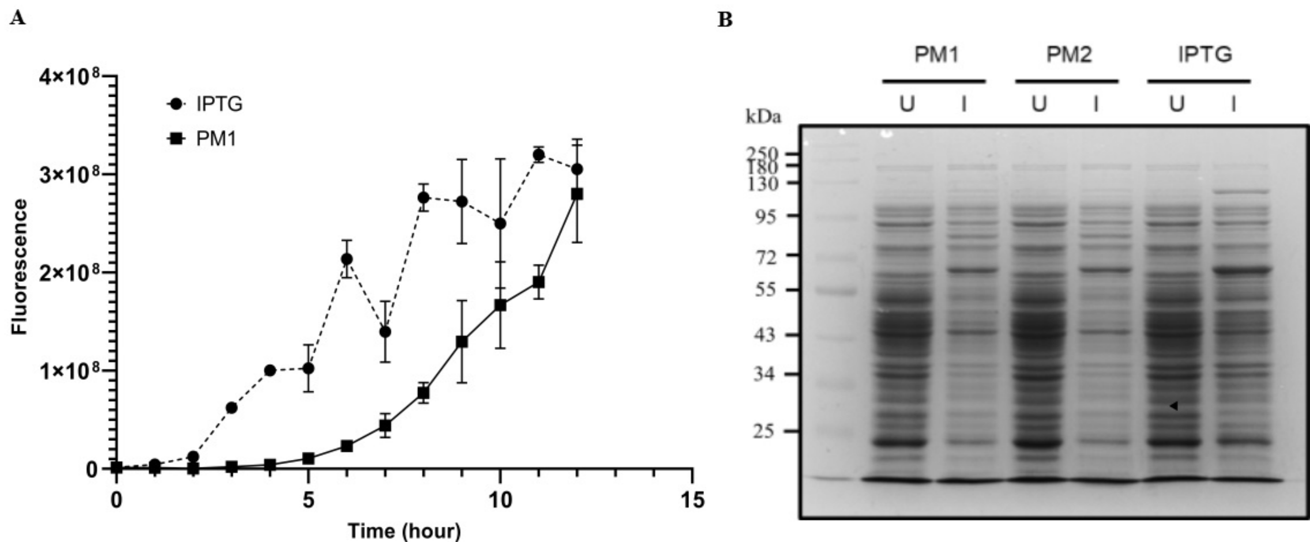


Figure 1: Induction of recombinant BstLF expression via powdered whole milk. (A) Time-course fluorescence expression of sfGFP-producing *E. coli* BL21 (DE3) cells induced with IPTG and powdered whole milk (PM1). All treatment groups were performed in triplicate. (B) SDS-PAGE analysis of uninduced (U) and induced (I) lysates of BstLF-producing *E. coli* BL21 (DE3) cells using two brands of 1.0% (w/v) powdered whole milk (PM1, Bear Brand™ and PM2, Birch Tree™) and 1 mM IPTG as inducers.

Subsequently, we evaluated the use of egg whites as a substitute for lysozyme, alongside the replacement of IPTG with powdered full cream milk. To quantify the yield of purified BstLF, we employed the Bradford assay. As illustrated in Fig. 2A, the protein yield was higher in cultures supplemented with PM1 (9.70 µg BstLF/L culture) and PM2 (11.06 µg BstLF/L culture) compared to the IPTG setup (8.95 µg BstLF/L culture). Statistical analysis indicated no significant difference between the yields observed in the PM1 and IPTG setups. In a separate experiment, we assessed the extraction yield using egg whites compared to pure lysozyme. Fig. 2B shows that the extraction yield with 10.0% (v/v) egg whites EW (8.85 µg BstLF/L culture) is comparable to that of lysozyme LZ (8.94 µg BstLF/L culture), with statistical testing confirming no significant difference between the yields obtained using the two lysis agents.

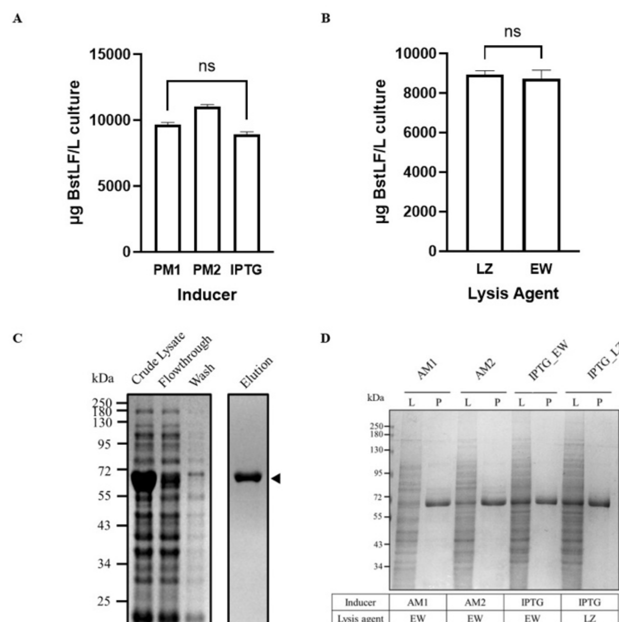


Figure 2: Simplified Ni-NTA Purification of BstLF. (A) Comparison of the purified BstLF yield obtained via induction with either PM1, PM2, or IPTG. (B) Comparison of purified BstLF obtained via lysis using raw egg white versus lysozyme. (C) SDS-PAGE of representative purification fractions eluted with 100 mM imidazole. The putatively purified BstLF is the ~69 kDa recombinant protein indicated in the figure. (D) SDS-PAGE of crude lysates and purified fractions using alternative reagents. The lysis agents used were (EW) - 10% (v/v) raw egg white and (LZ) - 0.2 mg/mL lysozyme. The inducers used were 1.0% (w/v) powdered whole milk (PM1, Bear Brand™ and PM2, Birch Tree™) and 1 mM IPTG. For (A) and (B), Bradford assay was used to quantify the proteins, with N = 3 replicates. Statistical analysis was performed using an unpaired two-tailed T- test; NS indicates P > 0.05

Most home-brewed BstLF is purified using heparin agarose, which aids in the removal of nucleic acids and other contaminating proteins. Heparin, a member of the glycosaminoglycan family, is commonly used as an anticoagulant and is immobilized in resins for routine purification experiments. Heparin resins function as cation exchangers due to their high concentration of anionic sulfate groups (Bolten et al., 2018). Several studies involving home-brewed BstLF have included an additional heparin purification step prior to the RT-LAMP assay. In this study, we present an optimized, alternative method for purifying BstLF using a one-step affinity purification process. Initial optimizations resulted in highly purified BstLF at a 100 mM imidazole concentration, as evidenced by the SDS-PAGE analysis of collected fractions (Fig. 2C). The traditional method typically yields 10 mg of BstLF from 1 L of culture. Notably, BstLF produced using the alternative methods AM1 and AM2, where powdered milk PM1 and PM2 were used respectively were also successfully purified with this method (Fig. 2D).

Nucleic acids present in a purified recombinant protein can hinder downstream processes (X. P. Zhang and Heyer, 2011). To assess potential nucleic acid contamination that could interfere with RT-LAMP, we measured the purity of the samples (Supplementary Table 1). A260/A280 nm values of less than 0.60 are indicative of low nucleic acid contamination. The absence of nucleic acid contamination in the produced BstLF was confirmed using the RT-LAMP assay, where no amplification was observed in the no template control (NTC).

The structure of the recombinant BstLF was modeled using I-TASSER, a homology modeling server designed to predict protein structure and function (Zhao et al., 2012). The top-ranked in silico model (Supplementary Fig. 2A) revealed a predominantly helical structure, with approximately 53% of the modeled BstLF exhibiting alpha-helical conformation. Further characterization of the home-brewed BstLF was performed using a JASCO J-1000 CD spectropolarimeter, which displayed CD spectra between 200 to 240 nm, closely resembling the spectra of Bst 2.0 DNA polymerase (Supplementary Fig. 2B). The CD spectra were deconvoluted to estimate the secondary structure using Yang's reference spectra (Sreerama and Woody, 2000). The secondary structure analysis of the home-brewed BstLF (Supplementary Fig. 2C) confirmed a predominantly alpha-helical structure, consistent with the calculated secondary structure from in silico models. This finding indicates that the expressed BstLF has properly folded.

RT-LAMP of home-brewed BstLF

The BstLF expressed, extracted, and purified through alternative methods was utilized in the RT-LAMP assay to evaluate its activity. A minimum effective concentration (MEC) of BstLF in RT-LAMP was established through initial scouting, which indicated that working concentrations of 12.0 ng/µL and 15.7 ng/µL were effective (Matute et al., 2021). Lower concentrations, specifically 6.0 ng/µL and 9.0 ng/µL, were also evaluated. RT-LAMP assays demonstrated that the 9.0 ng/µL BstLF setup exhibited a similar amplification profile (Supplementary Fig. 3A) and comparable cycle threshold (Cq) values (Supplementary Fig. 3B) to those obtained with the commercial Bst 2.0 DNA polymerase.

Based on these preliminary results, a working concentration of 9.0 ng/µL BstLF was selected for subsequent RT-LAMP assays. The functionality and performance of BstLF produced using milk induction were tested through RT-LAMP. The amplification profiles of BstLF produced with milk induction closely mirrored those of IPTG-induced BstLF, as shown in Fig. 3A, with similar Cq values depicted in Fig. 3B. Specifically, the Cq value for BstLF induced with PM1 was 24.41 ± 0.61, while BstLF induced with PM2 yielded a Cq of 25.90 ± 1.37, and the Cq for IPTG-induced BstLF was 20.70 ± 0.66. As indicated in Table 1, PM1 was chosen as the milk source for the alternative method (AM), as its Cq value was closest to that of IPTG-induced BstLF. Notably, no amplification was observed in the no template control (NTC) of the RT-LAMP assay, suggesting that the home-brewed BstLF may be free of nucleic acid contamination.

Table 1: Cq values of RT-LAMP assay using homebrewed BstLF induced with powdered whole milk and IPTG

Inducer	Cq[d]	N
AM1[a]	24.41 ± 0.61	3
AM2[b]	25.90 ± 1.37	3
OM[c]	20.70 ± 0.66	3

[a]Inducer used during recombinant expression was 1.0 % (w/v) Bear Brand™ powdered full cream milk
[b]Inducer used during recombinant expression was 1.0 % (w/v) Birch Tree™ powdered full cream milk
[c]Inducer used during recombinant expression was 1 mM IPTG
[d]Cq or cycle threshold from fluorescent qRT-LAMP reaction of 200 copies Omicron SARS-CoV-2 amplified through N2 primer set with 9.0 ng/µL BstLF

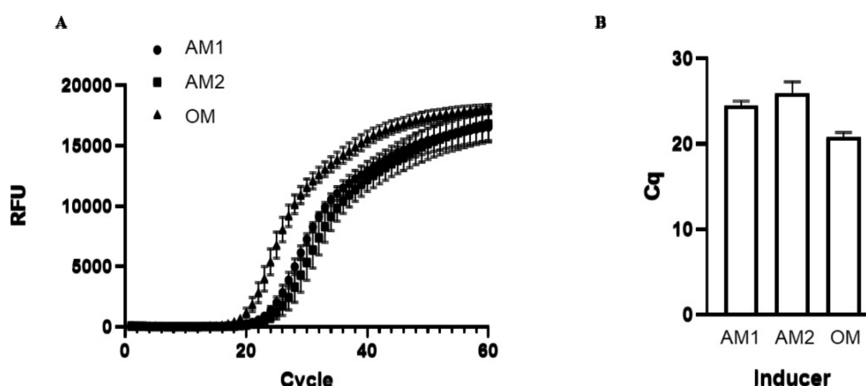


Figure 3: Functional Characterization of BstLF Produced via an Alternative Method through RT-LAMP. (A) Comparison of fluorescent qRT-LAMP amplification curves of BstLF produced through the old method versus the alternative methods AM1 and AM2. The template used consisted of 200 copies of Omicron SARS-CoV-2 amplified using the N2 primer set, with 9.0 ng/µL BstLF and 0.3 U/µL NEB reverse transcriptase (RTx) per reaction. No amplification was observed in the no template control (NTC). (B) Comparison of fluorescent qRT-LAMP Cq values for BstLF produced via the old method (OM), which used lysozyme as a lysis agent and IPTG as an inducer, versus BstLF produced using the alternative methods (AM1 and AM2), where egg whites at 10.0% (v/v) were used as lysis agents and powdered whole milk (PM1 and PM2) were used as inducers, respectively. All treatment groups were performed in triplicate. Statistical analysis was conducted using an unpaired two-tailed T-test, $P < 0.05$.

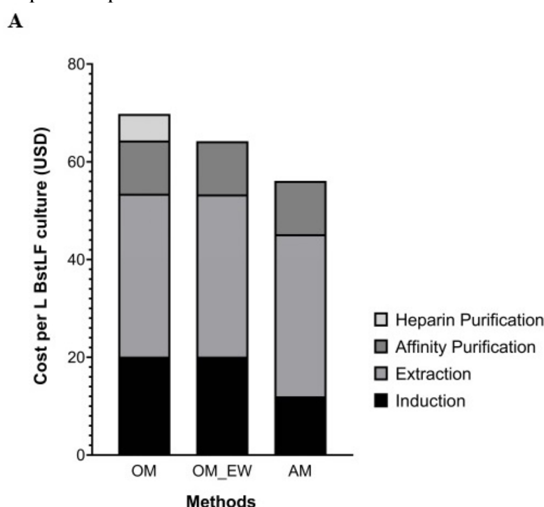
Additionally, the stability of BstLF during liquid storage was evaluated through RT-LAMP assays, with the longest storage period tested being 10 months at -80°C. The amplification profiles of BstLF stored for both 1 month and 10 months (Supplementary Fig. 4A) were similar, as were their corresponding Cq values (Supplementary Fig. 4B). Statistical analysis revealed no significant difference between the Cq values of the 1-month and 10-month BstLF stocks. These consistent amplification curves and Cq values suggest that home-brewed BstLF is stable during liquid storage.

Cost Analysis of a simple home-brewed BstLF and an RT-LAMP mastermix

Price calculations were performed to quantify the cost of producing home-brewed BstLF through alternative methods compared to conventional techniques and its commercial counterparts. Updated prices from 2024 were used in the calculations, incorporating every aspect of the process, including equipment usage, reagents, and consumables.

The cost of producing home-brewed BstLF from a 1 L culture is illustrated in Fig. 4A, showing three approaches: OM (old method), OM_EW (old method with egg white instead of lysozyme), and AM (alternative method). The alternative method encompasses induction using powdered full cream milk (PM1), protein extraction with 10% (w/v) egg white, one-step affinity purification, and a reduced concentration of BstLF (9.0 ng/µL) for the RT-LAMP assay. The overall cost of producing home-brewed BstLF using this methodology was calculated to be $0.18 USD per reaction. Additionally, the cost of an RT-LAMP mastermix incorporating home-brewed BstLF and home-brewed MMLV-RT (with a MEC of 68 ng/µL) was determined to be $0.23 USD per reaction.

A



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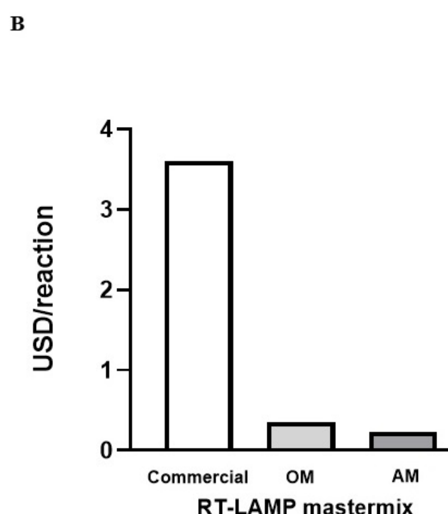

Figure 4: Cost Analysis of an Alternative Method for Home-Brewed BstLF Production. (A) Cost reduction associated with the alternative method per 1 liter of BstLF culture. OM represents the old method of producing home-brewed BstLF, while OM_EW indicates the old method that uses 10% (w/v) egg white as a lysis agent. AM refers to the alternative method, which incorporates all proposed alternative components and methodologies outlined in this study. (B) Cost comparison of RT-LAMP mastermixes: the commercially available NEB WarmStart® LAMP Kit, OM (the home-brewed RT-LAMP mastermix using the old method), and AM (the home-brewed RT-LAMP mastermix produced through the alternative method).

Comparative analysis revealed that the costs of the home-brewed one-step RT-LAMP mix from the OM (old method) were compared to the cost from the work of Matute et al., which amounted to $0.36 USD per reaction (Matute et al., 2021). The RT-LAMP mix produced using the alternative method was calculated to be $0.21 USD per reaction. It is anticipated that home-brewed BstLF intended for commercial use will undergo extensive quality assessment tests, including third-party verification, mass spectrometry for protein identification and purity determination, and additional tests necessary to ensure the enzyme's quality before commercialization. Quality assurance processes, which encompass functional and structural testing, account for 74.77% of the production cost for 1 L of BstLF culture (Supplementary Fig. 5). However, the limited availability of laboratories that can provide such services poses challenges for those looking to enter the enzyme production field.

Overall, the calculated cost of the RT-LAMP mix using home-brewed BstLF was $0.21 USD per reaction. When comparing the prices of the home-brewed kit to the NEB WarmStart® LAMP Kit, which costs approximately $3.60 USD per reaction, significant cost savings are evident. Figure 4B displays a comprehensive comparison of RT-LAMP mixes made with (1) commercial Bst 2.0 DNA polymerase, (2) the home-brewed BstLF approach outlined by Matute et al. (the OM), and (3) the two home-brewed BstLF methods, which demonstrate a cost reduction of 93.61% and 94.17% compared to commercial Bst 2.0 DNA polymerase.

CONCLUSION

Overall, this study proposes a low-cost home-brewed BstLF produced through an alternative process that incorporates full cream powdered milk induction, protein extraction using egg whites as a lysis agent, one-step affinity purification, and a reduced BstLF concentration in the RT-LAMP assay. The home-brewed BstLF demonstrated performance comparable to that of commercial Bst 2.0 DNA polymerase, exhibiting similar amplification profiles and stability in liquid storage for up to 10 months.

A cost analysis revealed that the expense of one RT-LAMP reaction utilizing the research-teaching home-brewed BstLF is $0.21 USD. In contrast, the commercial Bst 2.0 WarmStart® LAMP Kit costs approximately $3.60 USD per reaction, resulting in a 94.17% reduction in cost when using the home-brewed BstLF in RT-LAMP assays. The implementation of the home-brewed RT-LAMP kits can help fill gaps and expand diagnostic capabilities in the Philippines.

Future investigations that will focus on the optimization and quality control of BstLF produced using this low-cost method is recommended. This includes the impact of different buffers—both commercial and home-brewed—on enzyme functionality and overall cost. Furthermore, mass spectrometry can be employed for thermal degradation analysis and purity determination. Further tests regarding sensitivity and specificity of the home-brewed RT-LAMP kit can also be evaluated through testing with clinical samples and samples from other infectious diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

EAM served as the corresponding author and led the development of novel concepts presented in this study, including the use of alternative reagents and the design of a simplified purification protocol. He conducted experiments to demonstrate the efficacy of the alternative reagents, supervised the experimental procedures, validated the results, and most of the revisions during the peer review process. MVSP performed experiments related to BstLF production using traditional methods, conducted RT-LAMP assays, and conducted the physicochemical tests. He also carried out the cost analysis and wrote the initial draft of the manuscript. VAC was responsible for BstLF batch production, executed RT-LAMP assays, contributed to the manuscript, and assisted in verifying the experimental results. JG aided in the preliminary experiments, provided support for the cost analysis, and contributed to the development of the manuscript. CL assisted in conducting the experiments and contributed to the revision process of the manuscript. MZ played a key role in optimizing the RT-LAMP assays, refining the results, and proofreading the manuscript.

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