Original Article

Cost-effective in-house COVID-19 reverse transcription-polymerase chain reaction testing with yeast-derived *Taq* polymerase

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Abstract:

BACKGROUND: Despite the decline of the COVID-19 pandemic, there continues to be a persistent requirement for reliable testing methods that can be adapted to future outbreaks and areas with limited resources. While the standard approach of using reverse transcription-polymerase chain reaction (RT-PCR) with *Taq* polymerase is effective, it faces challenges such as limited access to high-quality enzymes and the presence of bacterial DNA contamination in commercial kits, which can impact the accuracy of test results.

METHODS: This study investigates the production of recombinant *Taq* polymerase in yeast cells and assesses its crude lysate in a multiplex RT-PCR assay for detecting the SARS-CoV-2 RNA-dependent RNA polymerase *(RdRP)* and *N* genes, with human *Ribonuclease P* serving as an internal control.

RESULTS: The unpurified yeast *Taq* polymerase demonstrates sensitivity comparable to commercially purified bacterial *Taq* polymerase and unpurified bacterial counterparts in detecting the *RdRP* and *N* genes. It exhibits the highest specificity, with 100% accuracy, for the *N* gene. The specificity for the *RdRP* gene closely aligns with that of commercially purified bacterial *Taq* polymerase and unpurified bacterial *Taq* polymerase.

CONCLUSIONS: The use of unpurified recombinant yeast *Taq* polymerase shows promise as a cost-effective approach for conducting in-house COVID-19 RT-PCR testing. By eliminating the need for chromatography purification steps, the production of RT-PCR kits can be streamlined, potentially improving accessibility and scalability, especially in resource-limited settings and future pandemics.

Keywords:

COVID-19, DNA contamination, recombinant yeast *Taq* polymerase, reverse transcription-polymerase chain reaction sensitivity, reverse transcription-polymerase chain reaction specificity, SARS-CoV-2, *Taq* polymerase

The COVID-19 pandemic has presented unprecedented challenges, highlighting the crucial need for accurate and efficient detection methods for SARS-CoV-2 infections. This has led to the development of robust testing approaches.[1-6] Among these approaches, reverse transcription-polymerase chain reaction (RT-PCR) has emerged as the gold standard due to its ability to sensitively and specifically identify viral RNA in patient samples.[1-3,7-12] However, the precision of RT-PCR results can be compromised by potential contamination of commercial *Taq* polymerases with small amounts of bacterial DNA, which can result in false-positive results.[13-16]

The issue of DNA contamination is a common challenge in the production

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of recombinant thermostable DNA polymerases for commercial use. This is primarily due to the complexities involved in the manufacturing processes and incomplete purification. Although these polymerases are typically produced using *Escherichia coli*, the contaminating DNA may originate from various bacterial sources, which poses a persistent problem.[17] Despite the use of physical, chemical, and enzymatic treatments to eradicate this contamination,[18-20] a universally applicable method for completely eliminating bacterial DNA from thermostable DNA polymerase preparations has yet to be established by the scientific community. Furthermore, the use of numerous decontamination procedures may negatively impact the performance and sensitivity of the polymerase, leading to a decrease in the accuracy of RT-PCR results.

Yeast genome organization is distinct from that of viruses and bacteria, with unique features such as the presence of stable genome structure.[21] This organization is influenced by physical tethering, volume exclusion, and DNA-encoded nucleosome organization.[22,23] The yeast genome also exhibits a Rabl configuration and extensive regional and higher-order folding.[24] Genome evolution in yeasts is driven by mechanisms such as tandem gene repeat formation, segmental duplication, genome duplication, gene loss, displacements, and relocations.[25-27] Thus, to tackle the issue of bacterial DNA contamination in thermostable DNA polymerase preparations, a viable solution could be the use of recombinant yeast-derived thermostable DNA polymerase. This enzyme carries yeast DNA, differing from bacterial and viral DNA, providing a potential solution to contamination issues. Although prior studies have successfully purified thermostable DNA polymerase from yeast, its applicability in the context of viral RT-PCR detection, particularly for SARS-CoV-2, remains unexplored.[27] Therefore, a comprehensive examination is imperative to assess the accuracy and effectiveness of yeast-produced thermostable DNA polymerase in detecting SARS-CoV-2.

This study seeks to assess the efficacy of a recombinant yeast-derived thermostable DNA polymerase in a multiplex SARS-CoV-2 RT-PCR assay, comparing its performance with a bacterial enzyme and a commercially available master mix containing purified bacterial *Taq* polymerase. Such a comparative analysis aims to improve the accuracy and reliability of SARS-CoV-2 testing methodologies.

Methods

Preparation of bacterial *Taq* polymerase crude extract

The *Taq* polymerase gene (GenBank: J04639.1) was artificially synthesized and incorporated into the pD454-SR bacterial expression vector as previously described.[28] The enzyme pellet was dissolved in 2 mL of storage buffer (50 mM Tris-HCl, pH 7.9; 50 mM KCl; 0.1 mM ethylenediaminetetraacetic acid; 1 mM DTT; 0.5 mM PMSF; and 50% glycerol) and stored at −20°C.

Preparation of yeast Taq polymerase crude extract

Atum. bio (Newark, CA, USA) synthesized and cloned the *Taq* polymerase gene into the pD1204 (GAL1-Ura3) yeast expression vector. The plasmid vector was then transformed into *Saccharomyces cerevisiae* strain (ura3-52) using a yeast transformation kit from Sigma (Cat. Yeast1). The transformed yeast colonies containing the *Taq* polymerase gene were selected using SC selective medium containing 0.5% ammonium sulfate, 2% glucose, and 2% agarose, along with 0.17% yeast nitrogen base without amino acids. The isolated colonies were cultured and expressed in 200 mL of SC-galactose medium supplemented with 100 mg/L gentamicin and 50 mg/L kanamycin, at 28°C for 72 h. Yeast cells were then harvested by centrifugation at 5000 rpm for 10 min and washed three times with sterile water. To obtain the *Taq* polymerase enzyme, three grams of *Taq* polymerase-producing yeast cells were resuspended in 3 mL of ice-cold prelysis buffer (50 mM Tris-HCl, pH 7.5, and 50 mM KCl), and 6 g of glass beads were added. The cell lysate was agitated vigorously using a vortex mixer at 4°C. After centrifugation at 12,000 rpm for 20 min, the insoluble fraction was resuspended in 30 mL of lysis buffer, and sonication-freezing-thawing steps were performed. The lysate was heated, and the enzyme was precipitated using ammonium sulfate precipitation. The enzyme pellet was dissolved in 2 mL of storage buffer at −20°C.

Determination of *Taq* polymerase enzyme activity

As previously described,[29] the real-time PCR assay was utilized to determine the activity of the *Taq* polymerase enzyme. To compare the activity of the synthesized *Taq* polymerase enzyme with that of a commercial *Taq* DNA polymerase from Sigma-Aldrich (Saint Louis, MO, USA), multiple titrations were conducted. The assay employed oligonucleotide primer UPlong (TTCCCAGTCACGACGTT GTAAAACGACGGCCAGTG) and M13 mp18 Single-stranded DNA (New England Biolabs, NEB, Massachusetts, USA).

Assessment of bacterial and yeast *Taq* polymerase enzyme crude extracts by silver staining in SDS-PAGE analysis

Protein samples for SDS-PAGE were prepared using a modified standard protocol as described previously.[30] Protein concentration was determined by BCA assay. Each sample (20 μg) was mixed with sample buffer and

incubated. Electrophoresis was run at 15 mA using a Protean II chamber. After staining and fixing, protein bands were visualized and analyzed using a Bio-Rad ChemiDocTM Imaging System.

Assessment of bacterial and yeast *Taq* polymerase DNA contamination

To assess DNA contamination in eukaryotic (yeast) and prokaryotic (bacterial) *Taq* polymerase enzymes, PCR was conducted with specific primers targeting the bacterial 16S rRNA gene and yeast 18S rRNA gene [Table 1]. The 25 µL PCR master mix included ×1 PCR master mix, 0.4 µM of each primer, and 0.5 U of Taq polymerase. Thermal cycling involved initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C (16S) and 58°C (18S) for 1 min, and extension at 72°C for 1 min. A final extension step occurred at 72°C for 5 min. Positive controls with *E. coli* and *S. cerevisiae* DNA, along with a negative control, were included. Amplification used an Applied Biosystems thermal cycler, and agarose gel analysis followed PCR to detect DNA contamination.

SARS-CoV-2 real-time reverse transcription-quantitative polymerase chain reaction optimization

Nasal swab samples (105 samples) that had been preserved in universal transport medium tubes at −80°C at the Department of Pathology and Laboratory Medicine at King Abdulaziz Medical City, central laboratories in Riyadh, KSA, were utilized in this study. RNA extraction was carried out using the automated MagNA Pure 96 system (Roche, Germany) according to their protocol. The extracted RNA, obtained from 200 µL of the original sample, was then eluted in 50 µL and used as a template for SARS-CoV-2 detection using the RealStar SARS-CoV-2 RT-PCR kit from Altona Diagnostic (GmbH, Germany). The precision and effectiveness of both yeast and bacterial *Taq* polymerase enzymes in detecting SARS-CoV-2 were evaluated by calculating positivity and negativity rates. These rates were then compared to the results obtained using a commercially available master mix kit containing purified *Taq* polymerase (TaqPathTM from Life Technologies). To amplify three specific genes listed in Table 1, a previously validated multiplex SARS-CoV-2 real-time reverse-transcriptase polymerase chain reaction master mix was utilized.[28] This assay includes the amplification of the human *Ribonuclease P* (RP) gene as an internal control, in addition to two viral genes (RNA-dependent RNA polymerase [*RdRP*] and nucleocapsid [*N*]) for the detection of the virus. In the case of the in-house *Taq* polymerase, the enzymes were added to the master mix, resulting in a total volume of 20 µL. All assays were subjected to uniform thermal cycling conditions, which included a reverse transcription step at 50°C for 10 min, denaturation at 95°C for 2 min, and 40 cycles of denaturation at 95°C for 15 s, followed by annealing/extension at 58°C for 45 s. The amplification process was carried out using a Bio-Rad CFX96 Real-Time PCR Detection System, and the resulting Ct (Cycle threshold) values were carefully analyzed.

Ethics approval and sample collection

The study was approved by the Institutional Review Board of Alfaisal University (protocol code IRB-20035). A total of 105 RNA COVID-19 frozen samples were tested.

Statistical analysis

The sensitivity, specificity, and accuracy of the multiplex SARS-CoV-2 RT-PCR assay were evaluated using MedCalc statistical software (MedCalc Software Ltd, Belgium). True positives, true negatives (TNs), false positives (FPs), false negatives, Sensitivity (rate of true positives in SARS-CoV-2-positive samples), specificity (rate of TNs in SARS-CoV-2-negative samples), and accuracy (overall correctness of the results) were all calculated. Each calculated measure is accompanied by a 95% confidence interval (CI).

Table 1: List of primers used for the detection of SARS-CoV-2

Primer	Sequence (5'–3')	Target gene
16S-sense	GACCTCGGTTTAGTTCACAGA	16S rRNA
16S-antisense	CACACGCTGACGCTGACCA	
18S-sense	ATACCGTCGTAGTCTTAACCA	18S rRNA
18S-antisense	GTCAATTCCTTTAAGTTTCAGCCT	
RdRP-sense	GTGAAATGGTCATGTGTGGCGG	SARS-CoV-2, RNA-dependent RNA polymerase gene
RdRP-antisense	CAAATGTTAAAAACACTATTAGCATA	
RdRP-probe	CAGGTGGAACCTCATCAGGAGATGC	
N-sense	CTGCAGATTTGGATGATTTCTCC	SARS-CoV-2, nucleocapsid gene
N-antisense	CCTTGTGTGGTCTGCATGAGTTTAG	
N-probe	ATTGCAACAATCCATGAGCAGTGCTGACTC	
RP-sense	AGATTTGGACCTGCGAGCG	Human RNase P gene
RP-antisense	GAGCGGCTGTCTCCACAAGT	
RP-probe	TTCTGACCTGAAGGCTCTGCGC	

Results

Assessing bacterial and yeast *Taq* polymerase enzyme crude extracts using silver staining

The comparison between crude bacterial and yeast *Taq* polymerase enzymes and commercially purified bacterial *Taq* polymerase, with a focus on size and the presence of contaminating proteins, revealed significant differences. The use of heat denaturation and ammonium sulfate precipitation for crude yeast *Taq* polymerase resulted in a distinct pattern of contaminating proteins compared to crude bacterial *Taq* polymerase, as depicted in Figure 1. Even after chromatography purification, the commercial bacterial *Taq* polymerase still contained contaminating proteins and/or DNA, which raises concerns about the effectiveness of the purification methods used. This suggests that there may be limitations in removing contaminating proteins and/or DNA. These findings highlight the variability in composition and purity of *Taq* polymerase based on its source and the purification methods used. It is important to consider these differences when selecting a *Taq* polymerase for specific applications, as the presence of contaminating proteins and/or DNA may affect the enzyme's performance and reliability in subsequent assays or reactions.

Assessment of bacterial and yeast DNA contamination in *Taq* polymerase enzymes using 16S and 18S rRNA primers

Figure 2 displays the results of the assessment of bacterial DNA contamination in the bacterial and yeast *Taq* polymerase enzymes using 16S rRNA primers that amplify an amplicon with a size of 585 bp, targeting a conserved region in bacterial genomes. Lanes 2 and 3 represent the crude extract of bacterial *Taq* polymerase, with Lane 2 representing the presence of 1 ng of *E. coli* DNA and Lane 3 representing the absence of *E. coli* DNA. Lanes 4 and 5 represent the crude extract of yeast *Taq* polymerase, with Lane 4 representing the presence of 1 ng of *E. coli* DNA and Lane 5 representing the absence of *E. coli* DNA. The results indicate that the yeast *Taq* polymerase used in this experiment is not contaminated with bacterial DNA, as evidenced by the absence of a band at the expected size (585 bp) in Lane 5. In contrast, the bacterial crude extract of *Taq* polymerase has DNA contamination, as evidenced by the presence of a band at the expected size (585 bp) in Lane 3. Similarly, Figure 3 suggests that the bacterial *Taq* polymerase used in the experiment is not contaminated with yeast DNA. The presence or absence of a band at the expected size (147 bp) in each lane indicates the presence or absence of the 18S rRNA amplicon.

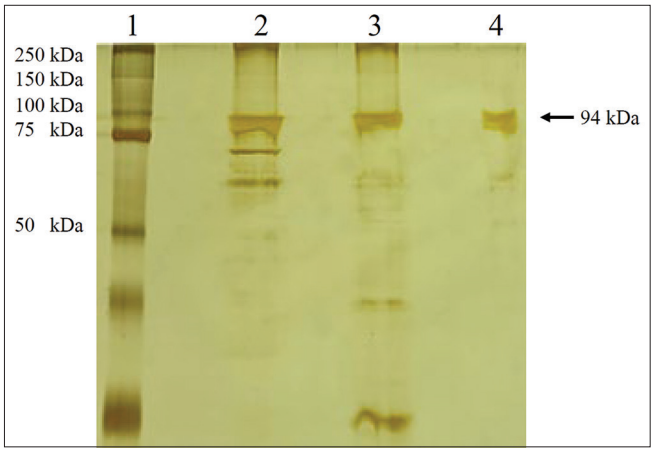

Figure 1: The SDS-PAGE analysis presented displays the bacterial and yeast *Taq* polymerase enzymes, as well as the commercial *Taq* polymerase obtained from Bio Basic Inc. (Ontario, Canada). Lane 1 of the gel represents the molecular weight marker, while Lane 2 and Lane 3 exhibit the crude extract of bacterial and yeast *Taq* polymerase, respectively. Lane 4 displays the purified commercial *Taq* polymerase from Bio Basic Inc. The analysis indicates that the heat denaturation and ammonium sulfate precipitation of yeast *Taq* polymerase resulted in distinct contaminating proteins and/or DNA when compared to crude bacterial *Taq* polymerase. The molecular weight marker used in this analysis is the Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards from Bio-Rad, with the catalog number 1610375

Comparing the performance of recombinant yeast and bacterial *Taq* polymerases with commercial purified *Taq* polymerase for SARS-CoV-2 detection

Supplementary Table 1 summarizes the performance of three distinct RT-PCR assays in the detection of SARS-CoV-2. In Table 2, the results for each assay are presented. The sensitivity of the crude recombinant yeast and bacterial *Taq* polymerases was found to be 73.68% (60.34%–84.46%), while the commercial PCR master mix exhibited a slightly higher sensitivity of 78.95% (66.11%–88.62%). However, all three enzymes demonstrated an exact specificity of 98.25% (90.61%–99.96%), indicating a high level of specificity in correctly

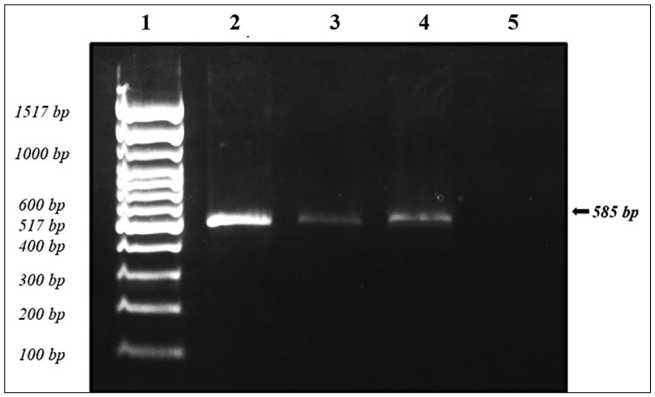

Figure 2: Evaluation of Bacterial DNA Contamination in *Taq* Polymerase Enzymes Using 16S rRNA Primers. Lane (1) displays the molecular weight marker from New England Biolabs #N3231. Lanes (2) and (3) illustrate the crude extract of bacterial *Taq* polymerase in the presence of 1 ng of *Escherichia coli* DNA (Lane 2) or its absence (Lane 3). Similarly, Lanes (4) and (5) depict the crude extract of yeast *Taq* polymerase in the presence of 1 ng of *E. coli* DNA (Lane 4) or its absence (Lane 5). The absence of a band at the expected size (585 bp) in Lane 5 indicates the absence of detectable 16S rRNA in the yeast *Taq* polymerase crude extract

identifying TNs. In contrast, the recombinant yeast *Taq* polymerase displayed a sensitivity of 98.25% (90.61%–99.96%) in the amplification of the *N* gene, comparable to the recombinant bacterial and commercial PCR master mix with purified bacterial *Taq* polymerase. Moreover, the recombinant yeast *Taq* polymerase achieved the highest specificity of 100.00% (92.60%–100.00%), correctly identifying all TNs without any FPs. On the other hand, the specificity of the recombinant bacterial *Taq* polymerase was 89.58% (77.34%–96.53%), and the commercial PCR master mix exhibited a specificity of 60.42% (45.27%–74.23%), indicating a higher rate of FPs. The sensitivity for human RP (IC) was comparable among the different *Taq* polymerases while the specificity could not be calculated as there is no FP detected in the three *Taq* polymerases tested. The performance differences among yeast *Taq* polymerase, bacterial *Taq* polymerase, and commercial *Taq* polymerase for each of the two genes were not statistically significant, as evidenced by highly overlapping CIs. Overall, the results suggest that the recombinant yeast *Taq* polymerase presents a viable alternative for SARS-CoV-2 RT-PCR assays with consistently high specificity despite slightly lower sensitivity in *RdRP* gene amplification compared to the commercial master mix. This indicates that the unpurified recombinant yeast *Taq* polymerase may be considered for SARS-CoV-2 detection, offering an advantageous option for assays where specificity is of paramount importance.

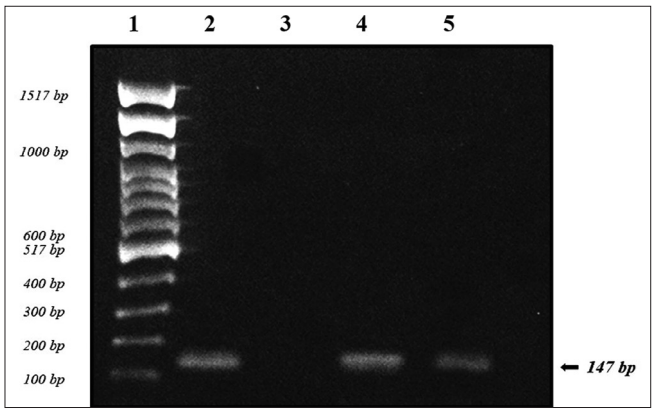

Figure 3: Evaluation of Yeast DNA Contamination in Bacterial and Yeast *Taq* Polymerase Enzymes Using 18S rRNA Primers. The selected primers target a conserved region in yeast genomes, aiming to amplify an amplicon sized at 147 bp. Lane 1: Molecular weight marker; Lane 2: Crude extract of bacterial *Taq* polymerase in the presence of 1 ng of yeast DNA; Lane 3: Crude extract of bacterial *Taq* polymerase without yeast DNA; Lane 4: Crude extract of yeast *Taq* polymerase in the presence of 1 ng of yeast DNA; Lane 5: Crude extract of yeast *Taq* polymerase without yeast DNA

Discussion

The presence of trace amounts of bacterial DNA contamination poses a significant challenge in achieving heightened sensitivity, reliable detection, and establishing unequivocal negative controls in PCR protocols that utilize commercially available *Taq* polymerases. This challenge arises due to the inadvertent amplification of bacterial DNA during the PCR process, which can lead to potential FPs and a subsequent reduction in detection thresholds.

The sources of bacterial DNA contamination in commercial *Taq* polymerases are multifaceted and can potentially originate from various elements within the laboratory environment during the complex protein purification process. Although the conventional production of thermostable DNA polymerase often involves *E. coli*, it is important to note that the contaminating DNA is not exclusively derived from the host cells. Instead, it may stem from diverse bacterial sources, which adds complexity to the identification and elimination of such contaminants. This multifactorial nature of contamination highlights the intricate challenges in maintaining the purity of commercial *Taq* polymerases for optimal PCR performance.

Currently, there is no universally applicable technique established for completely removing bacterial DNA from

Table 2: Comparison of the accuracy of yeast-derived *Taq* polymerase to a recombinant bacterial and a commercial *Taq* polymerase master mix, *Taq*Path™ from Life Technologies, for detection of SARS-CoV-2 RNA-dependent RNA polymerase and *N* genes in 105 subjects

	TP	TN	FP	FN	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
Yeast *Taq* polymerase-*RdRP*	42	48	0	15	73.68% (60.34%–84.46%)	100.00% (92.60%–100.00%)	85.71% (77.53%–91.78%)
Bacterial *Taq* polymerase-*RdRP*	42	48	0	15	73.68% (60.34%–84.46%)	100.00% (92.60%–100.00%)	85.71% (77.53%–91.78%)
Commercial master mix-*RdRP*	45	47	1	12	78.95% (66.11%–88.62%)	97.92% (88.93%–99.95%)	87.62% (79.76%–93.24%)
Yeast *Taq* polymerase-*N*	56	48	0	1	98.25% (90.61%–99.96%)	100.00% (92.60%–100.00%)	99.05% (94.81%–99.98%)
Bacterial *Taq* polymerase-*N*	56	43	5	1	98.25% (90.61%–99.96%)	89.58% (77.34%–96.53%)	94.29% (87.98%–97.88%)
Commercial master mix-*N*	56	29	19	1	98.25% (90.61%–99.96%)	60.42% (45.27%–74.23%)	80.96% (72.13%–87.96%)
Yeast *Taq* polymerase-RP (IC)	103	0	0	2	98.10% (93.29%–99.77%)		
Bacterial *Taq* polymerase-RP (IC)	102	0	0	3	97.14% (91.88%–99.41%)		
Commercial master mix-RP (IC)	105	0	0	0	100.00% (96.55%–100.00%)		

The TP, TN, FP, and FN results are shown for each assay, along with sensitivity, specificity, and accuracy, reported with their respective 95% confidence intervals. TP=True positive, TN=True negative, FP=False positive, FN=False negative, CI=Confidence interval, *RdRP*=RNA-dependent RNA polymerase, *N*=Nucleocapsid protein, IC=Internal control

preparations of thermostable DNA polymerase. Various approaches have been investigated in an effort to reduce bacterial DNA contamination in these preparations, including physical, chemical, and enzymatic treatments. These methods involve the use of techniques such as restriction endonuclease digestion, DNase I digestion, ethidium monoazide treatment, ultraviolet (UV) irradiation, psoralens with long-wave UV light, and ultrafiltration.[18-20,31] However, the effectiveness of these methods in consistently achieving decontamination results has been inconsistent, and some of them may negatively impact the performance of thermostable DNA polymerase, leading to reduced sensitivity and reliability of PCR amplification. As a result, there is an urgent need in the field of PCR for the development of reliable and efficient decontamination methods specifically designed for commercial *Taq* polymerases.

In contrast, thermostable DNA polymerases obtained from eukaryotes are produced using eukaryotic host cells and undergo purification using different techniques, which can potentially decrease the risk of bacterial DNA contamination. The addition of antibacterial drugs during the manufacturing process can be used to hinder or eliminate bacteria, thereby further reducing the presence of bacterial DNA in the final product. However, it is important to note that while antibacterial drugs can minimize bacterial DNA, they do not completely eliminate it. Therefore, careful consideration and timing are necessary during the manufacturing process. Previous studies have shown that the use of eukaryote-derived thermostable DNA polymerases results in an enzyme with reduced bacterial contamination. However, their effectiveness in applications such as viral or bacterial PCR detection has not been thoroughly evaluated thus far.[32] If proven successful, this approach could open up possibilities for the development of a wide range of powerful applications in various fields, enabling the sensitive and reliable detection of bacteria and viruses without the need for lengthy purification steps to obtain highly pure *Taq* polymerase.

The architectural blueprint of DNA varies significantly across different domains of life. Prokaryotes possess compact circular DNA molecules that lack histones and are anchored to the cell membrane.[33] In contrast, eukaryotes store their linear DNA within the nucleus, intricately wrapped around histone proteins.[34] Yeasts, a type of eukaryote, have a unique genome organization influenced by physical tethering, volume constraints, and DNA-encoded nucleosome positioning.[22] This organization is further shaped by forces such as tandem repeats, gene duplications, and gene loss.[35] Viruses, which lack essential life functions, can still undergo horizontal gene transfer, altering their genomes.[23] Eukaryote-made thermostable DNA polymerase has been proposed as a solution to minimize bacterial DNA contamination in conventional bacterially-made thermostable DNA polymerase, potentially enhancing the sensitivity and reliability of PCR in detecting viral infections such as SARS-CoV-2.[32] This is due to the different genome organization of eukaryotic cells compared to bacteria and viruses. However, further research is needed to evaluate the efficacy and practical applications of this approach. Other studies have focused on the isolation and characterization of thermostable DNA polymerases from various sources, including the archaeon; *Archaeoglobus fulgidus*,[36] the hyperthermophilic archaeum; *Thermococcus litoralis* Sh1AM,[37] and a viral metagenome.[38] These studies have highlighted the potential of these enzymes for PCR applications, but their specific role in reducing bacterial DNA contamination has not been directly addressed.

The results of our study indicate that crude recombinant yeast *Taq* polymerase shows potential as a feasible substitute for bacterial *Taq* in the detection of SARS-CoV-2, especially when targeting the N gene. Its exceptional specificity presents an opportunity to decrease the occurrence of FP results, which is crucial for precise diagnosis. Nevertheless, additional investigation is required to validate its effectiveness and broader applicability in the detection of SARS-CoV-2 and other pathogens. The utilization of locally assembled COVID-19 reverse transcription-quantitative polymerase chain reaction kits incorporating crude yeast *Taq* extracts could substantially reduce expenses, thereby paving the way for sustainable and accessible diagnostic testing for various infectious diseases.

Conclusions

Our investigation reveals that the crude yeast Taq polymerase exhibits comparable sensitivity and specificity to both purified and unpurified bacterial *Taq* polymerase in the detection of the *RdRP* gene in SARS-CoV-2 RT-PCR. Crude yeast *Taq* polymerase demonstrated 100% specificity for the *N* gene, which proves its value in minimizing false positive (FP) results. This not only simplifies the manufacturing process of testing kits but also reduces the occurrence of FPs for both bacterial and viral pathogens.

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Conflicts of interest

The authors state that they do not have any known financial interests or personal relationships that could

have been perceived to influence the findings reported in this paper.

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Supplementary Table 1

Supplementary Table 1: Ct values of three SARS-CoV-2 rRT-PCR assays using different Taq polymerases: Yeast, bacterial, and a commercially available Taq polymerase master mix

Sample#	RealStar SARS-CoV-2 RT-PCR kit Results	Results from TaqPathTM from Life Technologies			Bacterial Taq Polymerase			Yeast Taq Polymerase		
		IC	Rdrp	N	IC	Rdrp	N	IC	Rdrp	N
1	POSITIVE	24.0	26.0	24.5	23.7	25.7	23.4	23.9	25.6	23.3
2	POSITIVE	32.1	18.0	16.9	15.6	20.3	15.9	13.5	17.4	15.9
3	POSITIVE	25.9	>45	29.3	24.5	22.4	29.8	25.7	1.1	29.8
4	POSITIVE	27.3	18.5	17.5	13.7	17.2	17.1	14.0	18.4	17.1
5	POSITIVE	27.1	17.1	14.6	11.7	16.5	13.6	11.9	16.8	13.6
6	POSITIVE	23.4	19.3	18.4	27.5	18.0	17.2	28.1	18.2	17.2
7	POSITIVE	21.7	22.1	20.4	21.5	20.0	19.4	21.9	21.2	19.4
8	POSITIVE	27.5	18.4	15.1	12.5	18.3	14.1	12.5	18.5	14.1
9	POSITIVE	26.3	23.1	21.0	>45	22.6	19.5	>45	22.5	19.6
10	POSITIVE	24.5	17.5	15.1	12.4	16.7	13.9	12.7	16.8	13.8
11	POSITIVE	22.2	22.2	20.5	23.7	22.6	19.3	23.5	22.4	19.2
12	POSITIVE	23.6	17.9	15.3	12.6	17.4	14.3	12.7	17.4	14.1
13	POSITIVE	19.1	22.4	20.6	19.3	23.8	19.3	19.3	28.7	19.5
14	POSITIVE	26.5	15.3	13.4	10.9	14.8	12.5	11.3	14.9	12.6
15	POSITIVE	18.9	>45	23.2	19.0	>45	22.4	18.7	>45	22.4
16	POSITIVE	28.3	17.6	15.5	13.3	16.5	14.5	13.5	18.1	15.0
17	POSITIVE	19.9	>45	21.4	20.2	>45	>45	20.1	>45	20.5
18	POSITIVE	23.3	22.1	20.4	26.5	21.1	19.5	25.3	21.0	18.9
19	POSITIVE	23.0	>45	28.4	23.3	>45	30.5	23.4	>45	34.3
20	POSITIVE	23.0	26.1	23.9	23.2	26.3	22.2	23.4	26.6	22.3
21	POSITIVE	22.6	>45	27.4	23.0	>45	27.4	23.1	>45	28.0
22	POSITIVE	27.0	22.8	21.8	4.5	23.4	21.3	8.5	23.2	21.3
23	POSITIVE	24.3	35.7	26.9	25.1	>45	26.2	25.7	>45	26.7
24	POSITIVE	19.7	>45	31.6	20.4	>45	21.3	20.3	>45	20.6
25	POSITIVE	26.3	21.4	19.0	>45	20.4	18.1	>45	20.9	18.0
26	POSITIVE	25.5	17.8	15.5	12.7	16.9	14.7	12.9	17.4	14.7
27	POSITIVE	27.5	19.6	17.0	13.7	18.9	16.4	14.0	19.4	16.2
28	POSITIVE	25.4	16.3	14.0	11.4	15.3	13.3	11.6	15.8	13.2
29	POSITIVE	25.5	26.5	24.5	25.6	25.5	23.2	25.9	26.1	23.3
30	POSITIVE	25.7	26.4	24.2	26.4	9.7	9.1	25.3	25.9	23.3
31	POSITIVE	22.2	>45	29.1	21.8	>45	34.8	22.0	>45	9.8
32	POSITIVE	23.4	29.0	25.4	23.0	>45	24.4	22.9	>45	24.3
33	POSITIVE	28.4	20.9	18.6	13.9	19.6	17.6	14.6	20.4	17.6
34	POSITIVE	25.6	18.6	16.4	13.1	17.5	15.6	13.9	18.0	15.3
35	POSITIVE	24.5	19.2	16.7	33.0	18.0	15.9	40.3	18.2	15.9
36	POSITIVE	23.2	24.9	22.3	23.0	24.5	21.1	23.2	25.1	21.2
37	POSITIVE	23.1	>45	26.2	22.5	>45	25.4	22.4	>45	25.7
38	POSITIVE	23.3	21.3	18.9	24.4	19.8	18.1	24.6	20.2	18.1
39	POSITIVE	23.6	19.7	17.0	37.9	18.1	16.1	33.3	19.2	16.2
40	POSITIVE	25.1	19.3	16.8	38.3	17.9	16.0	14.5	18.3	16.1
41	POSITIVE	24.8	18.5	15.8	12.7	16.4	15.2	13.4	17.1	15.1
42	POSITIVE	24.5	20.4	17.8	31.1	18.1	16.7	31.9	18.7	17.0
43	POSITIVE	24.3	20.6	17.5	29.7	19.3	16.8	7.5	20.0	17.0
44	POSITIVE	26.6	20.7	17.8	38.2	19.1	16.8	15.1	19.9	16.9
45	POSITIVE	23.3	>45	27.7	22.5	>45	29.1	22.5	>45	31.6
46	POSITIVE	25.6	16.2	13.5	10.7	14.5	12.5	11.3	14.7	12.4
47	POSITIVE	24.7	21.0	18.3	28.9	19.8	17.3	30.0	20.3	17.3
48	POSITIVE	27.2	19.2	15.8	12.7	19.6	15.7	13.6	20.4	16.0
49	POSITIVE	24.7	20.2	17.4	31.1	18.5	16.5	36.9	19.3	16.5
50	POSITIVE	24.9	24.3	21.2	24.8	23.7	20.5	24.8	24.4	20.4

Contd...

Supplementary Table 1: Contd....

Sample#	RealStar SARS-CoV-2 RT-PCR kit Results	Results from TaqPathTM from Life Technologies			Bacterial Taq Polymerase			Yeast Taq Polymerase		
		IC	Rdrp	N	IC	Rdrp	N	IC	Rdrp	N
51	POSITIVE	22.3	>45	25.4	22.0	>45	25.7	22.0	>45	25.6
52	POSITIVE	23.0	>45	25.1	22.4	>45	24.5	22.2	>45	24.6
53	POSITIVE	20.6	>45	>45	20.1	>45	29.8	20.2	>45	>45
54	POSITIVE	21.8	27.8	24.5	21.7	>45	24.1	21.5	>45	24.3
55	POSITIVE	24.3	16.6	14.3	10.8	15.4	13.6	11.5	15.9	13.6
56	POSITIVE	23.9	12.3	28.6	24.0	>45	28.7	23.7	>45	6.2
57	POSITIVE	25.1	20.6	17.8	31.2	18.7	17.0	32.7	19.1	17.0
58	Negative	22.9	>45	32.4	22.5	>45	>45	23.1	>45	>45
59	Negative	23.2	>45	>45	23.3	>45	>45	23.4	>45	>45
60	Negative	22.9	17.9	>45	23.3	>45	>45	23.3	>45	>45
61	Negative	21.7	>45	31.7	21.9	>45	>45	22.6	>45	>45
62	Negative	19.9	>45	>45	20.3	>45	>45	19.8	>45	>45
63	Negative	23.3	>45	30.7	23.9	>45	>45	24.0	>45	>45
64	Negative	24.8	>45	>45	24.6	>45	>45	24.6	>45	>45
65	Negative	18.3	>45	>45	18.2	>45	>45	18.5	>45	>45
66	Negative	22.0	>45	29.8	21.6	>45	>45	22.1	>45	>45
67	Negative	22.6	>45	29.1	22.3	>45	>45	22.4	>45	>45
68	Negative	19.8	>45	29.8	20.5	>45	>45	21.0	>45	>45
69	Negative	24.8	>45	31.9	24.1	>45	>45	24.0	>45	>45
70	Negative	21.3	>45	27.6	21.1	>45	21.9	21.6	>45	>45
71	Negative	27.3	>45	31.9	27.2	>45	>45	27.3	>45	>45
72	Negative	23.9	>45	30.8	24.3	>45	>45	23.8	>45	>45
73	Negative	22.6	>45	33.2	23.0	>45	>45	22.6	>45	>45
74	Negative	20.4	>45	>45	20.2	>45	37.3	20.5	>45	>45
75	Negative	22.6	>45	>45	22.7	>45	23.3	22.6	>45	>45
76	Negative	23.0	>45	>45	23.6	>45	>45	23.4	>45	>45
77	Negative	25.3	>45	32.9	25.0	>45	>45	25.1	>45	>45
78	Negative	26.2	>45	32.5	26.1	>45	>45	26.1	>45	>45
79	Negative	22.2	>45	>45	21.9	>45	18.1	21.8	>45	>45
80	Negative	24.8	>45	29.6	23.9	>45	>45	24.0	>45	>45
81	Negative	22.6	>45	30.0	21.7	>45	>45	21.9	>45	>45
82	Negative	20.6	>45	>45	19.8	>45	>45	20.0	>45	>45
83	Negative	25.7	>45	>45	25.1	>45	>45	24.9	>45	>45
84	Negative	21.2	>45	>45	21.0	>45	>45	20.8	>45	>45
85	Negative	22.5	>45	29.1	22.1	>45	>45	22.0	>45	>45
86	Negative	25.6	>45	>45	24.6	>45	>45	24.8	>45	>45
87	Negative	24.6	>45	>45	24.3	>45	>45	24.4	>45	>45
88	Negative	24.2	>45	>45	23.9	>45	>45	24.0	>45	>45
89	Negative	23.0	>45	>45	22.2	>45	>45	22.3	>45	>45
90	Negative	30.3	>45	>45	>45	>45	>45	33.8	>45	>45
91	Negative	22.0	>45	>45	22.3	>45	>45	22.5	>45	>45
92	Negative	23.3	>45	29.7	22.8	>45	>45	22.8	>45	>45
93	Negative	24.8	>45	30.3	24.1	>45	>45	24.2	>45	>45
94	Negative	25.2	>45	>45	25.2	>45	>45	24.9	>45	>45
95	Negative	25.5	>45	34.6	25.2	>45	>45	25.1	>45	>45
96	Negative	23.3	>45	>45	22.9	>45	>45	23.1	>45	>45
97	Negative	21.6	>45	>45	21.9	>45	>45	21.8	>45	>45
98	Negative	24.2	>45	>45	23.9	>45	>45	24.0	>45	>45
99	Negative	20.4	>45	>45	20.6	>45	>45	20.1	>45	>45
100	Negative	22.1	>45	>45	22.0	>45	>45	21.8	>45	>45
101	Negative	19.6	>45	>45	19.4	>45	14.0	19.6	>45	>45
102	Negative	23.7	>45	>45	22.5	>45	>45	22.3	>45	>45
103	Negative	21.8	>45	>45	21.3	>45	>45	21.1	>45	>45
104	Negative	25.7	>45	>45	23.9	>45	>45	24.5	>45	>45
105	Negative	25.0	>45	>45	24.2	>45	>45	24.3	>45	>45