



Manufacturing and Formulation Development of Lyophilized Crude *Taq* DNA Polymerase Extract

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Abstract: Finding the optimal formulation for the lyophilization of biomolecules, such as antibodies and other proteins, is an essential component in drug development to preserve the drug product and increase its shelf life. In this study, we developed a lyophilization formulation for crude *Taq* polymerase, a common DNA polymerase enzyme used to amplify DNA through the Polymerase Chain Reaction (PCR) technique. Lyophilization is a standard process to stabilize and preserve biomolecules such as antibodies, enzymes, peptides, and small molecules. Through a formulation screen, we identified optimal lyophilization (lyo) formulations that resulted in a lyo cake with good physical characteristics while maintaining polymerase activity upon reconstitution. Subsequently, we found that our lyo formulation preserved the polymerase activity even after the *Taq* crude extract was stored at elevated temperatures for weeks.

Keywords: lyophilization (lyo), lyo cake, crude *Taq* polymerase, *Taq* crude extract, Polymerase Chain Reaction (PCR)

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Introduction

ASPIRE (Advanced Student-focused Project: Internship, Research, & Education) is a biotechnology program funded by NSF (NSF DUE 2100575) that provides internships in protein biomanufacturing and research opportunities for community college and high school students in the Greater Los Angeles Region. ASPIRE offers various collaborative research projects, including Tiny Earth [1], SEA-PHAGES [2], DNA Barcoding, and industry-relevant protein bio-manufacturing and process development projects. In collaboration with the DNA Learning Center (DNALC) of Cold Spring Harbor Laboratory, ASPIRE students have successfully optimized the production of *Taq* polymerase crude extract and the associated classroom materials (Standard Operating Procedure and Batch Record), disseminated locally and nationwide through the InnovATEBIO and the DNALC.

Taq polymerase is a DNA Polymerase I, an essential enzyme that creates copies of DNA with a miniscule error rate, which enables cells to pass genetic information from the parent to daughter cells. It was isolated from a thermophilic bacterium in the geysers of Yellowstone National Park, *T. Aquaticus*, in 1976 [3]. Since its initial discovery, *Taq* Polymerase has revolutionized the field of Molecular Biology [4]. Owing to its thermal stability, *Taq* Polymerase has become the ideal enzyme to create massive amounts of DNA copies in vitro through a process known as the Polymerase Chain Reaction (PCR), streamlining the DNA amplification process by eliminating the need to replace DNA polymerase that is destroyed in each PCR cycle [5]. PCR has become crucial to genetic engineering, forensics, and medical diagnostics, including the COVID-19 detection method [6].

Lyophilization, also colloquially known as freeze-drying, has become part of standard drug development workflows to preserve the Active Pharmaceutical Ingredient (API) by removing water through sublimation. Generally, samples are frozen and placed into a vacuum chamber to allow the water molecules to escape as gas, leaving the molecule of interest as a dry solid, commonly called the “cake.” Ideal lyo cake should rehydrate easily into a homogenous mixture within a reasonable period, usually within minutes, while preserving the biological activities of the biomolecules [7]. Finding the optimal chemical combination, also



known as excipients, that protects the biomolecules during lyophilization and produces a 'good cake' can be challenging. This has spurred the growth of specialist CDMOs (Contract Development and Manufacturing Organizations), offering services to develop the lyo formulation before the drugs are manufactured at large scale for clinical trials.

ASPIRE students produce *Taq* polymerase crude extract as a part of the Biotechnology training program; products and protocols are disseminated nationally through the DNALC and InnovATEBIO, the National Biotechnology Education Center. We developed a lyo formulation for crude *Taq* extract to minimize the distribution cost. Lyophilization of a Ready-to-Use RT-PCR mix with a similar enzyme requires a combination of Trehalose, Ficoll-400, and Gelatin [8]. Trehalose comprises two glucose molecules linked together at the reducing end (glycosidic bond), making it a nonreducing end and stable against acidic conditions. Trehalose is generally considered a good lyoprotectant (protects biomolecules from denaturation during the lyophilization) and bulking agent [9], owing to its chemical arrangement that resists degradation as well as its backbone flexibility which enables it to participate in an extensive hydrogen bond network [10, 11].

Although lyophilization for the *Taq* mixture has been attempted [8], the presence of other cytosolic *E. coli* proteins in the crude *Taq* extract may render the lyo formulation ineffective. Therefore, we seek to determine the optimal formulation for the lyophilization of crude *Taq* extract. In this study, we developed a formulation screen that led to the identification of optimal lyo formulation for the *Taq* crude extract. We then performed PCR to assess the polymerization activity of the preserved crude *Taq* extract upon reconstitution. Our results suggest that the lyophilized crude *Taq* extract maintains its polymerase activity after more than 10 weeks of storage at 50°C and two weeks at 72°C.

Methods

Protein Expression

An open-source *Taq* polymerase (pOpen*Taq*, FreeGenes #BBF10K_003493) construct, provided by the DNALC, was transformed into the BL21 *E. coli* expression strain. Protein expression is done using the standard protocol for bacterial protein expression systems. Briefly, cells were streaked onto LB Amp plates ([amp]: 50 µg/mL) and were incubated overnight (16 – 20 hours) at a 37°C incubator. An overnight (ON) culture was prepared by inoculating 5-10 colonies into 3 mL LB Amp broth ([amp]: 50 µg/mL), placed in the shaking incubator (250 rpm) at 37°C for 16 hours. Two mL of the ON culture was spun down to remove secreted β-lactamase in the supernatant. The pellet was resuspended and transferred into a fresh 80 mL LB Amp in a 250 mL baffled flask. The culture was brought to OD₆₀₀ 0.9 in a 37°C shaking incubator before induction with Isopropyl β-D-1-thiogalactopyranoside (IPTG) (final concentration of 0.5 mM) for 16 hours. The culture was spun down, and the supernatant was removed. The pellet can be used immediately or stored at -80°C for later use.

Crude *Taq* Extract Preparation

Crude *Taq* extract was prepared according to the previously published protocol [12] with some modifications. Briefly, the pellet was resuspended in TEN buffer (10 mM Tris-HCl pH 7.9, 1 mM EDTA, 100 mM NaCl), 10 mL per 1 g pellet. Cell lysis was done by sonication (Qsonica, 40% Amplitude, 1 sec on, 1 sec off, 1 min, repeat 3x); the sample was kept on wet ice for the entire sonication duration. The cell lysate was incubated at 75°C for 30 minutes to denature unwanted aggregated proteins and further lyse the cells, maximizing yield without risking enzymatic activity. Lysate is then spun down (14,000x g for 20 minutes) to remove any cellular debris, and the supernatant is collected as the crude *Taq* extract. Complete SOP and Batch records are made available through InnovATEBIO.

Polymerase Chain Reaction (PCR) to Assess DNA Amplification Activity

The prepared *Taq* extract's amplification activity was assessed using the standard λ DNA amplification developed by the Bay Area Bioscience Education Community (BABEC). Briefly, 0.25 ng of λ DNA was amplified in a 25 µL reaction using a 0.4 µM λ primer set (PC01: 5'-GATGAGTTCGTGTCCGTACAACCTGG-3', PC02: 5'-GGTTATCGAAATCAGCCACAGCGCC-3'). The thermal cyclers setting is as follows: Initial denaturation (95°C, 2 minutes), 25 cycles of amplification (95°C for 15 sec, 37°C for 15 sec, and 72°C for 30 sec), final elongation (72°C for 5 min), and storage (4°C, infinity).



The amount of *Taq* yield was determined qualitatively by comparing the polymerase activities to the commercially available recombinant *Taq* polymerase: *Taq* DNA Polymerase (New England Biolabs, M0273S, at 5 units per 25 μ L reaction). The qualitative assessment compared the relative DNA band size on the gel electrophoresis (0.8% Agarose).

0.25 T reaction uses 0.25 μ L of *Taq* crude extract in a 25 μ L PCR reaction.

0.5 T reaction uses 0.5 μ L of *Taq* crude extract in a 25 μ L PCR reaction.

1 T reaction uses 1 μ L of *Taq* crude extract in a 25 μ L PCR reaction.

2 T reaction uses 2 μ L of *Taq* crude extract in a 25 μ L PCR reaction.

10x *Taq* buffer was prepared in-house using the same recipe as the 10x Standard *Taq* Reaction buffer (NEB, Cat #B9014S).

10 mM dNTP mix: Promega, 1,000 μ L (Cat #U1515)

Preparation of Stock Solutions for Lyophilization Formulation Screen

D-(+)-Trehalose (Fisher BioReagent, BP2687100), Ficoll-400 (Thermo Scientific, AAB2209518), and Gelatin (VWR, 470301-132) were used in the lyophilization process of the *Taq* crude extract. The 50% stock solution for trehalose was made by diluting 10 grams of trehalose in 20 mL of diH₂O, the 50% stock solution for Ficoll-400 was made by diluting 10 grams of Ficoll-400 in 20 mL of diH₂O, and the 30% stock solution for gelatin was made by diluting 3 grams in gelatin in 10mL diH₂O (the gelatin had to be melted to allow for the 3 grams to dissolve fully). Sterilization of the trehalose was done by sterile filtration (Pall Corporation, 0.22 μ m), while Ficoll-400 and gelatin stock solutions were sterilized by autoclave. All components were mixed, and the volume was brought up to 1000 μ L with sterile distilled water in a deep-well 96-well plate to create the lyophilization formulation matrix and stored at -80°C before use.

Lyophilization Screen

20 μ L of diluted *Taq* crude extract and 20 μ L of each formulation mix were added to each well of a new 96-well assay plate; this mixing brings the effective *Taq* concentration to half as much compared to the original crude *Taq* extract. Upon thorough mixing, the 96-well assay plate was frozen at -80°C. Lyophilization was done using Labconco FreeZone 2.5L Benchtop Freeze Dryers following the standard lyophilization setting—briefly, the assay plate containing frozen *Taq* lyo. Mixes were put under a vacuum (0.01 Torr) in the drying chamber as the temperature was raised slowly to room temperature. After lyophilization, Lyo cakes had an initial visual inspection. The visually good lyo cakes were reconstituted using sterile distilled water for functional assay to assess the retention of DNA amplification activity.

Thermal Stability Study of Lyophilized *Taq*

Several aliquots of lyophilized *Taq* (at 8 μ L) were prepared for the stability study at six different temperatures (4°C, 25°C, 37°C, 50°C, 72°C, 95°C). Each temperature was strategically chosen to cover various conditions found in the lab. At 4°C, refrigeration conditions are practiced in labs, and 25°C represents room temperature. The physiological conditions at 37°C mirror the conditions of the human body. Then, a moderate increase in temperature to 50°C between 72°C mimics the condition of PCR amplification—lastly, 95°C, which is the denaturing temperature of the PCR reaction. One aliquot from each temperature was taken at each time point and used for comparative functional assay (λ DNA amplification) against both the non-lyophilized *Taq* mix and the crude extract that was incubated at each corresponding temperature for the same time. A sample is collected weekly from each incubation temperature for ten weeks (70 days), and its polymerase activity is assessed by PCR amplification of λ DNA, as described above.

We prepared several aliquots of 4 μ L of crude *Taq* extract, mixed with 4 μ L of B7 formulation, to make a total of 8 μ L of B7 mix in thin-walled PCR tubes. These mixes were then lyophilized overnight. Between 5 to 15 aliquots of the B7 mix were placed at the different incubation temperatures. Given that *Taq*'s half-life at 95°C is 40 minutes [13] and based on the preliminary temperature stability study done previously (data not shown),



we anticipated that the lyophilization would not extend its activity beyond a few days at this temperature hence, only 5 aliquots of lyophilized B7 mix were placed at 95°C. We prepared 15 aliquots for each of the other five temperatures (F.L.-Formulation Lyophilized). Aliquots of non-lyophilized crude *Taq* extract (C.E.) were also placed at 4°C, 25°C, 37°C, 50°C, 72°C, and 95°C. In addition, we also asked whether the B7 mix excipients themselves, without the freeze-drying process, are sufficient to protect polymerase activity. To address this, we prepared the B7 mix without subjecting it to the freeze-drying process; in other words, we kept it as a liquid. We also stored these non-lyophilized B7 mix (F.NL.-Formulation Non-Lyophilized) at 4°C, 25°C, 37°C, 50°C, 72°C, and 95°C.

Samples were collected at regular intervals, and polymerase activities were compared to the aliquot that had not undergone thermal stress (i.e., Day 0). F.L. was rehydrated with sterile dH₂O to bring it to the original 8 µL volume. C.E., F.L., and F.NL. were then used to prepare a 2x master mix for the PCR assay. We used a 2T recipe (Table 1) to prepare a 2x *Taq* master mix from F.L. and F.NL. to account for the ½ dilution with the lyo stock solution, 1T recipe (Table 1) was used for C.E. A commercially available NEB 2x Master Mix from New England Bio-Labs (Cat. #M0270S) was used as a benchmark. The polymerase activities of these master mixes were assessed by PCR amplification of λ DNA.

Results and Discussion

Preparation of *Taq* Polymerase Crude Extract

Crude *Taq* extract was prepared using a previously developed protocol [12] with some modifications described in the method section. We observed a protein band at around 90 kDa on a denaturing protein gel, SDS PAGE, consistent with the calculated *Taq* Polymerase's size, 94 kDa (Fig. 1a). We noticed several low molecular weight bands in the crude *Taq* extract, underscoring that it is not a completely pure enzyme. This gel also shows the presence of other proteins in the extract, such as the 37 kDa and 20 kDa bands, as well as other less prominent protein bands. This underscores the crude nature of this prep (Fig. 1a). Nevertheless, we can enrich the proportion of *Taq* polymerase in the mixture through this method. We then asked whether we could see polymerase activity in the *Taq* crude extract we prepared. To do this, we performed DNA amplification using the λ DNA PCR protocol provided by BABEC. We chose this method since it is standard and has been used by many high school students for years; the PCR protocol is described briefly in the method section. Due to other protein contaminants, it is difficult to predict the enzymatic activity simply by relying on protein concentration numbers. For this reason, we performed various dilution series to determine the amount of crude *Taq* extract needed to create a 2x master mix for PCR. The degree of DNA amplification of our master mixes is then qualitatively compared to the one obtained from the commercially available NEB 2x Master Mix (NEB, Cat. #M0270S).

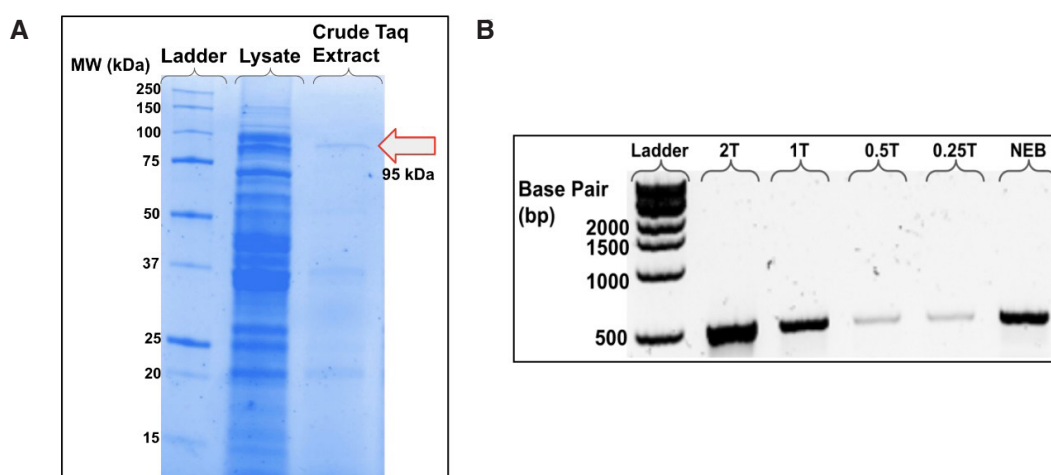


Fig. 1. (A) SDS PAGE comparing cell lysate and crude *Taq* extract reveals the enrichment of *Taq* Polymerase in the crude extract mixture. (B) Qualitative DNA amplification assay of crude *Taq* extract at various dilutions compared against commercially available *Taq* master mix.



The composition of our 2x master mix is shown in Table 1. Briefly, we prepared 50 μL of 2x master mix first. We then mixed 12.5 μL of the 2x master mix with 0.25 ng of λ DNA and the λ primer mix at a final primer concentration of 0.4 μM . We assigned this 1T to describe the 2x master mix composition with 4 μL of crude *Taq* extract in 50 μL of total volume. Given that each 25 μL PCR reaction only requires 12.5 μL of the 2x master mix, only 1 μL of crude *Taq* extract is effectively used in a 25 μL PCR reaction. In addition, we ran a PCR reaction using the NEB 2x Master Mix as a comparison at the same λ DNA and λ primer mix concentration.

Table 1. *Taq* Master Mix Concentration Dilutions

Master Mix	2T	1T	0.5T	0.25T
	(2 μL <i>Taq</i> /25 μL rxn)	(1 μL <i>Taq</i> /25 μL rxn)	(0.5 μL <i>Taq</i> /25 μL rxn)	(0.25 μL <i>Taq</i> /25 μL rxn)
10x <i>Taq</i> Buffer (μL)	10	10	10	10
dNTP (μL)	2	2	2	2
dH ₂ O (μL)	20	24	26	27
50% Glycerol (μL)	10	10	10	10
<i>Taq</i> Extract (μL)	8	4	2	1

Following the PCR reaction, we ran gel electrophoresis (0.8% agarose) to qualitatively compare our crude *Taq* extract at various dilutions to the NEB's *Taq* (Fig. 1b). Based on our data, we suggest that 1 μL of our crude *Taq* extract exhibits similar polymerase activity to the NEB 2x Master Mix. Given that approximately 12 mL of crude *Taq* extract was produced from an 80 mL *E. coli* culture; we produced enough *Taq* crude extract to support 12,000 PCR reactions from each manufacturing batch.

Developing Lyophilization Formulation for the Crude *Taq* Extract

Lyophilization is a common method used to preserve biomolecules. Therefore, we attempted to find the ideal lyophilization (lyo) formulation for our crude *Taq* extract. Previous attempts to lyophilize a qPCR mix have been successful [8]. Although the composition of the qPCR mix [8] is markedly different from our crude *Taq* extract, we hypothesized that the ideal lyo formulation for our crude *Taq* extract should consist of similar excipients, albeit at a different proportion. For this reason, we designed the lyo formulation screen in a 96-well format using the same excipient combination: trehalose, ficoll-400, and gelatin.

A combination of three concentrations of trehalose (10%, 20%, 30%), three concentrations of gelatin (0.2%, 2%, 6%), and seven concentrations of Ficoll-400 (2%, 6%, 9%, 12%, 15%, 18%, 20%) were chosen to create the lyo formulation screen matrix (Fig. 2a). Each stock solution was brought to a 1000 μL total volume using sterile nanopure water. For trehalose, columns 1-3 had no trehalose (0%), columns 4-6 had 10%, columns 7-9 had 20%, and columns 10-12 had 30%. For gelatin, columns 1, 4, 7, and 10 had 0.2%, columns 2, 5, 8, and 11 had 2%, and columns 3, 6, 9, and 12 had 6%. For Ficoll-400, row A had 0%, B-2%, C-6%, D-9%, E-12%, F-15%, G-18% and H-20%. Wells G11, H10, H11, and the entirety of column 12 could not be used as the percentages overshot the 1000 μL limit for the stock (Fig. 2a).

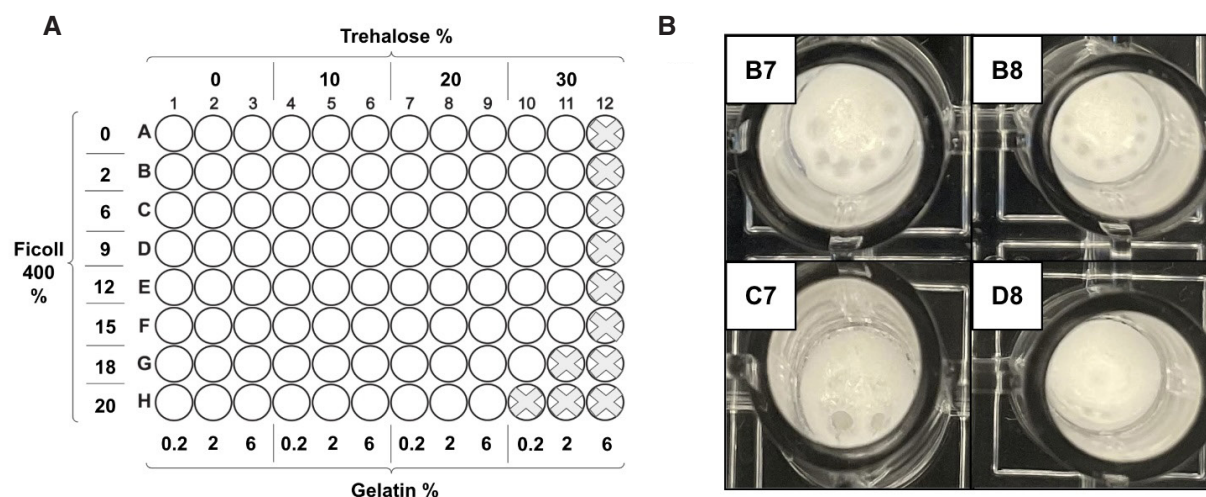


Fig. 2. (A) Lyo formulation screen design in a 96-well-plate format containing combinations of Trehalose, Ficoll-400, and Gelatin at various concentrations (B) Images of lyo formulations that yield desirable cake appearance: wells B7 mix, B8 mix, C7 mix, and D8 mix.

To start the lyophilization process, 20 μL of the crude *Taq* extract was added to each well of the new 96-well screen plate, and 20 μL of the solutions from the stock well plate was added to their corresponding wells. The screen plate was stored in a -80°C freezer while the lyophilizer was primed to reach -80°C . Once the lyo mixtures were completely frozen, the screen plate was transferred to the lyophilization vacuum chamber overnight to completely remove the water molecules through the sublimation process.

Once lyophilization was complete, a physical inspection was done to find the ideal cake, defined as white powdery solids that can be easily reconstituted within a few minutes [7]. Based on these criteria, four good cakes were identified out of the 85 formulations screened in this attempt: wells of mixtures B7 (10% Trehalose, 1% Ficoll 400, 0.1% Gelatin), B8 (10% Trehalose, 1% Ficoll 400, 1% Gelatin), C7 (10% Trehalose, 3% Ficoll 400, 0.1% Gelatin) and D8 (10% Trehalose, 3% Ficoll 400, 1% gelatin); all of them were white, dry, powdery, had minimal puffing on the surface, uniform structure throughout the cake and dissolved instantly when water was added to rehydrate them (Fig. 2b).

Lyophilized Crude *Taq* Extract Retained the DNA Amplification Capability Upon Reconstitution

To investigate the retention of polymerase activity of the lyophilized *Taq* crude extract, lyo cakes in wells B7, C7, B8, and D8 were rehydrated with 20 μL of sterile H_2O since only 20 μL of crude *Taq* extract was added to each well. We then prepared a 2x master mix using the 2T recipe (Table 1), an equivalent of 2 μL of crude *Taq* extract in a 25 μL PCR reaction, anticipating a reduction in polymerase activities, referred to as B7 mix, C7 mix, B8 mix, and D8 mix. As a comparison, we also prepared a 2x *Taq* master mix using crude *Taq* extract that has not undergone lyophilization using the 2T recipe, referred to as U (un-lyophilized). Lastly, we compared the polymerase activity of B7 mix, C7 mix, B8 mix, D8 mix, and U to the Master Mix created using NEB's *Taq* (Fig. 3).

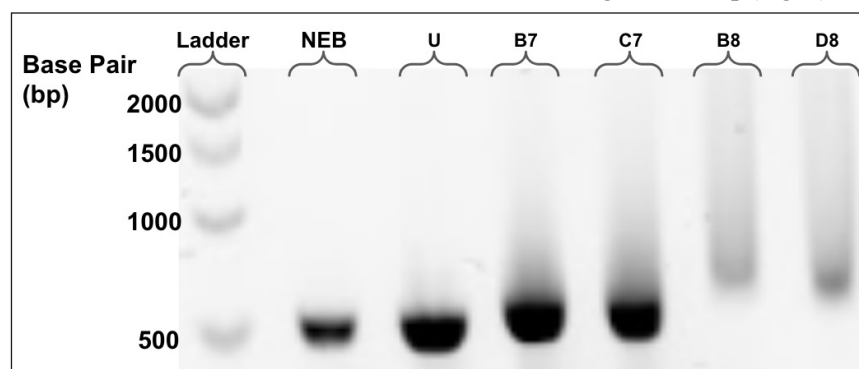


Fig. 3. PCR assay to assess the retention of polymerase activities of the lyophilized *Taq* crude extract.



Given that the 1T *Taq* master mix recipe exhibited comparable polymerase activity to the NEB (Fig. 1b), the non-lyophilized *Taq* master mix control at 2T recipe (U) displayed higher polymerase activity compared to the NEB 2x Master Mix control (1T recipe). Only the B7 and C7 mix showed polymerase activity comparable to the master mix prepared using non-lyophilized crude *Taq* extract (U). The B7 and C7 mixes contain a final concentration of 10% Trehalose and 0.1% Gelatin. There was no discernable difference in polymerase activity when varying the Ficoll 400 concentration between 1% (B7 mix) and 3% (C7 mix). Wells containing similar Trehalose and Gelatin concentrations (A7 mix and D7 mix), similar Trehalose and Ficoll 400 concentrations (C8 mix), and similar Gelatin and Ficoll 400 concentrations (B4 mix, C4 mix, B10 mix, and C10 mix) were not tested in this assay as they did not produce good cake (Supp. Fig. 1). While the cakes of B8 mix and D8 mix looked promising, their polymerase activities were almost non-existent compared to U, B7 mix, or C7 mix. The lyophilization process unaffected the PCR assay for the B7 mix and C7 mix as the polymerase activity is comparable to U. The B8 mix and D8 mix did not result in a high polymerase activity due to a non-optimal concentration of chemicals. We can conclude that the lyophilization process does not affect the retention of the polymerase activity.

Lyophilized Crude *Taq* Extract Retained Its Function Even After Prolonged Incubation at Higher Temperature

We performed a ten-week stability study to address whether the lyo formulation can protect the crude *Taq* extract activities against elevated temperature. The polymerase activity of lyophilized crude *Taq* extract was compared to the control of the non-lyophilized crude *Taq* extract after 10 weeks of incubation at various temperatures. Since the polymerase activity is comparable between B7 and C7 mixtures, only the B7 mix formulation was used in this stability study.

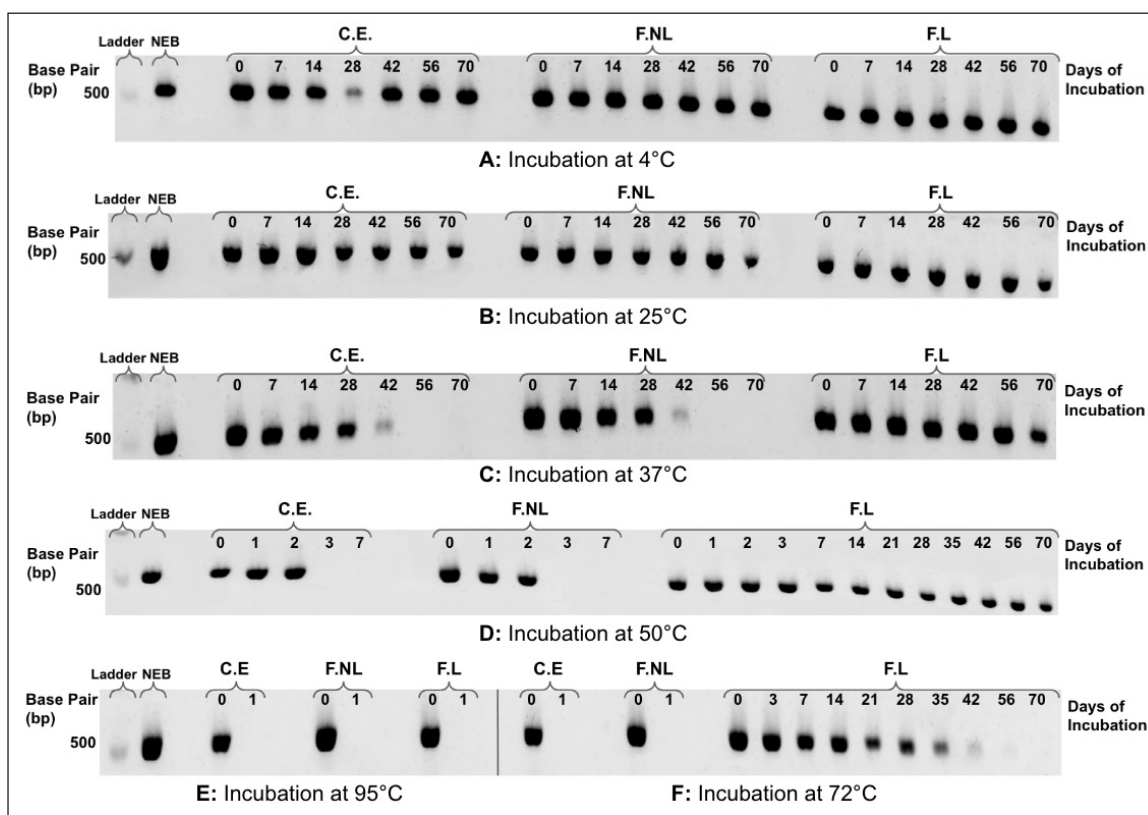


Fig. 4. DNA gel of the three different *Taq* polymerase solutions tested at six different temperatures.

Samples were run on 0.8% agarose gels (Fig. 4). *Taq* retained its polymerase activity after ten weeks of incubation at 4°C for C.E., F.N.L., and F.L. (Fig. 4: A). This is expected since 4°C is a common storage temperature for laboratory reagents. A similar trend is also observed for the incubation at 25°C (Fig. 4: B). This is somewhat surprising since enzymes, or any biomolecules in general, are typically stored at 4°C or



frozen to avoid the loss of enzymatic activity due to degradation or aggregation. This highlights crude *Taq* extract's resiliency, making it especially suitable for introductory Bioscience classrooms.

The lyophilized crude *Taq* extract (F.L.) continued to retain its polymerase activity for 70 days (10 weeks) at 37°C, while C.E. and F.N.L. displayed a loss of polymerase activity starting at day 42 (6 weeks) and beyond (Fig. 4: C). This suggests that the lyo mix alone, without the freeze-drying process, is insufficient to protect the polymerase activity of the crude *Taq* extract at this temperature. The difference in the retention of polymerase activity is even more prominent at higher incubation temperatures. Both C.E. and F.N.L. were stable for only 2 days, while the F.L. was stable for at least ten weeks (Day 70) at 50°C incubation (Fig 4: D). At 72°C, F.L. continued to display polymerase activity for about two weeks (Day 14) before showing a notable decline in enzymatic activity (Fig. 4: F). This is impressive since both C.E. and F.N.L. lost their polymerase activity at 24 hours of incubation at 72°C. Given the nature of our qualitative assay, however, we cannot detect the time when the polymerase activity starts to decline. This lyo formulation, however, is unable to save the crude *Taq* extract from a near-boiling temperature (95°C). All samples (C.E., F.N.L., and F.L.) lost their polymerase activity within 24 hours of incubation at 95°C (Fig. 4: E). Visual inspection of the F.L. samples stored at 95°C revealed a color change from white to yellowish powder (Fig. 5). A change in the color of the cake indicates possible chemical changes that affect the stability of the crude *Taq* extract cake, consistent with the results from the functional assay (Fig. 4: E).

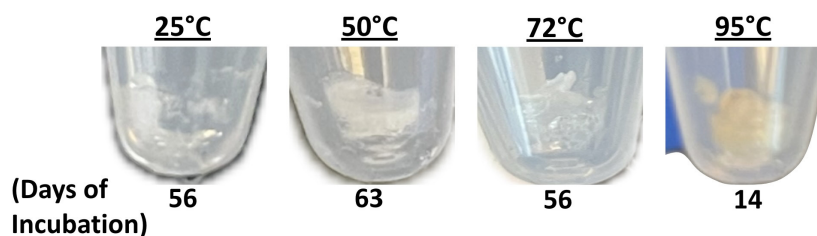


Fig. 5. Image of Lyo cakes of the crude *Taq* extract stored in various temperatures.

Conclusion

Overall, we developed an ideal lyo formulation that can preserve the polymerase activity of a crude *Taq* extract even after prolonged storage at elevated temperatures. In this study, we showed that non-lyophilized crude *Taq* extract maintains the polymerase activity even after being stored at room temperature for up to 10 weeks. However, polymerase activity has a qualitatively observable decline beyond 14 days of incubation (2 weeks). We also showed that our lyophilized crude *Taq* extract (F.L.) can maintain its activity even after being stored at 72°C for about two weeks before seeing a significant decline in activity. The additional cost associated with lyophilization reagent is calculated to be roughly 30 cents per 100 PCR reactions or less than 1 cent per 1 PCR reaction. This additional cost is negligible, considering that the cost of commercial *Taq* is roughly 50 cents per 1 PCR reaction. There are ongoing efforts to develop lyo formulation using even more affordable chemical combinations. Our results will enable ASPIRE and Biotechnology programs nationwide to distribute the crude *Taq* extract to high school and community college partners without the need for expensive cold shipping.

Acknowledgments. H.K. and I.P. contributed equally to this project. I.P. developed the lyo formulation screen and identified the ideal formulation for the crude *Taq* extract. H.K. optimized the qualitative functional assay. I.P. optimized and performed the qualitative functional assay to show that the lyophilized crude *Taq* extract maintains the polymerase activity upon reconstitution. H.K. designed and executed the 10-week temperature stability study (stress test). Q.M. and C.S. performed the preliminary temperature stability study for the non-lyophilized crude *Taq* extract. J.H. and B.N. provided the open-source *Taq* polymerase (pOpen*Taq*, FreeGenes #BBF10K_003493) construct and the DNALC protocols for the crude *Taq* extract preparation, providing us with a starting point. B.N. continues to provide scientific advice for this project. W.W. supervised ASPIRE interns in the early phase of this project. A.K. offered ongoing support and supervision of this project. H.K., I.P., and A.K. drafted the manuscript.

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Disclosures. The authors declare no conflict of interest.

Supplemental Materials. Please see <https://micronanoeducation.org/wp-content/uploads/2024/09/Supplementary-Figure-1-Doc-edit-returned.docx>

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