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Research Article

A scaled-down and simplified protocol for purifying recombinant *Taq* DNA polymerase

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Abstract. We previously described in a paper published in *BIOS* an undergraduate lab activity involving the gene cloning, expression, and purification of *Thermus aquaticus* (*Taq*) DNA polymerase, an enzyme used in the polymerase chain reaction (PCR). Based on the large number of requests for biological materials and questions about the protocols this paper invoked, we explored methods to simplify the protein purification portion of the published lab activity. A faster and simpler protocol would permit labs and classes with limited equipment or supplies the ability to produce active *Taq* DNA polymerase more easily for both teaching and research use. In the simplified protocol described here, bacterial cell lysis and enzyme purification is achieved in a small starting volume using only a hot water bath, a microcentrifuge, and one simple buffer. The purified enzyme from this protocol works well in PCR, and we additionally describe its use in a 2X master mix.

Introduction

Recombinant DNA polymerase purified from *E. coli* expressing the *DNA polymerase I* gene from *Thermus aquaticus* (*Taq)* is the most commonly used enzyme for amplifying DNA using the polymerase chain reaction (PCR). Numerous published reports have described purification schemes for recombinant *Taq* DNA polymerase (Engelke et al., 1990; Pluthero, 1993; Grimm and Arbuthnot 1995). We described a laboratory activity for undergraduate biology classes that involves cloning the *DNA polymerase I* gene from *Taq* genomic DNA, the expression of the *Taq* gene in *Escherichia coli* and purification of the recombinant enzyme (Ferralli et al., 2007).

In the three years following our initial *Taq* DNA polymerase publication, we received dozens of requests for biological materials and questions from individuals throughout the world-wide scientific community. Many of these requests came from teachers planning to incorporate the activity into their biology lab courses and also from research labs aiming to save money by making their own *Taq* DNA polymerase. Inquiries from teachers often questioned the purification portion of the activity, which we adopted from previously published protocols, that involves large starting volumes and uses three complex buffers containing three different detergents (Engelke et al., 1990). To address these frequently asked questions, we investigated ways to simplify the purification protocol so that labs that lack some of the reagents or equipment required in the original protocol could easily perform the purification. The scaled-down, simplified protocol described here should make *Taq* DNA polymerase

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purification more feasible for both undergraduate and high school laboratories.

Materials and Methods

Enzyme purification

Cell cultures of *E. coli* strain Fp251 (Ferralli et al., 2007) that contains the *Taq DNA polymerase I* expression plasmid were grown in LB media containing ampicillin (100 μg/mL) at 37°C with shaking at 200 rpm. A 30 mL culture was inoculated with 1 mL of a saturated overnight culture, and expression of the recombinant protein was induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to 0.2 mM when the cell density became cloudy, an optical density at 600 nm between 0.4-1.0. The culture was grown for an additional 10–16 hours at 37°C. Shorter growth periods resulted in low enzyme yields and longer growth periods have been reported to result in degraded protein products (Pluthero, 1993). After the induction period, the culture can be held on ice for a few hours if needed without enzyme yield loss.

One mL of induced culture was dispensed into a microcentrifuge tube and cells harvested by centrifugation (1 min at 15,000 x g). The cells were resuspended in 200 μl TEN buffer (10 mM Tris-HCl pH 7.9, 1 mM EDTA, 100 mM NaCl) and placed in a 75°C water bath for 30 min with brief vortex mixing every 10 min. Cell debris and denatured protein were removed from the lysate by centrifugation at room temperature (10 min at 15,000 x g). The supernatant, containing *Taq* DNA polymerase, was recovered to a fresh tube and used immediately in the PCR or stored in the refrigerator (4°C) for later use. For long term storage, glycerol was added to give a final glycerol concentration of 25% and the enzyme preparation stored in the freezer (-20°C).

PCR reactions

PCR was used to assay for *Taq* DNA polymerase activity. Reaction volumes were 25 μL and contained 25 ng plasmid template (pJET1 containing the cDNA for *Arabidopsis thaliana* gene At3g05090), 1 μM forward and reverse primers, 250 μM dNTPs, 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 1 μL *Taq* DNA polymerase preparation. PCR using commercially-prepared *Taq* DNA polymerase (Promega GoTaq DNA Polymerase, catalog #M3171; Fisher BioReagents *Taq* DNA Polymerase, catalog #FB-6000) for positive enzyme controls were conducted as above but using one manufacturer-defined unit of enzyme in its supplied buffer. Thermocycling conditions were 95°C initial melt for 5 min followed by 27 cycles of 95°C melting for 30 seconds, 54°C annealing for 45 seconds, and 72°C extension for 60 seconds; these cycles were followed by a final 72°C extension for 10 min. Amplification products were analyzed by electrophoresis using 1% agarose gels.

2X PCR master mix

The double-strength (2X) PCR cocktail mix (GullTaq) consisted of 20 mM Tris-Cl (pH 8.3), 100 mM KCl, 3 mM $MgCl_2$, 500 μM dNTPs (Fisher Scientific), 34% sucrose, 0.05% cresol red (Sigma catalog #114472) and purified recombinant *Taq* DNA polymerase (16 μL enzyme fraction per 100μL 2x cocktail mix solution). QIAGEN HotstarTaq Plus Master Mix (catalog #203643) was used as a reference control. PCR was conducted as above but with a different plasmid template and primer set. More detailed information on preparing this cocktail can be found at the Erickson Laboratory homepage at Salisbury University (http://faculty.salisbury.edu/~flerickson/).

Results and Discussion

Previously, published methods for *Taq* DNA polymerase purification were performed using large volumes, numerous complex buffers that included lysozyme and multiple detergents, and one or more high-speed centrifugation steps. To make this purification more practical for teaching laboratories, we determined the minimal steps and buffer ingredients required to purify active *Taq* DNA polymerase.

Our first goal was to determine if we could perform the purification starting with a smaller

cell culture volume so that all purification steps could be performed in a 1.5 mL microcentrifuge tube and using a bench-top microcentrifuge. Following our previously published purification protocol (Ferralli et al., 2007), but using only 1 mL instead of 30 mL of starting cell culture volume, we found we could purify enough *Taq* DNA polymerase activity to perform at least 100 polymerase chain reactions (data not shown).

After concluding that we could scale down the purification volumes, we focused on simplifying the buffer components and reducing the number of purification steps. Numerous purification trials were performed where we omitted various buffer ingredients like the lysozyme for breaking open the cells or the different detergents required in our original protocol. We found that a single buffer consisting only of Tris-HCl, EDTA and NaCl (TEN buffer) was sufficient and enzyme purification could be completed in less than one hour.

The steps of the new purification protocol are shown in Fig. 1 and described in more detail in the material and methods section. Cells from 1 mL of induced culture are harvested by centrifugation. After discarding the supernatant, the cell pellet can be frozen for short-term storage (at least 1 month) or processed immediately. The cells are resuspended in TEN buffer and placed in a hot water bath. This step efficiently lyses the cells and denatures any *E. coli* proteins, like proteases, that may reduce *Taq* DNA polymerase activity. Freezing the cell pellet before this step has been reported to aid cell lysis (Grimm and Arbuthnot, 1995) but we found freezing unnecessary. Cell debris and heat-denatured proteins are removed from the lysate by centrifugation at room temperature. The supernatant, containing *Taq* DNA polymerase, is recovered to a fresh tube and used immediately in the PCR or stored in the refrigerator for later use. For storage longer than a few weeks, glycerol can be added and the enzyme preparation stored in the freezer. Comparing the activity level of the *Taq* DNA polymerase fraction from the new protocol to commercially-prepared enzyme preparations in PCR showed that our preparations yielded about 100 units of *Taq* DNA polymerase in 200 µL (~0.5 units per µL), which is comparable to the activity present in commercial enzyme preparations (Fig. 2A).

Homemade 2X PCR master mix

Taq DNA polymerase is often purchased as concentrated cocktail mixes that contain the

1. **Inoculate** 30 mL of Luria broth containing 100 µg/mL ampicillin with 1 mL of saturated culture of *E. coli* strain containing *Taq DNA polymerase I* plasmid.
2. **Grow** culture at 37°C with shaking at 200 rpm until cloudy (A_{600}= 0.4-1.0).
3. **Induce** expression of *Taq DNA polymerase I* gene by adding IPTG to 0.2 mM.
4. **Grow** culture for an additional 10-16 hours.
5. **Dispense** 1 mL of induced bacterial culture into a microfuge tube.
6. **Pellet** cells by spinning for 1 min at maximum speed using a microcentrifuge.
7. **Decant** media supernatant and completely resuspend cell pellet with 200 µL TEN buffer.
8. **Heat** resuspended cells at 75°C for 30 min, with brief mixing every 10 min.
9. **Centrifuge** for 10 min at maximum speed in microcentrifuge to pellet cell debris.
10. **Transfer** supernatant containing recombinant *Taq* DNA polymerase to a fresh tube.

Figure 1. Steps of scaled-down and simplified *Taq* DNA polymerase purification scheme.

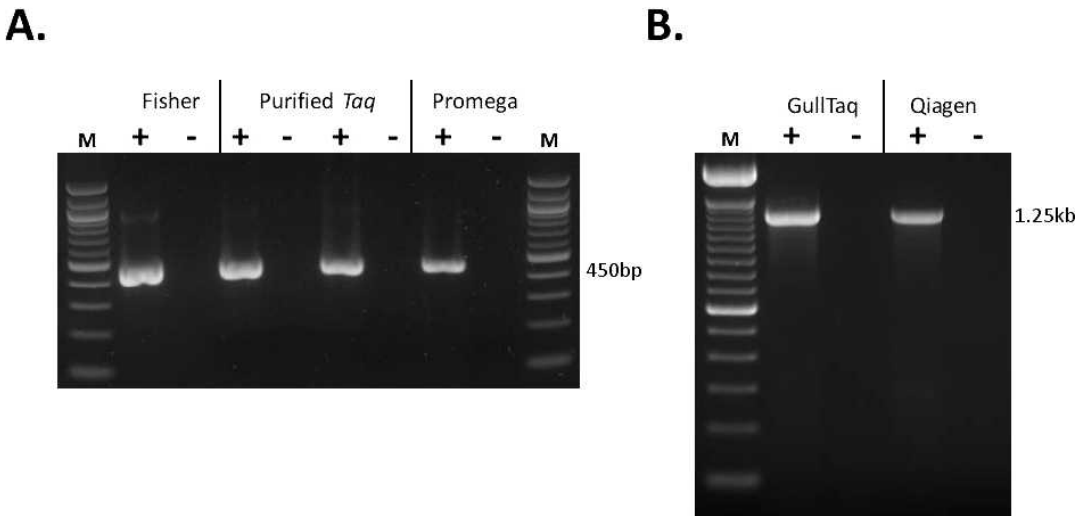

Figure 2. Enzyme activity of purified and commercially available *Taq* DNA polymerase in the PCR with (+) and without (-) plasmid DNA template. PCR was performed as described in material and methods and samples were separated by electrophoresis on 1% agarose gels. "M" denotes DNA size marker lanes. **(A)** Reactions using either 1 μL of purified or 1 unit of commercially-prepared *Taq* DNA polymerase (from Fisher or Promega). Successful amplification results in a single amplicon of 450 base pairs (bp). **(B)** 2X GullTaq master mix used in PCR. Commercially-prepared master mix from QIAGEN was used as a positive control. Successful amplification results in a single amplicon of 1.25 kilobase pairs (kb).

required buffer and reagents for use in PCR. Upon dilution of these cocktails, only primers, template DNA, and water need to be added. These convenient "master mixes" reduce PCR assembly steps and pipeting errors, and by including a densifying agent and an electrophoresis tracking dye, post-PCR alterations are not required before loading the reactions into an agarose gel for electrophoresis. Using our purified DNA polymerase fraction, we developed a double concentrated (2X) PCR master mix that we call "GullTaq", named after our school's mascot, the seagull. The inclusion of cresol red as the electrophoresis tracking dye gives GullTaq a maroon color, one of our school colors. The performance of GullTaq is shown in Fig. 2B, where it has a comparable activity level to a commercially-prepared PCR master mix. GullTaq is currently being used in numerous teaching and research labs at Salisbury University, saving hundreds of dollars per semester in reagent costs.

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