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## RESEARCH ARTICLE

## RAPID SIMPLIFIED PROTOCOL FOR PURIFICATION OF TAQ DNA POLYMERASE FRAGMENT EXPRESSED IN ESCHERICHIA COLI

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### Abstract

An economical and simple method, boiling lysis method was developed and used to purify 94kDa Taq DNA polymerase fragment from transformed BL21 (DE3) cells. It is based on conditions such as thermostable properties, boiling time, removal of nucleic acids to achieve a high yield and activity of Taq DNA polymerase fragment on one cycle of boiling for 60mins. The centrifugation protocol followed was ideal for the purification, followed by streptomycin sulphate treatment to remove nucleic acids. The clear supernatant containing heat resistant Taq DNA polymerase was separated and stored at -70°C. The activity of enzyme was compared with commercial Taq DNA polymerase, stored in buffer containing 50% glycerol, at -20°C. The purified enzyme has a molecular weight of 94kDa, as predicted by SDS-PAGE and yielded appropriate enzyme activity comparing to the commercial Taq DNA polymerase. The whole process of purification was achieved within 3hrs. A total 53mg of Taq DNA polymerase enzyme was purified from 1 liter of bacterial culture emphasizing the fact that our method was economical and simple.

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### Introduction:-

Taq DNA polymerase is an enzyme procured from an heat stable bacteria called *Thermus aquaticus* having a molecular weight of 94,000 Daltons<sup>1</sup>. Taq polymerase is the most commonly used enzyme in almost every biological sciences research laboratory around the globe for the past three decades<sup>2,3</sup>. This powerful tool for amplification of DNA has seen a major breakthrough in its isolation, modification, purification, and activity<sup>3</sup>. A lot of work done in this aspect even today for better exploitation of this enzyme for commercial usage<sup>4</sup>. Many articles have described purification methods for Taq DNA polymerase<sup>5,6</sup>, purification of biologically active enzyme from complex mixture remains as one of the most challenging aspect in biotechnology. Previously published protocols involves use of large starting culture volumes and use of complex buffers containing many detergents<sup>7</sup>. By addressing these issues we have developed simple and rapid purification protocol which can be carried out in labs that lacks some of the reagents and equipment required in original protocol. Boiling lysis and nucleic acid removal methods yielded an approx 53mg of Taq enzyme within 3hrs from 1-liter *Escherichia coli* cell culture. The scale-down, simplified, rapid method described here would enable Taq DNA polymerase purification more feasible for research and diagnostic laboratories.

### Materials and method:-

#### Enzyme purification:-

The colony-transformed BL21 cells were cultured overnight at 37°C in 5ml of LB (Luria-Bertani) (Titan Biotech, India) medium supplemented with ampicillin (100µg/ml) (HiMedia, India). Large scale cultures were then initiated

by adding 5ml of overnight culture to 100ml of LB medium with the addition of ampicillin (100µg/ml). These cultures were grown at 37°C until the broth had reached an attenuation ( $D_{600}$ ) of 0.6. At this point, IPTG was added to a concentration of 0.5mM and the culture was grown for another 12-18h. The bacterial cells were harvested by centrifugation for 10min at 8000g, then resuspended in 4X Taq storage buffer (20mM Tris/HCL), pH 8.0, 10mM EDTA, 100mM KCL, 0.5% Nonidet P40 and 0.5% Tween 20) to one-twentieth of the original volume by vigorous vortex-mixing. Suspension of cells was disrupted by sonication on ice (5mins, 10-S pulse with 10-S pause intervals) and centrifuged at 12000g, 15mins, 4°C. The supernatant was incubated at 75°C for 1 h, cooled on ice for 10min, centrifuged at 13000g, 15min, 4°C to remove the denatured proteins. Nucleic acids was removed by addition of streptomycin sulfate solution (40%) to a final concentration of 4mg/ml. The mixture was gently stirred for 1 h at 4°C and centrifuged at 13000g, 15min, 4°C. The purified Taq polymerase fragment was then stored at -20°C. It required proper and careful diluted before use.

#### **Protein estimation and activity assay:-**

The Taq Protein concentration was determined by measuring  $A_{595}$  as described by Bradford<sup>8</sup>. The Taq protein content was qualitatively visualized by SDS-PAGE on denaturing polyacrylamide gel [4.5% (w/v) stacking gel 12% (w/v) separating gel] stained with coomassie brilliant blue by loading 10µl aliquot of the supernatant containing Taq protein after nucleic acid treatment.

The activity of the purified Taq DNA polymerase fragment was determined using a PCR amplification assay by titration against the commercial enzyme preparation (Fermentas, Pittsburgh, United States). For the enzyme activity assay, the purified Taq polymerase was diluted 1:10, 1:20, and 1:30 with 1X Taq storage buffer before adding it to the reaction mixtures. Titration of the enzyme was performed in 50µl reaction mixtures with 0.5µl of diluted enzyme added immediately before starting the temperature cycling. The reaction mixture contained 75µM of each dNTP (NEB), 2.5mM MgCl<sub>2</sub>, 0.02µM each of Upstream and Downstream Primers (Sigma Aldrich, Bengaluru), (5'-GATGTTGATGCCGACAAATG-3' and 5'-ACCATAGCCAGGTTGTTTGC-3') that amplifies a 615-bp fragment of the *chuA* gene encoding outer membrane heme/hemoglobin receptor, and 100ng of template DNA isolated from uropathogenic *E. coli* culture in 1X PCR reaction buffer 1X Taq Buffer containing (42.5mM Tris-HCl (pH 8.2 at 25°C), 50mM KCl, 20mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.08% (v/v) Nonidet P-40, 0.01% (v/v) Tween 20), 2.5 units of Taq DNA polymerase (Bhat Biotech India Pvt Ltd, Bengaluru). PCR amplification was started and was run for 30 cycles under the conditions of 3 min at 95°C, 30 sec at 94°C, 30 sec at 57°C, 30 sec at 72°C and final extension 10 min at 72°C in an DNamp (Bhat Biotech India Pvt Ltd, Bengaluru) thermo cycler. After PCR cycle was completed, aliquots of 10µl of PCR products were analyzed by electrophoresis in 1.5% (w/v) agarose gel in the presence of TAE (Tris/acetate/EDTA) buffer. Proteins were stained with 5ng/µl propidium iodide and a SM0311 ladder (Fermentas) was used as a size marker. To test the ability of the purified Taq enzyme to amplify different lengths of DNA sequences, the enzyme was applied to amplify 615bp and 246bp fragments.

#### **Results:-**

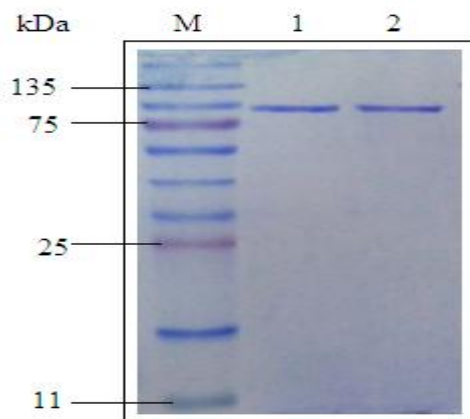
In our experiment, one cycle treatment consisted of 60 min boiling lysis and subsequent centrifugation at 12000g for 15min at 4°C. As demonstrated in Figure 1, Taq polymerase was released in high yield in the supernatant after boiling (total period of 60 min). The figure also indicates that almost no other protein remained in the supernatant after boiling.

To check for contamination with nucleic acids, 3µl of boiled lysate supernatant was tested on agarose gel electrophoresis, as shown in Figure 2. Using our protocol, the Taq preparations showed the presence of nucleic acid unless the streptomycin treatment was used (lane 1-2 in Figure 2). To remove this nucleic acid contamination, the enzyme was treated with streptomycin sulphate solution (40%) to a final concentration of 4mg/ml. The mixture was gently stirred for 1 h at 4°C and centrifuged at 12000g for 15 min at 4°C. The supernatant was stored at -20°C for several months. This step was completely effective in the removal of nucleic acid contamination (see lane 3-4 in Figure 2).

The activity of the purified Taq DNA fragment was quantified by a PCR-amplification reaction titrated against commercial enzyme (Figure 3). The concentration of 2µl of product of the 0.6kb fragment amplified using 1:10-diluted Taq polymerase (lane 10) was nearly equivalent to that of 2µl of product amplified using commercial enzyme (lane 1). Thus the 1:10 diluted Taq enzyme showed double activity compared with the commercial enzyme (Figure 3). The 1:30 diluted Taq enzyme prepared using our protocol showed an activity of 4units/µl. Consequently, the activity of the typical concentration of the final purified fragment of undiluted stocks reached a value of 40

units/ $\mu\text{l}$ . In the present study, 1 litre of bacterial cultures may produce amounts of Taq polymerase that is equivalent to  $1.06 \times 10^7$  units, a value in contrast with that pertaining to the commercial enzyme. We also confirmed that at a of 1:30 dilution Taq obtained by our method showed an equivalent activity with that of commercial enzyme. We concluded that the activity value of 1 unit quantified by PCR assay contained the equivalent of 3.8ng of active Taq protein, as verified by the Bradford method (0.38mg/ml,-(undiluted)).

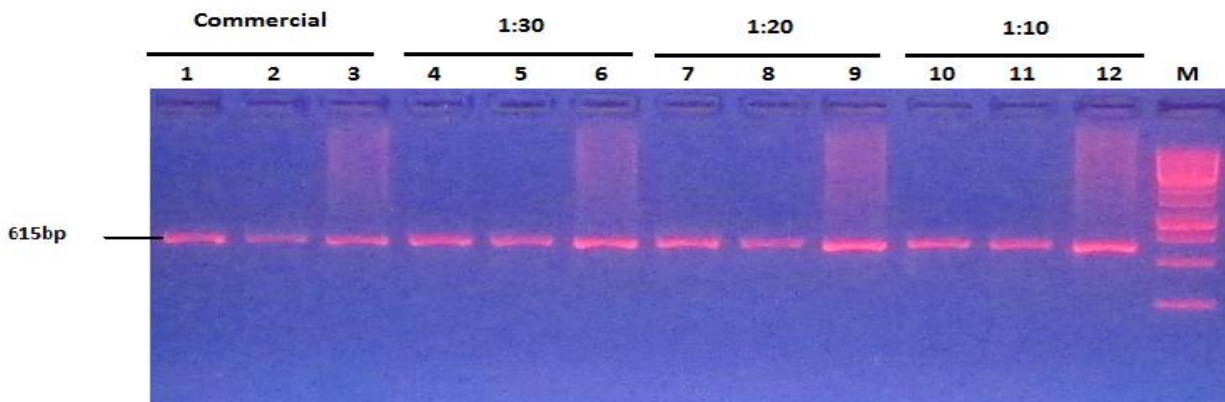
To confirm the effectiveness of the Taq enzyme purified by our protocol, we tested the ability of the enzyme to amplify different length of DNA sequences (Figure 4). When 1:30 dilutions of purified Taq enzyme and commercial enzyme (Fermentas, Pittsburgh, United States) were used to amplify DNA fragments of lengths 615bp and 246bp under the same conditions, results indicated that the purified Taq enzyme performed with an efficiency equivalent to that of the commercial enzyme.



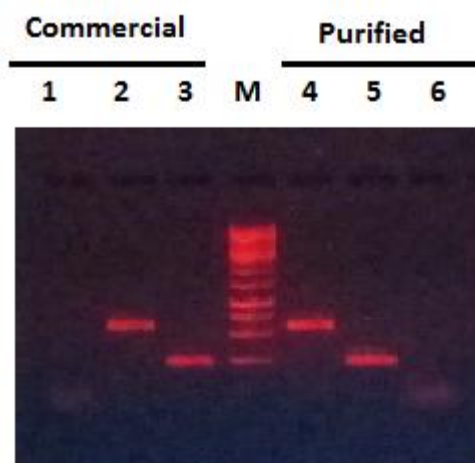
**Figure 1:-** Assessment of Taq DNA fragment by SDS/12%-PAGE with Coomassie Blue staining when boiling cycle were applied. Lane M contains protein marker, Lane 1 and 2 show the Taq protein in the supernatant after boiling for 60 minutes and treatment with streptomycin sulphate respectively (loading with approx.  $18.5 \mu\text{g}$  per lane). The expected Taq DNA polymerase (94kDa) was released with high yield and purity after boiling, streptomycin treatment and centrifugation.



**Figure 2:-** Identification of the nucleic acid contamination in the supernatant of boiled lysate with or without streptomycin sulphate treatment. Lane M contained 100bp (Fermentas) marker. Lanes 1-2 show the presence of nucleic acid contamination with one cycle boiling and centrifugation. Lane 3-4 indicates the absence of nucleic acids in the supernatant extracted using the streptomycin sulphate treatment.



**Figure 3:-** PCR amplification of *ChuA* gene using the purified Taq polymerase in different dilutions (1:10, 1:20 and 1:30). The expected amplified DNA fragment is marked with an arrow. Lane 1-12 shows the amplification products using commercial enzyme and Taq polymerase purified using our method. A 2 $\mu$ l portion of PCR products was loaded in lanes 1,4,7, and 10, a 3 $\mu$ l portion in lanes 2,5,8 and 11, and a 5 $\mu$ l portion in lanes 3,6,9, and 12.



**Figure 4:-** PCR amplification of different lengths of DNA fragment using the commercial enzyme and purified Taq polymerase. Lane M contains SM0311 Marker. Lanes 2 and 4 show the amplification products of the *chuA* gene (615bp), lanes 3 and 5 show the products of the *chuA* gene (246bp), Lanes 1 and 6 are negative controls.

### Discussion:-

Inventions in the field of molecular biology in present era especially the isolation & purification of enzymes is at the rate of minutes. New protocol for isolation and purification of commercially important enzymes is designed and developed across the globe every minute. With high speed technologies, research in PCR application and DNA sequencing techniques based on the use of Taq DNA polymerase, many protocols have been followed for purification of this enzyme to meet the current research and commercial requirements. Of them, most commonly used are heat treatment and freeze thawing. Unfortunately all these methods involves complex processes, which are time consuming and requiring sophisticated laboratories, in addition to the use of expensive and toxic chemicals. In the current study we formulated a simple protocol for the purification of full length Taq polymerase enzyme using boiling lysis method, which is based on its thermostability. Our experimentation for enzyme purification takes only 2-3hrs, using only one kind of storage buffer, no specialized instrumentation required nor usage of toxic chemicals. In our experiments, we isolated and purified 53mg of Taq polymerase enzyme from one liter of crude bacterial culture. The activity of our isolated enzyme was found to be  $1.8 \times 10^7$  units/ml as assessed and confirmed by PCR assay and the yield was compared with 5U/ $\mu$ l (Fermentas) of Taq DNA polymerase.

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