Original Research Article

High-level expression and purification of DNA and DNase free *Taq* DNA polymerase

ABSTRACT

Aim: *Taq* DNA polymerase from *Thermus aquaticus* is a key enzyme in the field of molecular biology that has been mostly used in polymerase chain reaction (PCR). Our aim is to produce standard grade *Taq* DNA polymerase free from censorious impurities like DNase, DNA and other contaminating protein.
Place and Duration of Study: The experiments were performed at Molecular Diagnostic Division of Bhat Biotech India (P) Ltd., Bangalore from February 2017 to January 2018.
Methodology: The recombinant *Taq* DNA polymerase clone was confirmed by PCR and DNA sequencing followed by BLAST analysis. The recombinant protein is in soluble form and was expressed in *E. coli* DH5α strain. The enzyme was extracted using boil-lysis method and followed by purification with ion exchange chromatography and silica column chromatography to remove the contaminating protein, DNase and DNA. The yield of the protein was also calculated.
Results: In our laboratory high quality *Taq* DNA polymerase was purified using ion exchange chromatography columns and silica column, with a resulting yield of about 45-50 mg/L and the activity was found to be 1.5 U/µL.
Conclusion: The use of silica column to remove the residual DNA is a remarkable step in obtaining an unequalled quality of *Taq* DNA polymerase.

Keywords:
Taq DNA polymerase; Thermus aquaticus; DNase; PCR; BLAST;Chromatography.

1. INTRODUCTION

Polymerase Chain Reaction (PCR) and its expanding variants have revolutionized the field of molecular biology and biotechnology and are indispensable in the field of recombinant DNA technology, molecular diagnosis, genetic analysis, forensic studies, etc., This technique ubiquitously requires a thermostable enzyme, *Taq* DNA polymerase, which has a deoxyribonucleic acid polymerization ability at high temperature thus, it has a huge market potential.

Taq DNA polymerase was primarily isolated from a thermophilic bacterium, *Thermus aquaticus*. In *T. aquaticus* the expression of native *Taq* DNA polymerase is quite low but the applications and demand are tremendous hence, later it was cloned into expression vectors which facilitate the production of large amount of protein in bacterial expression system [1]. The *Taq* DNA polymerase gene has 2499 base pairs, the full length protein has about 832 aminoacids and its predicted molecular weight is nearly 94 kDa [2]. Several articles are available for the over expression of *Taq* DNA polymerase in *E. coli* expression system. Many researchers have also worked on the purification system and tried to obtain pure enzyme, for that several methods has been followed by them to obtain high grade purified enzyme from the heat treated clarified lysate. Their methods included precipitation with polyethyleneimine followed by ion exchange chromatography [3]; precipitation with ammonium sulphate subsequently dialyzed with storage buffer [4]; precipitation with ethanol and dissolved in the storage buffer [5], nucleic acid precipitation with polymin P, followed by phenyl sepharose and heparin-sepharose column chromatography [6]; freezing and high temperature thawing of the culture filtrate followed by dialysis against storage buffer [7].

The pitfalls in the *Taq* DNA polymerase purification system with many researchers are the time consumption, low yield, host contaminating protein and DNA and sometimes nuclease activity. All these strategies must be considered for developing a novel purification protocol to get excellent

quality of *Taq* DNA polymerase. An attempt has been made in our laboratory to purify high quality *Taq* DNA polymerase using ion exchange chromatography columns, with a resulting yield of about 45- 50 mg/L.

2. MATERIAL AND METHODS

2.1 CONFIRMATION OF CLONE

The *Taq* DNA polymerase gene cloned in pLoxGentrc vector was transformed by heat shock method into *E. coli* DH5α competent cells that were prepared by $CaCl_2$ method [15]. The transformed cells were plated on Luria Bertani (LB) agar plates containing 100 µg / mL ampicillin and incubated at 37°C overnight. The insert of a clone was confirmed by PCR with gene specific primers followed by sequencing. The sequences of the forward and reverse gene specific primers were 5' ATGAGGGGGATGCTGCCCCTCT 3' and 5' TCACTCCTTGGCGGAGAGCCAG 3' respectively. The 2499 bp amplicon was visualized on a 1% agarose gel. The amplicon was gel eluted using Geneasy gel extraction kit (Bhat Biotech India Pvt. Ltd.) and sequenced. The sequence was further confirmed by NCBI BLAST analysis.

2.2 EXPRESSION AND PURIFICATION OF *Taq* DNA POLYMERASE

The transformed colony was inoculated in 200 mL of LB broth supplemented with 100 µg / mL ampicillin, incubated overnight at 37°C in an incubator shaker and used as a primary culture. This culture was used as pre-inoculum to inoculate 2 litre of LB broth containing 100 µg / mL ampicillin in the ratio 1:10 and incubated at 37°C until the broth reached an OD_{600} of 0.6. The culture was induced with 1.0 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) and incubated for 16 h at 37°C. The culture was harvested by centrifugation at 8000 rpm for 10 min and washed with 200 mL of Taq buffer A (20 mM Tris, pH 7.9; 50 mM Glucose; 1 mM EDTA). The cells were resuspended in 40 mL of Taq buffer A supplemented with 4 mg / mL lysozyme and incubated at 37°C for 15 min. After cell lysis, cell lysate was loaded onto 12% SDS-PAGE and observed the presence of *Taq* DNA polymerase by coomassie brilliant blue (CBB) staining [15]. Cell lysate was subjected to DNase treatment for 30 min at 37°C. Further, 40 mL of Taq buffer B (10 mM Tris, pH7.9; 50 mM KCl; 1 mM EDTA; 0.5% Tween; 1 mM PMSF) was added and incubated for 1h at 80°C. After that cell lysate was centrifuged at 10,000 rpm for 20 min at 4°C to remove the cell debris. The supernatant was collected and passed through Q-sepharose column pre-equilibrated with Taq Q buffer (25 mM Tris, pH 7.9; 1mM EDTA; 0.5% Tween 20; 10% glycerol; 1 mM dithiothreitol (DTT); 1 mM phenylmethane sulfonyl fluoride (PMSF)) supplemented with 25 mM KCl. The Q-sepharose column was washed with Taq Q-buffer containing 25 mM KCl. The protein was eluted with stepwise gradient of KCl concentration from 25 - 500 mM in Taq Q buffer. The collected fractions were checked on 12% SDS-PAGE followed by silver staining [15] for the presence of *Taq* DNA polymerase. The fractions containing the protein were pooled and dialyzed in Taq S buffer (20 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), pH 6.9; 1 mM EDTA; 0.5% Tween 20; 10% glycerol; 1 mM DTT; 1 mM PMSF) containing 25 mM KCl. The dialyzed sample was loaded onto S-sepharose column pre-equilibrated with Taq S buffer containing 25 mM KCl. The S-sepharose column was washed with 60 mL of Taq S buffer with 25 mM KCl. The protein was eluted with stepwise gradient of KCl concentration varying from 25 - 500 mM in Taq S buffer. At each step, 2 mL fractions were collected and checked using 12% SDS-PAGE followed by silver staining [15] for the presence of *Taq* DNA polymerase.

2.3 PURIFICATION OF *Taq* DNA POLYMERASE BY SILICA COLUMN CHROMATOGRAPHY

The fractions containing the protein were pooled and passed through the silica column. The flow through was collected and analyzed for the presence of *Taq* DNA polymerase using 12% SDS-PAGE. The flow through was further dialyzed against Taq storage buffer (50 mM Tris, pH 8.0; 100 mM NaCl; 0.1 mM EDTA; 1% Triton X 100; 5 mM DTT; 50 % glycerol). The in-house purified protein along with the commercial *Taq* DNA polymerase was loaded onto 12% SDS-PAGE and silver stained [15] to examine the purity of the purified protein as compared to commercial.

2.4 PCR ASSAY FOR BACTERIAL DNA CONTAMINATION

A PCR reaction was set up using universal primer for bacterial 16S rDNA and *malB* (maltose binding protein) primer to check for the *E. coli* genomic DNA contamination. The 16S rDNA bacterial primers generated a 1487 bp amplicon using forward primer 5' AGAGTTTGATCCTGGCTCAG 3' and reverse primer 5'GGTTACCTTGTTACGACTT 3'. The PCR was performed using following conditions; initial denaturation at 97°C for 3 min followed by 35 cycles of 94°C denaturation for 1 min, 55°C annealing for 1 min, 72°C extension for 90 sec followed by final extension at 72°C for 10 min. The forward 5' GATGCGTGCACCTGTTTTTA 3' and reverse primer 5' ACACCACGAATTCACCTTCA 3' amplified a target of 491 bp fragment of *malB* in *E. coli* genome. The PCR conditions for the amplification of *malB* included initial denaturation at 97°C for 3 min followed by 35 cycles of 94°C denaturation for 45 sec, 57°C annealing for 45 sec, 72°C extension for 45 sec followed by final extension at 72°C for 10 min.

2.5 DNase ACTIVITY ASSAY

DNase activity assay was carried out with purified *Taq* DNA polymerase to ensure that the purified protein were free from DNase enzyme. The plasmid was taken as the template to determine the DNase activity of the purified protein. The assay consisted of the following discrete reactions with appropriate controls as follows; (i) plasmid alone as control, (ii) plasmid incubated with DNase buffer as control, (iii) plasmid incubated with DNase enzyme and buffer, (iv) plasmid incubated with DNase buffer and purified in-house *Taq* DNA polymerase enzyme and (v) plasmid incubated with DNase buffer and commercial *Taq* DNA polymerase enzyme. All the reaction tubes were incubated overnight at 37°C.

2.6 ACTIVITY ASSAY AND UNIT DETERMINATION OF *Taq* DNA POLYMERASE

This study was carried out using standard commercial *Taq* DNA polymerase with various concentrations ranging from 0.25 U/µL to 2 U/µL. Each 50 µL PCR reaction was setup using cloned plasmid containing 164 bp of HCV genome as template. The samples were loaded and run on a 1.5% of agarose gel and the band intensity of amplicon generated using commercial *Taq* DNA polymerase was calculated using ImageJ software. A standard curve was prepared using peak area produced by the software for various concentrations of commercial enzyme. Using this standard curve, the units were extrapolated for in-house *Taq* DNA polymerase.

2.7 ACTIVITY ASSAY WITH DIFFERENT CLINICAL SAMPLES

The enzyme activity assay of the purified *Taq* DNA polymerase was performed using isolated DNA from different clinical samples. The *Acinetobacter* spp. was tested in the clinical sample using primers of *rpoB* gene of amplicon size 397 bp. The presence of uropathogenic *E. coli* (UPEC) was assessed in the urine sample by amplifying a 615 bp region of *chuA* gene which encodes outer membrane heme utilization receptor protein in UPEC. The presence of *Mycobacterium tuberculosis* was examined in the sputum sample by amplifying a 236 bp fragment of early secretory antigen *Esat6* gene.

3. RESULTS

The presence of *Taq* DNA polymerase gene within the recombinant plasmid was confirmed by PCR with gene specific primers and 2499 bp amplicon was visualized on the agarose gel (Fig. 1). Further the clone was sequenced and BLAST analysis showed sequence similarity with the *Taq* DNA polymerase of *Thermus aquaticus* (GenBank: D32013.1).

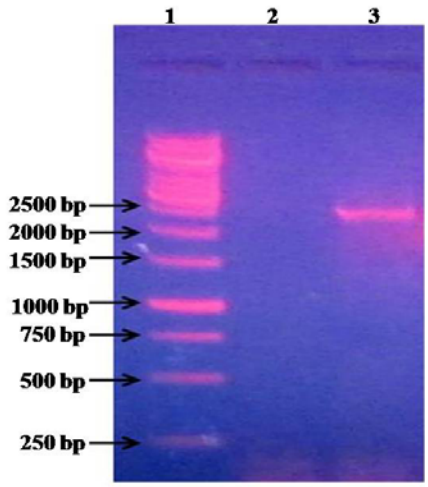

Fig. 1: PCR confirmation of recombinant plasmid containing *Taq* DNA polymerase gene. Lane 1: 1 kb ladder; Lane 2: Blank; Lane 3: Amplified PCR product of *Taq* DNA polymerase gene.

3.1 EXPRESSION AND PURIFICATION OF *Taq* DNA POLYMERASE

The *Taq* DNA polymerase was expressed in *E. coli* DH5α. The culture was induced with 1 mM IPTG, harvested and the cell disruption was facilitated with lysozyme to begin purification of soluble protein.

The thermostable nature of *Taq* DNA polymerase was utilized to eliminate majority of cellular proteins, by incubating the cell lysate at 80°C for 1 h. This led to the denaturation and precipitation of the said proteins, thus making it possible for their removal by centrifugation. However, in *E. coli* there are about 15 other cellular proteins that are known to be more or less thermostable thus, highly likely to be present in the supernatant [1, 8]. Hence, ion exchange chromatography was performed to remove these contaminating proteins from the heat treated supernatant. The isoelectric point (pI) of *Taq* DNA polymerase is 6.03; hence the soluble fraction had undergone through the anion exchange chromatography in Tris buffer at pH 7.9. At pH 7.9 the net charge of protein is negative thus these proteins electrostatically bind to the anion exchanger Q-sepharose and are eluted by increasing the concentration of KCl.

A majority of contaminating proteins were eliminated during the anion exchange chromatography but still some contaminating proteins were still present in the eluted fractions of the protein. For further purification all the fractions were pooled together and desalted for the succeeding purification steps. The desalted protein had undergone through the cation exchange chromatography in S sepharose. HEPES buffer (pH 6.9) and KCl was used for washing the column and the elution of protein with step gradient method. During the purification most of the *Taq* DNA polymerase binds to the matrix electrostatically and most of the co-purified contaminating proteins do not bind to the cation exchanger and goes into the flow through. The bound fraction of *Taq* DNA polymerase was eluted with stepwise gradient of KCl and the purity of eluted protein was observed on SDS-PAGE (Fig. 2c).

Extra precautions had to be taken to remove the residual DNA/RNA/plasmid from the purified *Taq* DNA polymerase because that might interfere during the diagnostic purpose or bacterial barcoding studies. Hence, the pooled fractions from the S sepharose column were passed through the silica matrix. Any residual nucleic acid would bind to the silica matrix and the purified protein comes in the flow through. The flow through containing the purified *Taq* DNA polymerase was dialysed in Taq storage buffer and stored at – 20°C. The purified protein along with the commercial enzyme was compared on silver stained gel and the purity was examined (Fig. 2d).

The result was analyzed on 12% SDS-PAGE and a single band at approximately 94 kDa was observed (Fig. 2c, 2d), there was no trace of non-specific or contaminating protein in purified *Taq* DNA polymerase.

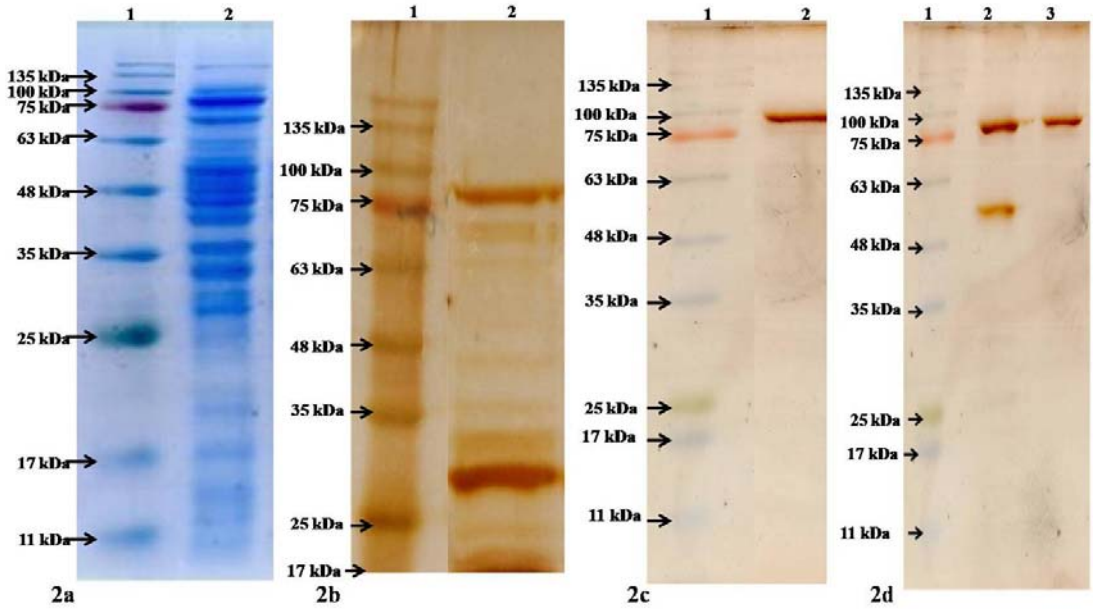

Fig. 2: Expression and purification of in-house *Taq* DNA polymerase and comparison with commercial enzyme. 2a: Lane 1: Prestained protein marker; Lane 2: *E. coli* cell lysate; **2b:** Lane 1: Prestained protein marker; Lane 2: Soluble supernatant fraction after heat treatment; **2c:** Lane 1: Prestained protein marker; Lane 2: Purified *Taq* DNA polymerase after ion exchange chromatography and silica column chromatography; **2d:** Lane 1: Prestained protein marker; Lane 2: Commercial *Taq* DNA polymerase; Lane 3: Purified *Taq* DNA polymerase.

3.2 PURITY TESTING

The *E. coli* cells were lysed with lysozyme to obtain a clear lysate, which was used for the downstream processing. In *Taq* DNA polymerase preparations, DNA and DNase are the two common impurities that are cumbersome to remove. However, in order to obtain standard grade enzyme we aim to get rid of these contaminants.

The DNA contamination in *Taq* DNA polymerase may originate from the *E. coli* genome and the expression plasmid. This DNA can show false positive amplification with the primers designed for the amplification of bacterial genome as well as common plasmid. It is crucial that higher grade *Taq* DNA polymerase should not have DNA contamination hence, for the elimination of these contaminants, the cell lysate was treated with DNase enzyme.

The DNase enzyme is also harmful for PCR reactions as it degrades the template DNA and primer before reaching the primary denaturation step; for this reason it is utterly essential to remove or inactivate the DNase from the *Taq* DNA polymerase. The thermostable nature of *Taq* DNA polymerase provides an advantage to precipitate and inactivate almost all the contaminating proteins including DNase at higher temperature (80°C for 1 hr). The other smaller contaminants were removed by dialysis.

For ultra-pure preparation of *Taq* DNA polymerase an additional purification step was performed with ion-exchange chromatography, to remove the residual contaminating protein and the fragments of small nucleic acid. The ion-exchange chromatography provides an additional protection against residual contamination of DNA and DNase. The theoretical isoelectric point of *Taq* DNA polymerase is 6.03 where as DNase and DNA are 5.2 and < 5 respectively ergo these contaminants bound more strongly with anion-exchange resin than *Taq* DNA polymerase. Finally, these leftover contaminants were removed from *Taq* DNA polymerase. Therefore, the ultra-grade preparation was doubly safeguard against DNA and DNAse and it is also known as double DNA and DNase free preparation.

The DNA contamination in purified *Taq* DNA polymerase was tested directly by PCR. The universal bacterial 16S ribosomal DNA and *malB* gene primers were used for this purpose to detect the bacterial and *E. coli* genomic DNA contamination in purified *Taq* DNA polymerase. Similarly, recombinant plasmid contamination was checked by PCR using specific primer for partial *Taq* DNA polymerase gene.

These three sets of primers enabled to gauge the quality of the purified *Taq* DNA polymerase in terms of nucleic acid contamination. In all these three cases, amplification was not observed when purified *Taq* DNA polymerase was used as a source of DNA template (source of contaminating genomic or recombinant plasmid DNA) (Fig. 3a, 3b and 3c). Hence, the purified *Taq* DNA polymerase was free from DNA contamination.

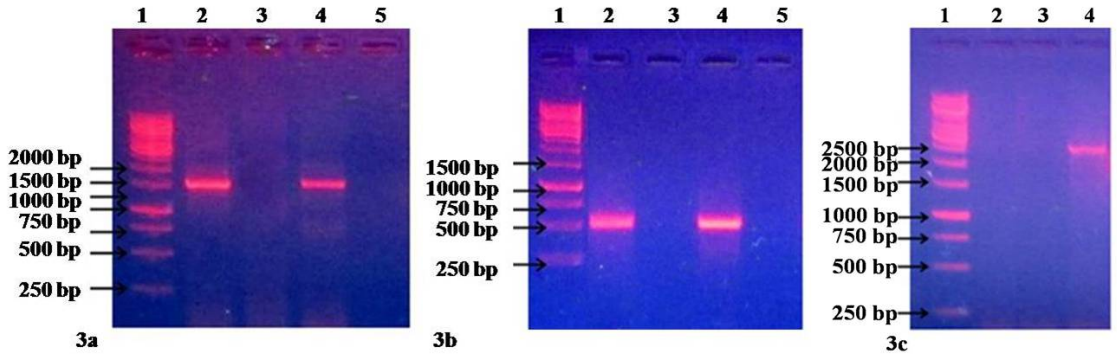

Fig.3: With respect to the DNA contamination, the purity of *Taq* DNA polymerase enzyme was examined by PCR and compared with commercial *Taq* DNA polymerase. 3a: PCR amplification with universal bacterial 16S ribosomal DNA primers using purified *Taq* DNA polymerase. Lane 1: 1kb ladder; Lane 2, 4: Positive control assay; where the bacterial genomic DNA was used as template for purified *Taq* and commercial *Taq* DNA polymerase respectively; Lane 3, 5: Purity assay; where purified *Taq* and commercial *Taq* DNA polymerase were used as source of DNA template as well as enzyme respectively; **3b: PCR amplification with *malB* gene specific primers using purified *Taq* DNA polymerase.** Lane 1: 1kb ladder; Lane 2, 4: Positive control assay; where the bacterial genomic DNA was used as template for purified *Taq* DNA polymerase and commercial *Taq* DNA polymerase respectively; Lane 3, 5: Purity assay; where purified *Taq* DNA polymerase and commercial *Taq* DNA polymerase were used as source of DNA template as well as enzyme respectively. **3c: PCR amplification with gene specific primers for *Taq* DNA polymerase to check plasmid contamination.** Lane 1: 1kb ladder; Lane 2 and 3: 1 and 5 µL purified *Taq* DNA polymerase used as source of DNA template respectively; Lane 4: Positive control for the assay.

For the study of DNase activity or DNase contamination of purified *Taq* DNA polymerase, the assay was performed by incubating it with the plasmid DNA and comparison was done with commercial enzyme (Fapon Biotech Inc). The amount of plasmid remained same after overnight incubation with in-house *Taq* DNA polymerase as well as the commercial enzyme (Fig. 4). The DNase activity was not evident in the purified protein. Again the standard preparation methods met our requirement to produce DNase free *Taq* DNA polymerase.

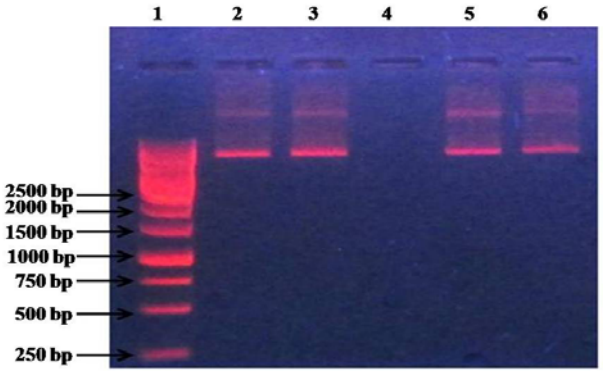

Fig. 4: With respect to the DNase contamination, DNase activity assay performed at 37°C for overnight with purified *Taq* and compared with commercial *Taq* DNA polymerase. Lane 1: 1 kb ladder; Lane 2: Plasmid only; Lane 3: Plasmid in DNase buffer; Lane 4: Plasmid and DNase enzyme in DNase buffer; Lane 5: Plasmid and purified *Taq* DNA polymerase enzyme in DNase buffer; Lane 6: Plasmid, commercial *Taq* DNA polymerase enzyme in DNase buffer.

3.3 ACTIVITY ASSAY

It is essentially required to know the unit of an enzyme before performing any enzyme assay, because the quantity of an enzyme available or used in an assay is very tough to identify in absolute term like grams or milli grams, since its purity is usually poor and some part of an enzyme may be in an inactive or partially active state. Most applicable parameters for any enzyme are its functional activity. These activities are generally calculated in terms of enzyme unit (U), for a *Taq* DNA polymerase 1 Unit is defined as the amount of enzyme that incorporates 10 nmol of deoxyribonucleoside triphosphates into acid-insoluble material in 30 min at 72°C in standard assay conditions.

The most authentic assay for identification of enzyme unit is radio labelled nucleotide incorporation during PCR amplification. But this method is undesirable due to the usage of radioactivity. There are several alternative methods to identify the enzyme unit and one among them being standard curve preparation method. Here standard curve preparation method was used for calculating the enzyme unit.

The assay was carried out to determine the unit of in-house purified *Taq* DNA polymerase for which standard curve was created using an authentic standard *Taq* DNA polymerase. For the preparation of standard curve, different unit of standard *Taq* DNA polymerase was used in the PCR reaction and the intensity of amplified PCR product was used to create standard curve.

ImageJ software was used to calculate the intensity of amplicon band where peak area represents the intensity of band on the agarose gel. The unit of standard *Taq* DNA polymerase enzyme vs peak area was used for standard curve preparation. There was a linear increase in peak area against the amount or unit of enzyme taken (Fig. 5b).

In order to determine the unit of in-house enzyme, a known volume of in-house purified *Taq* DNA polymerse was used in PCR and the peak area produced against the band intensity of amplified PCR product was extrapolated on the standard curve and finally enzyme unit of purified *Taq* DNA polymerase was calculated (Fig. 5b). The standard curve was precise with a R2 value of 0.981. 0.5 µL of in-house purified enzyme was used in a PCR reaction (Fig. 5a; Lane 9) and the unit was found to be 1.54 U/µL. Thus the purified *Taq* DNA polymerase is on par with commercial *Taq* DNA polymerase.

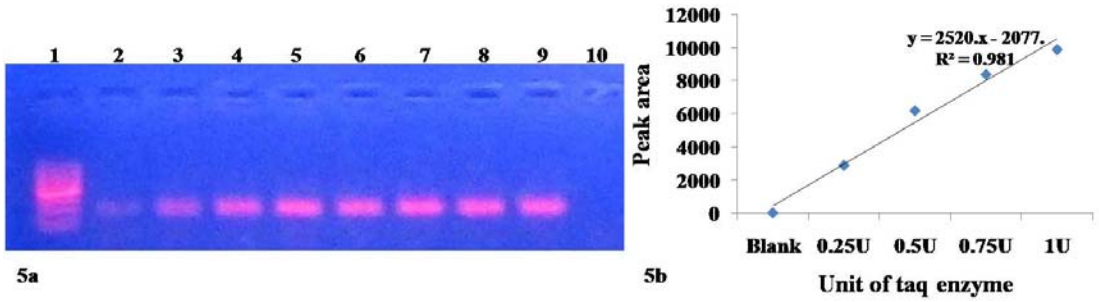

Fig. 5: Enzyme unit determination for purified *Taq* DNA polymerase. 5a: Agarose gel showing 164 bp amplification band of cloned HCV fragment with commercial *Taq* DNA polymerase. Lane 1: 100 bp ladder; Lane 2: 0.25 U; Lane 3: 0.5 U; Lane 4: 0.75 U; Lane 5: 1.0 U; Lane 6: 1.25 U; Lane 7: 1.5 U; Lane 8: 2 U; Lane 9: 0.5 µL of purified *Taq* DNA polymerase; Lane 10: Blank. **5b: Standard curve made with enzyme unit of commercial *Taq* DNA polymerase vs peak area measured using ImageJ software.**

3.4 VALIDATION OF IN-HOUSE *Taq* DNA POLYMERASE WITH CLINICAL SAMPLE

DNA was isolated from distinct clinical samples and used to test with purified *Taq* DNA polymerase. Excellent amplification was observed with the target gene in each clinical sample such as *Esat6* in *M. tuberculosis* (Fig. 6a), *chuA* in uropathogenic *E. coli* (Fig. 6b) and *rpoB* in of *Acinetobacter* spp. (Fig. 6c).

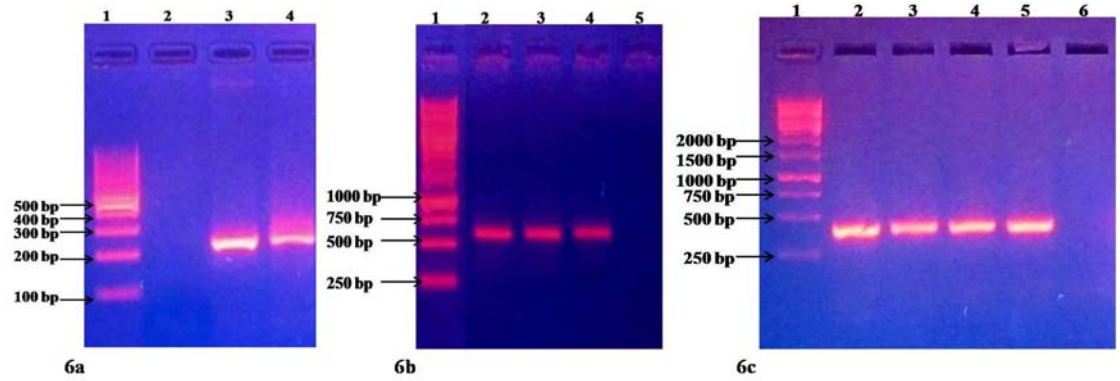

Fig. 6: Validation of In-house *Taq* DNA polymerase with various clinical samples. 6a: Amplification of 236 bp of *Esat 6* gene of *M. tuberculosis*. Lane 1: 100 bp ladder; Lane 2: Blank; Lane 3,4: DNA isolated from two different clinical samples used as template. **6b: Amplification of 615 bp *chuA* gene of uropathogenic *E. coli*.** Lane 1: 1 kb ladder; Lane 2,3,4: DNA isolated from three different clinical samples used as template; Lane 5: Blank; **6c: Amplification of 397 bp *rpoB* gene of *Acinetobacter* spp.** Lane 1: 1 kb ladder; Lane 2,3,4,5: DNA isolated from four clinical samples used as template; Lane 6: Blank.

4. DISCUSSION

Taq DNA polymerase is an indispensable enzyme in research field such as molecular biology and diagnostics. The patent for *Taq* DNA polymerase has expired and hence many companies and organization are involved in the manufacturing of the enzyme. Different vectors have been used by different investigators to increase the yield of *Taq* DNA polymerase such as pTTQ18 [3], pUC18 [9], pTZ57R [10], pET 15b [11], pTrc99A [12], pET28b [13]. Similarly different *E. coli* expression strains like INV1alphaF' [4], DH1, BL21 (DE3), TOP10 have been used.

There are two major aspects to produce standard grade *Taq* DNA polymerase commercially they are, yield and quality of the expressed protein. To increase the yield of protein four vital factors need to be taken under consideration namely, the expression strain, vector, concentration of IPTG and induction time period [4]. In our present study, we have transformed the clone in *E. coli* DH5α strain, induced with 1mM IPTG at 37°C for 12 – 16 h. This combination has increased the expression several fold than reported earlier. At present the yield of *Taq* DNA polymerase enzyme was about 45 - 50 mg/L. The purification protocol from previous researchers [3, 4, 13] found traces of contaminating protein from *E. coli*. During the crude purification of *Taq* DNA polymerase there are chances of cellular protein, DNase and DNA contamination in purified protein. There are fifteen thermostable cellular proteins that have chances to purify along with *Taq* DNA polymerase even after heat treatment and these proteins are, outer membrane lipoproteincarrier protein, transcription pausing factor L, maltose-binding proteins, putative EscN protein, FK-506-binding protein, adenylate kinase, ribosomal protein S19, DnaK, galactose glucose binding protein, D-ribose binding protein, trigger factor, GroES etc [1, 8]. These proteins may reduce the activity of the enzyme as there may be few inhibitors present [14]. The purified *Taq* polymerase has a fair chance of DNA contamination which might give false positive amplification when primers designed for the amplification of bacterial genome or common plasmid. Hence, it is necessary to get rid of the DNA contamination. Likewise, the *Taq* polymerase may also be contaminated with DNase enzyme which may not give an accurate result especially in case of clinical samples, where the template concentration is very low. It may result in false negative result. Hence, it necessitates the removal of DNase enzyme from the purified protein completely. Thus, in terms of purity and activity, our *Taq* polymerase is exceptionally good when compared to the other commercial enzyme. In the aspect of efficiency the purified *Taq* DNA polymerase supposed to work with wide range of samples including clinical samples. Here, the purified enzyme was tested with the DNA isolated from different types of clinical samples such as serum and sputum. The in-house purified enzyme worked efficiently with different samples by amplifying the specific target sequences.

5. CONCLUSION

In this study, we have optimized the expression and purification protocol in which the contaminating macromolecules including proteins and nucleic acid were completely removed and finally commercially high grade enzyme was purified. The in-house purified enzyme has an activity of 1.54 U/μL which is similar to most of the commercial polymerases. The enzyme is also compatible with a few clinical samples which were tested. Thus, the in-house purified *Taq* DNA polymerase can be used in a wide range of applications. This purified *Taq* DNA polymerase has proved to be of utmost purity and at par with other commercial enzymes, thus making headway towards its commercialization as Geneasy *Taq* DNA polymerase.

Ethical approval:

As per international standard or university standard ethical approval has been collected and preserved by the authors.

Consent: NA

REFERENCES

1. Kim SG, Park JT. Production of DNA polymerase from *Thermus aquaticus* in recombinant *Escherichia coli*. CNU Journal of Agricultural Science. 2014;41:245-49. DOI: 10.7744/cnujas.2014.41.3.245

2. Lawyer FC, Stoffel S, Saiki RK, Chang SY, Landre PA, Abramson RD, *et al.* High-level expression, purification, and enzymatic characterization of full-length *Thermus aquaticus* DNA polymerase and a truncated form deficient in 5'to 3'exonuclease activity. Genome Res. 1993;2:275-87. doi:10.1101/gr.2.4.275

3. Engelke DR, Krikos A, Bruck ME, Ginsburg D. Purification of *Thermus aquaticus* DNA polymerase expressed in *Escherichia coli*. Anal Biochem. 1990;191: 396-400. doi.org/10.1016/0003-2697(90)90238-5

4. Pluthero FG. Rapid purification of high-activity *Taq* DNA polymerase. Nucleic acids Res. 1993;21:4850-51. doi.org/10.1093/nar/21.20.4850

5. Chen S, Zheng X, Cao H, Jiang L, Liu F, Sun X. A simple and efficient method for extraction of Taq DNA polymerase. Electron J Biotechnol. 2015;18: 343-46. doi.org/10.1016/j.ejbt.2015.08.001

6. Lawyer FC, Stoffel S, Saiki RK, Myambo K, Drummond R, Gelfand DH. Isolation, characterization, and expression in *Escherichia coli* of the DNA polymerase gene from *Thermus aquaticus*. J Biol Chem. 1989;264:6427-37. PMID: 2649500

7. Grimm E, Arbuthnot P. Rapid purification of recombinant Taq DNA polymerase by freezing and high temperature thawing of bacterial expression cultures. Nucleic acids Res. 1995;23:4518. PMID: 7501478

8. Thapa A, Shahnawaz M, Karki P, Dahal GR, Sharoar MG, Shin SY, *et al.* Purification of inclusion body-forming peptides and proteins in soluble form by fusion to *Escherichia coli* thermostable proteins. Biotechniques. 2008;44:787-98. doi: 10.2144/000112728.

9. Desai UJ, Pfaffle PK. Single-step purification of a thermostable DNA polymerase expressed in *Escherichia coli*. Biotechniques. 1995;19:780-82. PMID: 8588916

10. Sadeghi HMM, Rabbani M, Moazen F. Amplification and cloning of Taq DNA polymerase gene from *Thermus Aquaticus* strain YT-1. Res Pharm Sci. 2007;1:49-52.

11. Moazen F, Rastegari A, Hoseini SM, Panjehpour M, Miroliaei M, Sadeghi HMM. Optimization of Taq DNA polymerase enzyme expression in *Escherichia coli*. Adv Biomed Res. 2012;1:82. doi: 10.4103/2277-9175.103004

12. Farazmandfar T, Rafiei A, Hashemi-Sotehoh MB, Valadan R, Alavi M, Moradian F. A simplified protocol for producing Taq DNA polymerase in biology laboratory. Res Mol Med. 2013;1:23-6.

13. Fang N, Zhong N, Yang Y, Guo Y, Ji S. Data of expression and purification of recombinant Taq DNA polymerase. Data Brief. 2016;9:81-4. doi: 10.1016/j.dib.2016.08.032

14. Mohammad R, Hamid G. Cloning and Expression of *Thermus aquaticus* DNA polymerase in *Escherichia coli*. Jundishapur Journal of Microbiology. 2008;1:1-5.

15. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press; 2001.