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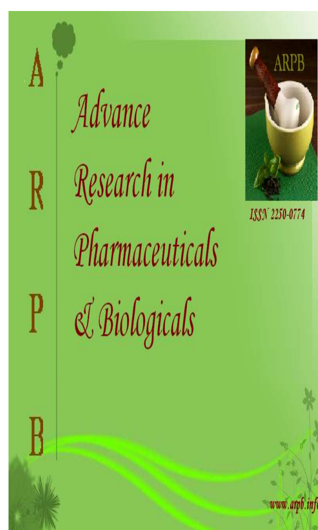

PRODUCTION OF *TAQPOLYMERASE* FROM *E. COLI*: A TREMENDOUS APPROACH OF CLONING AND EXPRESSION OF *TAQ*POLYMERASE-I GENE IN *E. COLI*

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ABSTRACT:

The thermostable properties of *Taq*DNA polymerase from *Thermusaquaticus* have contributed greatly to the yield, specificity, automation, and utility of the polymerase chain reaction method for amplifying DNA. *Taq*polymerase is widely used enzyme for DNA amplification in PCR techniques and highly applicable in molecular biology and biotechnology. The highly thermo stable DNA polymerase from *T. aquaticus* is ideal for both manual and automated DNA sequencing because it is fast, highly processive, has little or no 3'-exonuclease activity, and is active over a broad range of temperatures. Nowadays, the enzyme is produced from versions of the *Taq*gene that have been engineered so as to obtain high levels of expression in *E. coli*. Most of these alterations involve modification of the DNA sequences that precede and immediately follow the initiating ATG codon. The prominent features of *E. coli* i.e. fast replication, high expression of genes, ease of isolation, characterization and culturing in large scale, the complete knowledge of its genomic sequence which allow researchers to use this organism in many broad aspects. More than 50 DNA polymerase genes have been cloned and sequenced from various organisms including thermophiles by PCR cloning technique, whereby the gene encoding this enzyme was cloned into the expression vectors that produce recombinant *Taq*polymerase gene has facilitated for this enzyme production.

KEYWORDS: Thermusaquaticus, *Taq*polymerase, Sequencing, Purification.

INTRODUCTION

Taq, a thermo-stable DNA-dependent DNA polymerase, was first isolated from the thermophilic prokaryote *Thermusaquaticus* (*T. aquaticus*)[1]. *T. aquaticus*is a bacterium that lives in hot springs and hydrothermal vents, and *Taq*polymerase, which is identified as an enzyme able to withstand the protein-denaturing conditions (high temperature) required during polymerase chain reaction (PCR). Therefore, it replaced the DNA polymerase from *Escherichia coli* (*E.coli*) originally used in PCR[2]. The DNApolymerase from *T.aquaticus* is homologous to *E. coli* DNA polymerase I (*Pol*-I) and likewise has domains responsible for DNApolymerase and 5'nuclease activities. The structures to the polymerase domains of *Taq*polymerase and of the Klenowfragment (KF) of *Pol*-I are almost identical.

The structure of *Taq*DNA polymerase in different frame obtained from protein data bank (PDB) shown in Fig1**.** It is an enzyme having a molecular weight of about 6.6×10^4–9.4×10^4 Daltons[3] and it is a very important enzyme for molecular biological and biotechnological studies such as DNA amplification and DNA sequencing by the PCR[4,5]. *Taq's* temperature optimum for activity is 75-80°C, with a half-life of 9 minutes at 97.5°C, and can replicate a 10^3 base pair strand of DNA in less than 10 seconds at 72°C[6,7]. *Taq*DNA polymerase catalyzes the incorporation of dNTPs into DNA. It requires a DNA template, a primer terminus, and the divalent cation Mg^{2+} [8]. The recombinant *Taq*DNA polymerase expressed in *E. coli* shows identical characteristics to native *Taq* from *T. aquaticus* with respect to activity, specificity, thermostability and performance in PCR [9]. The enzyme became famous for its use in PCR[3, 9] and was designated in 1989 as Molecule of the Year for whatever that may mean[10]. The gene for *Taq* encodes an 832 amino acid protein (Mol. wt. = 93.9 kD) consisting of two domains[9]. The thermal stability of *Taq*DNA polymerase is thought to result from increased hydrophobicity of the core of the enzyme, improved stabilization of electrostatic forces, and enhanced interaction with solvent molecules, due to the presence of additional proline residues on the surface of the enzyme[10].

*Taq*limitation and challenges

A limitation in using *Taq*polymerase is its low fidelity rate compared to other types of polymerases. It is possible, however, that a form of *Taq* with a higher fidelity rate could be developed[11]. The challenge is fulfillment of rising demand of *Taq*DNA polymerase variants that add modified nucleotides for efficient DNA amplification and labeling, this challenge can be answered by using the techniques employed in protein engineering like random mutagenesis and site directed mutagenesis which will create various variants of existing *Taqpolymerase* and screening to find out the best one which fits the above requirements[7].

New England Biolab has expanded its DNA polymerase screening program to select for *Taq*DNA polymerase variants with desired properties. While *Taq* is a standard product used in 90% of molecular biology labs today, scientists still must spend time optimizing their PCR reactions, which can be time-consuming and tedious[4].

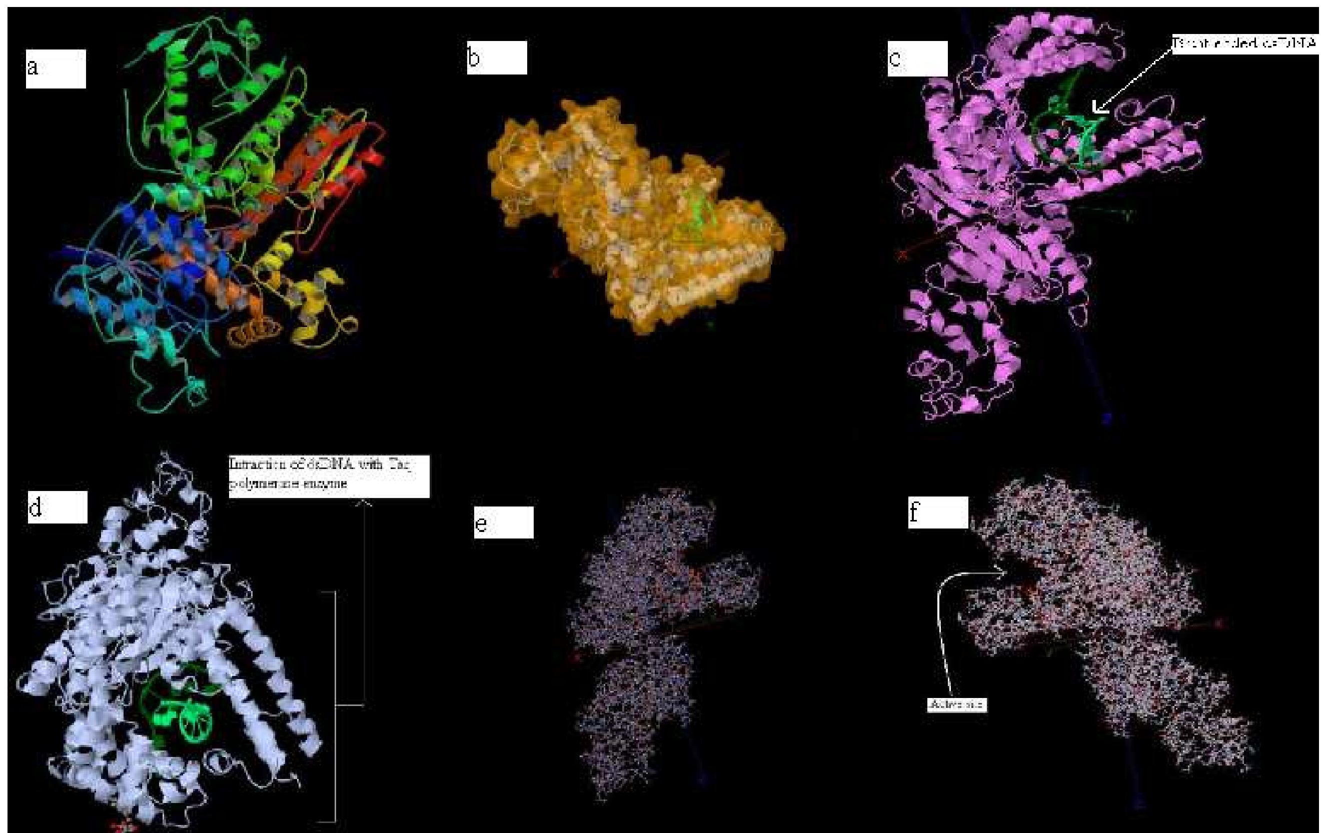

Fig1 The structure of *Taq*DNA polymerase in different frame obtained from protein data bank (PDB) provide better opportunity to understand the structure and function of*Taq*polymerase, 'a' represent protein model of the enzyme, 'b' molecular surface structure, 'c' and 'd' cartoon structure, 'e' and 'f' represent stick and "ball and stick" model respectively. The binding of dsDNA (blunt ended) in active site of enzyme is shown in 'b' 'c' and'd'. The active site of *Taq*polymerase is shown in 'e' and 'f', in some picture x, y and z represent the axes of enzyme. Structure model of *Taq*DNA polymerase obtained from PDB using jmol software. The URL is http://www.pdb.org/pdb/explore/explore.do?structureId=1TAU http://www.pdb.org/pdb/explore/jmol.do?structureId=1TAU&bionumber=1

Why *E. coli*?

E. coli has been the subject of intensive research interventions since the advent of molecular biology and genetic engineering[12]. It exhibits certain characters that exemplify it as the classical model organism[7]. The prominent features i.e. fast replication, high expression of genes, ease of isolation, characterization and culturing in large scale, the complete knowledge of its genomic sequence, and well established & standardized protocols for its manipulation at every level allow researchers to use this organism in many broad aspects[13]. In *T. aquaticus*, *Taq*polymerase expresses at such low levels (0.01-0.02% of the cellular protein) that commercial production is not a

viable proposition[6]. Most of alterations in *Taq*gene involve modification of the DNA sequences that precede and immediately follow the initiating ATG codon[10,14]. Since the clones used by various commercial manufacturers may be engineered in different ways and since the protocols used for purification of the enzyme may be different, preparations obtained from different manufacturers do not necessarily deliver identical results[4]. However, homemade *Taq*polymerase is consistently of high quality and shows little batch-to-batch variation[15]. A number of commercial preparations of *Taq* are available that lack 5'-3' exonuclease activity. These include the Stoffel fragment (Perkin-Elmer), a number of deletion variants, and a number of site-directed mutants[16].

Isolation of *Taq*Polymerase gene

Genomic DNA of *T. aquaticus* and plasmid has isolated by a Sambrookmethod[17]. A 2.6 Kb fragment containing the whole *T. aquaticus* DNA polymerase gene was prepared by PCR amplification with the *T. aquaticus* genomic DNA using primers[7]. Unique *Eco*RI and *Sal*I restriction sites respectively at each end of the amplified DNA fragment. The fragment ligated into the expression vector pET that had digested before with *Eco*RI and *Sal*I, giving a closed circular fusion molecule (The constructed vector called pTTQ). The ligate was transformed into competent *E. coli* strain, TOP 10 by $CaCl_2$ using heat shock method at 42°C for 45 seconds[18]. Most of scientist used protocol for isolation of *TaqPol* gene as illustrated in Sambrook method[17]. Nevertheless, some other scientist uses his or her own made protocol for isolation[9].

Amplification

The analysis of specific nucleotide sequences, like many analytic procedures, hampered by the presence of extraneous material or by the extremely small amounts available for examination[19]. Saiki et al. (1988) has recently described a method, the polymerase chain reaction, which overcomes these limitations[19,20]. This technique is capable of producing a selective enrichment of a specific DNA sequence by a factor of 10^6, greatly facilitating a variety of subsequent analytical manipulations. PCR which used in: - (i) Examination of nucleotide sequence variations[21] and chromosomal rearrangements[22] (ii) For high-efficiency cloning of genomic sequences[23] (iii) Direct sequencing of mitochondrial DNA[24] and genomic DNAs[25] (iv) Detection of viral pathogens[26]. PCR amplification involves two oligonucleotide primers that flank the DNA segment to be amplified and repeated cycles of heat denaturation of the DNA, annealing of the primers to their complementary sequences, and extension of the annealed primers with DNA polymerase[25]. These primers hybridize to opposite strands of the target sequence and are oriented so DNA synthesis by the polymerase proceeds across the region between the

primers, effectively doubling the amount of that DNA segment[22]. Moreover, since the extension products are also complementary to and capable of binding primers, each successive cycle essentially doubles the amount of DNA synthesized in the previous cycle. This results in the exponential accumulation of the specific target fragment, approximately 2^n, where n is number of cycles[27]. *Taq*genomic DNA has been used to amplify the open-reading-frame of the *Taq*DNA polymerase Igene (Gene Bank accession J04639)[28]. By means of PCR and gene-specific DNA primers have designed (Table1).

The upstream primer includes an *Eco*RI restriction site and the downstream primer includes a *Bgl*II restriction site. PCR is performed in a 25 µl volume containing 12.5 µl of 2× PCR master mix , 10 pmol each primer, and 250 ng of *Taq*DNA. PCR performed in a thermocycler programmed at 94°C for 2 minutes followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 3 minutes. A portion of the PCR is analyzed by gel electrophoresis to confirm the presence of a 2500 base-pair amplicon[27].

Table 1 Primers and buffers: The underlined sequences within the primers are the added restriction enzyme digestion sites of *Eco*RI and *Bgl*II respectively.

Storage Buffer	50 mMTris-HCL pH 7.9, 100 mMNaCl, 0.5 mM DTT, 1% Triton X-100 detergent, and 50% Glycerol
Buffer A	50 mMTris-HCL pH 7.9, 50 mM Dextrose, 1 mM EDTA
Buffer B	10 mM Tris- HCL pH 7.9, 50 mM KCL, 0.5% Tween 20 detergent, 0.5% Nonidet P40 detergent
Upstream primer	5’CACGAATTCGGGGATGCTGCCCCTCTTTGAGCCCAAG-3’
Downstream primer	5’GTGAGATCTATCACTCCTTGGCGGAGAGCCAGTC-3’

The following set of primers, forward one 5’ - CGG AAT TCT GAG GAG GTA ACA TGA GGG -3’ and the reverse sequence 5’-CGT CGA CTA GAT CAC TCC TTG GCG GAG AG -3’ having unique *Eco*RI and *Sal*I restriction sites respectively at each end of the amplified DNA fragment. The fragment was ligated into the expression vector *pET* giving a closed circular fusion molecule called *pTTQ*. Ligate is then transformed into competent *E. coli* strain, TOP 10 by $CaCl_2$ using heat shock method at 42°C for 45 seconds[7].

Cloning and gene expression

Several groups of scientists have reported the cloning and expression in *E. coli* of genes from thermophiles: malate dehydrogenase (*mdh*) from *T. flnvus*[29], P-isopropylmalate dehydrogenase *(leuB)* from *T. thermophiles*[30] and the Tag1 restriction-modification system from *T. aquaticus*[31]. The *mdh* gene from a *T. flnvus*partial *Hind*III library in pBR322 by screening crude extracts of pools of independent library transformants at 60°C for malate dehydrogenase activity[32]. Slatko*et al*. (1987) also preferred in a straight line for expression of *Taq*Imethylase in *Taq*pBR322 libraries[31]. However, *Taq*I endonuclease appeared not to be active at 37°C in *E. coli,* since clones with only the restriction gene were viable in the absence of modification. In another approach Kelley *et al.* (1977) cloned the structural gene for *DNA pol* I from *E. coli* in λ-bacteriophage, [33] while T4 DNA polymerase has been cloned and expressed in *E.* coli[34]. Different vectors are used for the cloning of *TaqPol* gene in *E. coli* strains for instance *XL1* Blue together with plasmid *pTZ57R* [28], *JM109* together with plasmid *pTTQ18*[27] and strain *DG98, DG116, N6590* by means of plasmid *pBS*$^+$, *pFC54T*, and *pDG160* respectively[6].

Quite a lot of researchers used new technique for gene cloning well-known by Universal TA cloning method. TA cloning is one of the simplest and most efficient methods for the cloning of PCR products[35]. Procedure exploits the terminal transferase activity of certain thermophilic DNA polymerases, including *T. aquaticus* polymerase. *Taq*polymerase has non-template dependent activity which preferentially adds a single adenosine to the 3'-ends of a double stranded DNA molecule, and thus most of the molecules PCR amplified by *Taq*polymerase possess single 3'-A overhangs. The use of a linearized "T-vector" which has single 3'-T overhangs on both ends allows direct, high-efficiency cloning of PCR products, facilitated by complementarities between the PCR product 3'-A overhangs and vector 3'-T overhangs[36]. The TA cloning method can be easily modified so that the same T-vector can be used to clone any double-stranded DNA fragment, including PCR products amplified by any DNA polymerase, as well as all blunt- and sticky-ended DNA species[37].

The directional cloning is made possible by appropriate hemi-phosphorylation of both the T-vectors and the inserts. With a single T-vector at hand, any DNA fragment can be cloned without compromising the cloning efficiency[38]. The complementarities between the vector 3'-T overhangs and PCR product 3'-A overhangs allows direct ligation of *Taq*amplified PCR products into the T-vector, and this strategy is commonly referred to as "TA cloning." The TA cloning method, originally designed to facilitate the cloning of PCR products, is easily converted to a universal cloning method[39]. Several methods have now been developed for the cloning of

PCR-amplified DNA molecules, including cohesive-end cloning by the introduction of restriction sites at the 5'-ends of PCR primers,[40] blunt end cloning,[41] and ligation-independent cloning[42].

Sequencing of *Taqpol* Igene

DNA sequencing by the Sanger dideoxynucleotide method[43] has undergone significant refinement in recent years, including the development of additional vectors, base analogs, enzymes, and instruments for partial automation of DNA sequence analysis[44]. The basic procedure involves (i) hybridizing an oligonucleotide primer to a suitable single- or denatured double-stranded DNA template[45,46] (ii) extending the primer with DNA polymerase in four separate reaction mixtures, each containing one labeled dNTP, a mixture of unlabeled dNTPs, and one chain-terminating ddNTP[47] (iii) resolving the four sets of reaction products on a high resolution poly-acrylamide/urea gel[48,49]and (iv) producing an auto-radiographic image of the gel, which can be examined to infer the DNA sequence[50]. The current commercial instruments address non-isotopic detection and computerized data collection and analysis[51]. The PCR product can then either be sub-cloned into a vector suitable for sequence analysis or, alternatively, purified PCR products can be sequenced[52]. A little of research papers are available that explain the sequencing of *TaqPol* gene of *T. aquaticus*[5,53]. Some scientist compared the DNA sequence of *TaqPol* I with that of *E.* coli DNA *pol I.* At the DNA level, the two genes lack any significant regions of homology. In regions where the amino acid sequences are homologous, the DNA sequences diverge, especially in third positions of codons. The longest stretch of DNA sequence identity is 19 bases in Table 2.[9]

Table: 2 Nucleotide sequence coordinates for *TaqPol*I and for *E.* coli Pol I adapted from Gen-Bank along with the matching accuracy of amino acid based on amino acid identity.

DNA Sequence identity of*TaqPol I* and *E. coli Pol I*				
	Location of nucleotide sequence	Identity of nucleotide	Identity of amino acid	Matching accuracy of amino acid based on amino acid identity (%)
Taq Pol I Pol I	190-208 178-196	19/19	6/6	100
Taq Pol I Pol I	1730-1757 2015-2041	23/28	9/9	100
Taq Pol I Pol I	2260-2277 2545-2562	17/18	6/6	100
Taq Pol I Pol I	2344-2363 2635-2654	17/20	7/7	100

The restriction map of *TaqPol* I gene shown in Fig-2 and DNA sequence and deduced amino acid sequence of the *TaqPol* I gene (described by Lawyer *et al.*,1989)[9] are

used to generate codon bias table by predicting amino acid sequence of *TaqPol* I. There is a heavy bias toward G and C in the third position (91.8% C and G) as would be expected for GC-rich organisms and as others have observed for other *Thermus*genes: 95% C and G for the gk24 gene encoding L-lactate dehydrogenase of *T. caldophilus,* [54] 94.8% for *mdh* from *T. jlauus*[32] and 89% for *leuB*from *T. thermophiles*[55].

Significant amino acid sequence similarity exists between *TaqPol* I, *E.* coli *Pol* I, and bacteriophage T7 DNA polymerase. There are two major regions of *TaqPol I* and one region of T7 DNA polymerase that show extensive sequence similarity compared to *E. coliPolI*. The first region of *TaqPol-I* extend from the N-terminus to approximately residue 300. The second region extends from approximately residue 410 to the C terminus of *TaqPol I*. The N-terminal region of *TaqPol* I corresponds to the N-terminal domain of *E. coli Pol I* shown to contain the 5'-3' Exonuclease activity[56]. The C-terminal regions of *TaqPolI* and T7 DNA polymerase correspond to the *E. coli Pol I* domain to contain DNA polymerase activity[57]. The X-ray structure of the KF shows that this domain contains a deep cleft believed to be responsible for DNA binding[58].

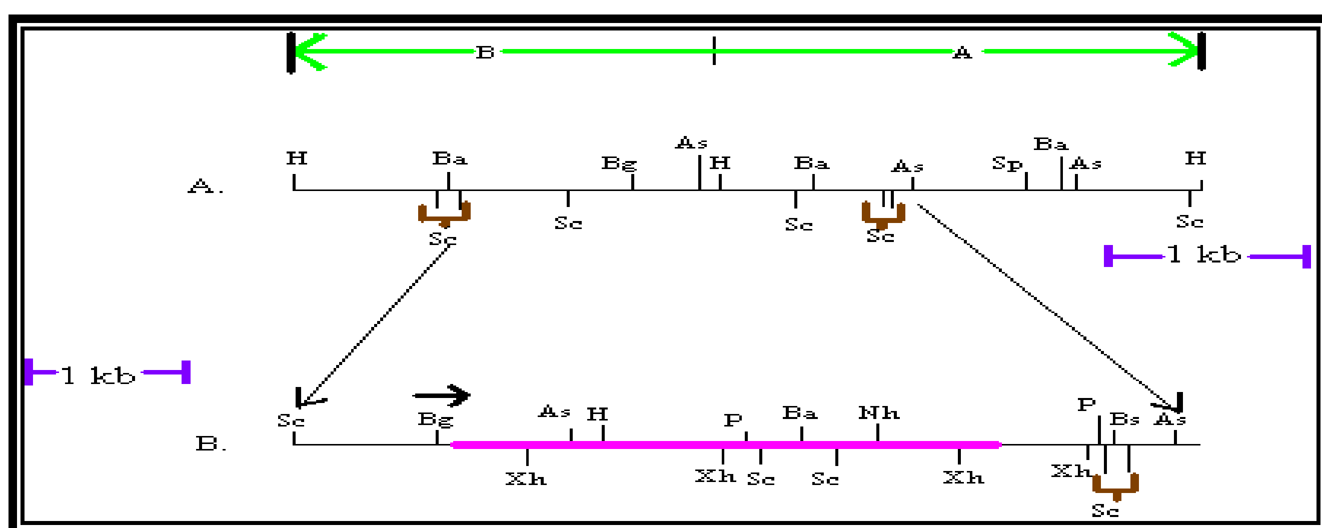

Fig-2 Restriction maps of DNA fragments containing the *TaqPol* I gene. The 4.5-kb *Hind*III B fragment and the 8.0-kb *Hind*III A fragment. Restriction sites are: *Hind*III*(H),* Sac1 (SC), *Bam*HI*(Ba), Bgl*II (Bg), Asp718 (As), and SphI*(Sp),* Bexpansion showing the *TaqPol I* coding region (bold pinkline), Arrow (indicates N terminus of the gene). Restriction sites are as above and *BstE*II*(Bs), Xho*I*(Xh) Pst*I*(PI),* and *Nhe*I (Nh).[9]

Sequence homology between *E. coli Pol I* and T7 DNA polymerase has been previously noted. The T7 DNA polymerase sequences to be conserved between *TaqPolI* enzyme and *E. coli Pol I* enzyme[13]. Most of the conserved residues are found in structural features that form the DNA binding cleft of the enzyme. Although short segments of T7 DNA

polymerase sequence in the 1 to 334 region are similar to regions in *E. coli Pol I* and *TaqPol I*, the overall sequence similarity in this region, ignoring the first 300 residues of *E. coli Pol I* and *TaqPol I* that form the 5'-3' Exonuclease domain, is poor. A complete and unambiguous sequence alignment for this region cannot be assigned. It should be noted that although T7 DNA polymerase also shows little similarity to *E. coli Pol I* in the region of the 3'-5' Exonuclease domain, T7 DNA polymerase has to be reported to display significant 3'-5' Exonuclease activity[59] The conserved sequences are found in polymerases from herpes simplex virus type 2, human *cytomegalovirus*, *Varicella-zoster* virus, *Epstein-Barr* virus, *Vaccinia* virus, adenovirus type 2, killer plasmid from *Kluveromyceslactis,* maize mitochondrial particle, bacteriophage 429, bacteriophage T4, bacteriophage *PRD1,* and yeast plasmids. Neither *E.* coli *Pol I* nor *TaqPol I* nor bacteriophage T7 DNA polymerase contains the conserved sequences noted in the polymerases from that sources[60].

Chemical modification and inactivation studies of *E.* coli *Pol* I have resulted in the identification of many amino acid residues believed to be important or essential for polymerase activity[61]. Analyses of the effects of various mutations in the *E.* coli *Pol* I gene upon enzymatic activity have also been used to define amino acid residues important for polymerase activity[62]. For example, a Gly to Arg mutation at position 850 *(polA5)* results in a polymerase that is less processive on the DNA substrate[63], an Arg to His mutation at position 690 *(polA6)* results in a polymerase that is defective in DNA binding[64]. Both Gly-850 and Arg-690 are conserved residues in *TaqPol* I. *E. coliPol* I characterized a number of mutants defective in 5’-3’ Exonuclease activity, amusingly the four mutations Y77C *(polA107),* G103E (*polA4113*), G184D (*polA480ex*), and G192D (*polA214*), these all four mutation occur at amino acid residues that are conserved in *TaqPolI*[65].

Enzyme from a thermophilic organism, *TaqPol* I is considerably more thermostable than *Pol* I from *E. coli*[66]. Many researchers have attempted to explain enzyme thermo stability by an analysis of amino acid content[67]. Several features of thermostable enzymes have been noted in such studies. Among those features are increased ratios of Arg to Lys residues, Glu to Asp residues, Ala to Gly residues, Thr to Ser residues, and a reduced Cys content. Comparing *TaqPol*I to *E. coli Pol* I, the Ala to Gly and Thr to Ser ratios are smaller for *TaqPol* I than for *E. coliPol* I[68].

Purification

The purification of *Taq*DNA polymerase from *E. coli* is relatively simple because of high expression levels and the thermostable nature of the enzyme, which allows a “heat-cut” to denature contaminating host proteins[27] Robert *et al*. The method *Taq*polymerase purification, characterization

and analysis laboratory series represents a successful approach to the laboratory training of undergraduates[69]. Methods for purification of *Taq*protein from bacterial cultures involve selective precipitation and ion exchange chromatography[27,7]. The novel implified protocol for purifying *Taq* polymerase which is suitable for PCR and DNA sequencing. The method exploits the thermostable properties of *Taq*polymerase contaminating macromolecules of the host *E. coli* (strain BL21 used here) are conveniently precipitated after their denaturation by freezing and high temperature thawing[70]. *E. coli* of the strain *BL21* was transformed with p*Taq*. These bacteria contain the *pLysS* plasmid, which makes them susceptible to lysis by freeze-thawing for the release of recombinant proteins. By changing the thawing temperature from room temperature to 75°C, most of the host *E. coliBL21* proteins were denatured and they were easily removed from the lysate as a precipitate[71]. The yield of enzyme activity was 20 U/pl in the dialyzed lysate or 400 U/ml of culture broth. This freeze-thawing method for the purification of *Taq*polymerase is simpler than previously described techniques, and the enzyme yield is high. Standard purification was done with 81 batches of *E. coli* culture, which produced average yields well in excess of 10^6 Units of *Taq*polymerase per liter of starting culture[9]. The final product of the purification showed a 90 kD band on SDS-PAGE and few contaminating protein bands were evident, even after silver staining[18]. The enzyme was highly concentrated and had to be diluted (usually by a factor of 100) before use, since *Taq*polymerase shows a decrease in activity at high concentrations[4].

Activity of *Taq*protein

The activity of the purified *Taq*polymerase was determined by titration against commercial preparations in a DNA amplification assay[4]. Typical concentration of activity of final purification product being 100 units/µl[15]. Exonuclease activity tested by incubating working concentrations of enzyme with cut plasmid DNA at 37°C overnight, and no evidence of nuclease activity was seen when the DNA was run on an agarose gel. The expected 300 base-pair fragment is produced by both the purified and commercial *Taq*DNA polymerase preparations[27]. Based on the relative intensity of the amplicons in the stained gel, 2 µl of 1/10 diluted supernatant fraction is at least equivalent to 2 units of commercial *Taq*DNA polymerase indicating the prepared protein fraction has about 10 units/µl of DNA polymerase activity[7].

CONCLUSION

DNA polymerase from *T. aquaticus* has become a common reagent in molecular biology because of its utility in DNA amplification and DNA sequencing protocols. Simplified methods have been discussed here for cloning, expression and purification of recombinant *Taq*enzyme.

One of the major achievements in recombinant DNA (rDNA) technology has been the high level expression (over production) of protein of interest in *E. coli*. The same operations need to be applied to the nucleotide sequence of *Taq*gene (one of the factors strongly influencing translation efficiency) for example, optimizing the codon usage and developing a dedicated host-vector system, to overcome the problem of deficient expression. A milestone would be achieved if we can develop optimized protocols for customizing protein of interest according to R&D/industrial needs, once we have an adequate quantity. The future of PCR based research lies in incorporating the desirable features of higher fidelity and thermostable DNA polymerases - *Vent* and *Pfu*, into *Taq* itself. This can therefore be achieved by altering the native gene of *Taq*Pol I to make mutant forms with improved expression in *E. coli*. *Taq*polymerase, therefore, though being one of the earliest revolutionary molecules of biotechnological research carries tremendous opportunity for investigation and improvement for cost and quality improvements in industries and laboratory settings.

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REFERENCES

1. A. Chien, D. B. Edgar and J. M. Trela. Deoxyribonucleic acid polymerase from the extreme thermophile *Thermusaquaticus*, Journal of Bacteriology 127: 1550-1557 (1976).
2. A. S. Kaledin, A. G. Sliusarenko and S. I. Gorodetskii. Isolation and properties of DNA polymerase from extreme thermophylic bacteria *Thermusaquaticus* YT-1, Biokhimiya 45: 644-651 (1980).
3. R. K. Saiki, D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis and H.A. Erlich. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase, *Science* 239: 487-491 (1988).
4. D. R. Engelke, A. Krikos, M. E. Bruck and D.Ginsburg. Purification of *Thermusaquaticus* DNA polymerase expressed in *Escherichia coli*, Analytical Biochemistry 191: 396-400 (1990).
5. M. A. Innis, K. B. Myabo, D. H. Gelfand and M. A.Brow. DNA sequencing with *Thermusaquaticus* DNA polymerase and direct sequencing of polymerase chain reaction amplified DNA, *Proceeding of the*

Natural Academy of Sciences of the USA 85: 9436-9440 (1988).

6. F. C. Lawyer, S. Stoffel, R. K. Saiki, S. Y. Chang, P. A. Landre, R. D. Abramson and D. H. Gelfand. 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, *PCR Methods and Applications* 2:275-287 (1993).
7. R. Mohammad and G. Hamid. Cloning and expression of *Thermusaquaticus* DNA polymerase in E. coli, Jundishapur Journal of Microbiology 1(1): 1-5 (2008).
8. K. Mullis, F. Faloona, S. Scharf, R. Saiki, G. Horn and H. Erlich. Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction, Cold Spring Harbor Symposium on Quantitative Biology 51(1): 263–373 (1986).
9. F. C. Lawyer, S. Stoffel, R. K. Saiki, K. Myambo, R. Drummond and D. H. Gelfand. Isolation, characterization, and expression in *Escherichia coli* of the DNA polymerase gene from *Thermusaquaticus*, Journal of Biological Chemistry 264: 6427-6437 (1989).
10. D. E. Koshland. The molecule of the year, *Science* 246: 1541 (1989).
11. Y. Kim, S. H. Eom, J. Wang, D. S. Lee, S. W. Suh and T. A. Steitz. Crystal structure of *Thermusaquaticus* DNA polymer-polymerase, *Nature*. 376:612-616 (1995).
12. S. Korolev, M. Nayal, W. M. Barnes, E. D. Cera and G.Waksman. Crystal structure of the large fragment of *Thermusaquaticus* DNA polymerase I at 2.5-A resolution: Structural basis for thermostability, Proc. Natl. Acad. Sci. 92: 9264-9268 (1995).
13. D. L. Ollis, C. Kline and T. A. Steitz. Domain of *E. coli* DNA polymerase I showing sequence homology to T7 DNA polymerase, Nature 313: 818-819 (1985).
14. Y. Ishino, T. Ueno, M. Miyagi, T. Uemori, M. Imamura, S. Tsunasawa and I. Kato. Overproduction of *Thermusaquaticus* DNA polymerase and its structural analysis by ion- spray mass spectrometry, Biochem. 116: 1019-1024 (1994).
15. F. G. Pluteo. Rapid purification of high activity of *Taq*DNA polymerase, Nucleic Acid Research 21: 4850-4851 (1993).
16. L. S. Merkens, S. K. Bryan and R. E. Moses. Inactivation of the 5'-3' exonuclease of *Thermusaquaticus* DNA poly-polymerase, Biochem. Biophys. Acta, 1264:243-248 (1995).
17. J. Sambrook, E. F. Fritsch and T. Maniatis. Molecular cloning: A laboratory manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press, 2001, pp. 20-25.
18. E. Grimm and P. Arbuthnot. Rapid purification of recombinant *Taq*DNA polymerase by freezing and high temperature thawing of bacterial expression cultures, Nucleic Acids Research 23: 4518-4519 (1995).

19. K. B. Mullis and F. A. Faloona. Specific synthesis of DNA *invitro* via a polymerase-catalyzed chain reaction, Methods Enzymol, 155: 335-350 (1987).
20. R. K. Saiki, S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich and N. Arnheim. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia, Science 230: 1350-1354 (1985).
21. R. K. Saiki, T. L. Bugawan, G. T. Horn, K. B. Mullis and H.A. Erlich. Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes, Nature (London), 324 (6093): 163-166 (1986).
22. M. S. Lee, K. S. Chang, F. Cabanillas, E. J. Freireich, J. M. Trujillo and S. A. Stass. Detection of minimal residual cells carrying the (14; 18) by DNA sequence amplification, Science 237: 175-178 (1987).
23. S. J. Scharf, G. T. Horn and H. A. Erlich. Direct cloning and sequence analysis of enzymatically amplified genomic sequences, Science 233: 1076-1078 (1986).
24. L. A. Wrischnik, R. G. Higuchi, M. Stoneking, H.A. Erlich, N. Arnheim and A.C. Wilson. Length mutations in human mitochondrial DNA: direct sequencing of enzymatically amplified DNA, Nucleic Acids Res, 15: 529-542 (1987).
25. G. McMahon, E. Davis and G. N. Wogan. Characterization of c-Ki-ras oncogene alleles by direct sequencing of enzymatically amplified DNA from carcinogen-induced tumors, Proc. Natl. Acad. Sci. U.S.A. 84: 4974-78 (1987).
26. S. Kwok, D. H. Mack, K. B. Mullis, B. Poiesz, G. Ehrlich, D. Blair, A. Friedman-Kien and J. J. Sninsky. Identification of Human Immunodeficiency Virus Sequences by Using In Vitro Enzymatic Amplification and Oligomer Cleavage Detection, J. Virol. 61: 1690-1694 (1987).
27. P. Ferralli, J. D. Egan and F. L. Erickson. Making *Taq*DNA polymerase in the undergraduate biology laboratory, Bios 78(2): 69-74 (2007).
28. H. M. M. Sadeghi, M. Rabbani and F. Moazen. Amplification and cloning of *Taq*DNA polymerase gene from *Termusaquaticus* strain YT-1, Research in Pharmaceutical Science 1: 49-52 (2006).
29. S. Iijima, T. Uozumi and T. Beppu. Molecular Cloning of Thermusflavus Malate Dehydrogenase Gene, Agric. Biol. Chem. 50: 589-592 (1986).
30. K. Nagahari, T. Koshikawa and K. Sakaguchi. Cloning and expression of the leucine gene from Thermusthermophilus in Escherichia coli. Gene (Amst.), 10: 137-145 (1980).
31. B. E. Slatko, J. S. Benner, I. Jager-Quinton, L. S. Moran, T. G. Simcox, E. M. Vann Cott and G. G.Wilson. Cloning, sequencing and expression of the *Taq I* restriction-

modification system, Nucleic Acids Res. 15: 9781-9796 (1987).

32. M. Nishiyama, N. Matsubara, K. Yamamoto, S. Iijima, T. Uozumi and T. Beppu. Nucleotide Sequence of the Malate Dehydrogenase Gene of *Thermusflavus* and its Mutation Directing an Increase in Enzyme Activity, J. Bwl. Chem. 261: 14178-14183 (1986).
33. W. S. Kelley, K. Chalmers and N. E. Murray. Isolation and characterization of a XpolA transducing phage. Proc. Natl. Acad. Sci. U. S. A. 74: 5632-5636 (1977).
34. T. C. Lin, J. Rush, E. K. Spicer and W. H. Konigsberg. Cloning and expression of T4 DNA polymerase, Proc. Natl. Acad. Sci. U. S. A. 84: 7000-7004 (1987).
35. M. Y. Zhou, E. Celso and Gomez-Sanchez. Universal TA cloning, Curr. Issues Mol. Biol. 2(1): 1-7 (2000).
36. T. A. Holton and M. W. Graham. A simple and efficient method for direct cloning of PCR products using ddT- tailed vectors, Nucleic Acids Res. 19: 1156-65 (1991).
37. D. Marchuk, M. Drumm, A. Saulino and F. S. Collins. Construction of T- vectors, a rapid and general system for direct cloning of unmodified PCR products, Nucleic Acids Res. 19: 1154-62 (1991).
38. D. A. Mead, N. K. Pey, C. Herrnstadt, R. A. Marcil and L.M. Smith. A universal method for the direct cloning of PCR amplified nucleic acid, Bio/Technology 9: 657-663 (1991).
39. M. Y. Zhou, S. E. Clark and C. E. Gomez-Sanchez. Universal cloning method by TA strategy, *BioTechniques* 19: 34-35 (1995).
40. V. Jung, S. B. Pestka and S. Pestka. Cloning of polymerase chain reaction-generated DNA containing terminal restriction endonuclease recognition sites, Methods in Enzymology 208: 357-362 (1993).
41. J. S. Crowe, H. J. Cooper, M. A. Smith, M. J. Sims, D. Parker and D. Gewert. Improved cloning efficiency of polymerase chain reaction (PCR) products after proteinase K digestion, Nucleic Acids Res.19: 184-192 (1991).
42. A. Rashtchian. Novel methods for cloning and engineering genes using the polymerase chain reaction, Current Opinion in Biotech. 6: 30-36 (1995).
43. F. Sanger, S. Nicklen and A. R. Coulson. DNA sequencing with chain-terminating inhibitors, Proc. NatI. cad. Sci. USA 74: 5463-5467 (1977).
44. C. Yanisch-Perron, J. Vieira and J. Messing. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors, Gene 33: 103-119 (1985).
45. D. R. Mills and F. R. Kramer. Structure-independent nucleotide sequence analysis, Proc. Natl. Acad. Sci. USA 76: 2232-2235 (1979).

46. P. J. Barr, R. M. Thayer, P. Laybourn, R. C. Najarian, F. Seela and D.R. Tolan. 7-Deaza-2'-deoxyguanosine-5'-triphosphate: enhanced resolution in M13 dideoxy sequencing, BioTechniques 4(5): 428-432 (1986).
47. S. Tabor and C. C. Richardson. DNA sequence analysis with a modified bacteriophage T7 DNA polymerase, Proc. Natl. Acad. Sci. USA 84: 4767-4771 (1987).
48. L. M. Smith, J. Z. Sanders, R. J. Kaiser, P. Hughes, C. Dodd, C. R. Connell, C. Heiner, S. B. Kent and L.E. Hood. Fluorescence detection in automated DNA sequence analysis, Nature (London) 321: 674-679 (1986).
49. J. M. Prober, G. L. Trainor, R. J. Dam, F. W. Hobbs, C. W. Robertson, R. J. Zagursky, A. J. Cocuzza, M. A. Jensen and K. Baumeister. A system for rapid DNA sequencing with fluorescent chain-terminating dideoxynucleotides, Science 238:336-341 (1987).
50. W. Ansorge, B. Sproat, J. Stegemann, C. Schwager and M. Zenke. Automated DNA sequencing: ultrasensitive detection of fluorescent bands during electrophoresis, Nucleic Acids Res. 15: 4593-4602 (1987).
51. D. R. Engelke, P.A. Hoener and F. S. Collins. Direct sequencing of enzymatically amplified human genomic DNA, Proc. Natl. Acad. Sci. USA 85: 544-548 (1988).
52. E. S. Stoflet, D. D. Koeberl, G. Sarkar and S. S. Sommer. Genomic amplification with transcript sequencing, Science 239: 491-494 (1988).
53. U. B. Gyllensten and H. A. Erlich. Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA-DQA locus, Proc. Natl. Acad. Sci. USA 85: 7652-7656 (1988).
54. K. Kunai, M. Machida, H. Matsuzawa and T. Ohta. Nucleotide sequence and characteristics of the gene for L-lactate dehydrogenase of *Thermuscaldophilus* GK24 and the deduced amino-acid sequence of the enzyme, Eur. J. Biochem.160: 433-440 (1986).
55. Y. Kagawa, H. Nojima, N. Nukiwa, M. Ishizuka, T. Nakajima, T. Yasuhara, T. Tanaka and T. Oshima. High Guanine plus Cytosine Content in the Third Letter of Codons of an Extreme Thermophile, J. Biol. Chem. 259: 2956-2960 (1986).
56. D. Brutlag, M. R. Atkinson, P. Setlow and A. Kornberg. An active fragment of DNA polymerase produced by proteolytic cleavage, Biochem. Biophys. Res. Commun. 37:982-989 (1969).
57. P. S. Freemont, D. L. Ollis, T. A. Seitz and C. M. Joyce. A domain of the Klenow fragment of *Escherichia coli* DNA polymerase I has polymerase but no exonuclease activity, Proteins Struct. Funct. Genet. 1: 66-73 (1986).

58. D. L. Ollis, P. Brick, R. Hamlin, N. G. Xuong and T. A. Steitz. Structure of large fragment of *Escherichia coli* DNA polymerase I complexed with dTMP, Nature 313: 762-766 (1985).
59. S. Tabor and C. C. Richardson. Selective oxidation of the exonuclease domain of bacteriophage T7 DNA polymerase, J. Biol. Chem. 262: 15330-15333 (1987).
60. A. Bernad, A. Zaballos, M. Salas and L. Blanco. Structural and functional relationships between prokaryotic and eukaryotic DNA polymerases, EMBO J. 6: 4219-4225 (1987).
61. A. Basu and M. J. Modak. Identification and amino acid sequence of the deoxynucleoside triphosphate binding site in *Escherichia coli* DNA polymerase I. Biochemistry 26: 1704-1709 (1987).
62. V. N. Pandey, K. R. Williams, K. L. Stone and M. J. Modak. Photo affinity labeling of the thymidine triphosphate binding domain in *Escherichia coli* DNA polymerase I: identification of histidine-881 as the site of cross-linking, Biochemistry 26: 7744-7748 (1987).
63. S. W. Matson, F. N. Capaldo-Kimball and R. A. Bambara. On the Processive Mechanism of Escherichia coli DNA polymerase I, J. Biol. Chem. 253: 7851-7856 (1978).
64. W. S. Kelly and N. D. F. Grindley. Pol A6 a mutation affecting the DNA binding capacity of DNA polymerase I, Nucleic Acids Res. 3: 2971-2984 (1976).
65. C. M. Joyce, D. M. Fujii, H. S. Laks, C. M. Hughes and N. D. Grindley. Genetic mapping and DNA sequence analysis of mutations in the *polA* gene of Escherichia coli, J. Mol. Biol. 186: 283-293 (1985).
66. R. Singleton and R. E.Amelunxen. Proteins from Thermophilic Microorganisms, Bacteriol. Reu. 37: 320-342 (1973).
67. P. Argos, M. G. Rossmann, U. M. Grau, H. Zuber, G. Frand and J. D. Tratschin. Thermal stability and protein structure, Biochemistry 18: 5698-5703 (1979).
68. P. K. Ponnuswamy, R. Muthusamy and P. Manavalan. Amino acid composition and thermal stability of proteins, Int. J. Bwl. Macromol.4: 186-190 (1982).